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CONTRIBUTIONS

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No. 1.

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MALARIA.[1]

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Malaria, in my opinion, is the most disastrous scourge, considering both the past and the present, that has ever afflicted the human race. The destruction of that wonderful civilization of Greece is now practically proved to have been due, according to Jones, not so much to the Macedonian and the Roman as to the great tyrant which now holds half the world—Malaria. Choosing its victims not from among the weak and undesirable, and thus tending to a survival of the fittest, but attacking in countries where the disease is rife all, good and bad, strong and weak. Malaria has been called the most incapacitating disease to which man is liable, and it is this peculiarity that makes it so dangerous and so disastrous to those countries where it is common, sapping not only the vitality, but the intellect and morals of the inhabitants, increasing the death rate and reducing the birth rate. The disease has been known and recognized for centuries, and we must admit that Galen and Celsus seem to have had a better conception of the disease as an entity and of its clinical picture than many of our present physicians. Galen divided, for example, malarial fevers into quartans and tertians, and also recognized mixed and double infections, and states that a fever with attacks recurring every day is liable to be diagnosed by the uninitiated as a quotidian. But (and let me lay especial emphasis on this sentence) if a man take pains and have a genuine interest in medicine, he will not forget that the same effect can be produced by two tertians or three quartans. Galen and Celsus both speak of a malignant or semi-tertian differing from the simple tertian, which resembles the quartan except in its periodicity, the semi-tertian being a much more malignant variety. I will speak later of the recent rediscovery a few years ago of this same malignant tertian malaria.

[1] Read at a Symposium on Protozoan Diseases before the Pathological Society of Philadelphia, January 26, 1911

An example of our present lack of knowledge and lamentable lack of what Galen would call, I suppose, a genuine interest in medicine, has been mentioned by Craig, who states that the "colossal mistake," to use his own words, of diagnosing hundreds of cases of typhoid fever as malaria was responsible for the terrible epidemic at Chickamauga in the Spanish-American war.

Laveran, in 1880, in Algeria, discovered the cause of malaria, and announced to the world that he had found certain parasites in the blood of patients with malaria. These parasites were called by Laveran the Plasmodium malariæ. This discovery, like many another important one, was not received at once with much credence. The term Plasmodium used by Laveran was an unfortunate one, as the parasite is not, zoölogically speaking, a plasmodium, but a sporozoa, and has been placed in the order of hemosporidia. For a time the plasmodium was divided into three varieties—the Plasmodium vivax causing the tertian type of fever, the Plasmodium malariæ causing the quartan type, the Plasmodium falciparum causing the estivo-autumnal type. These terms were, however, not universally adopted, many other appellations being also used. In Philadelphia tertian malaria is much the commonest variety observed. Estivo-autumnal malaria comes next in point of frequency, but is usually imported. The quartan variety I have never seen except in imported cases, and then only three or four times. Golgi had, in 1885, isolated the estivo-autumnal variety as a distinct type, and in 1891 Marchiafava and Bignami described a subvariety, the malignant tertian, and now it is almost universally admitted to consist of two varieties—the malignant tertain and the quotidian, differing not only clinically but also in the morphology of the parasite; so that at present we speak of four varieties of the malarial parasite:

The benign tertian.
The quartan.
The malignant tertian.
The quotidian.

This classification is not admitted by all authorities. Scheube, for example, in his stupendous work on Die Krankheiten der Warmen Länder, 1910, omits the quotidian described by Marchiafava and Bignami, and believes that this is probably identical with the parasite of malignant tertian. Craig, however, in this country stoutly defends the

division of estivo-autumnal malaria into these two varieties. Certainly the few cases of estivo-autumnal malaria seen here cannot easily be differentiated clinically at any rate into malignant tertians and quotidians, as the majority have practically no fever at all. There is no doubt that previous to Laveran's discovery the parasites had been seen by different investigators who failed to grasp the meaning or importance of what they saw; for example, in 1866, my father, Dr. William Pepper, reported before this very Society three cases of malaria, in the description of one of which he stated that the blood drawn from the finger during life had shown the red corpuscle pale, many of them crenated with several large black pigment masses, and in another case he had found in the blood a deficient number of red corpuscles, which were crenated and running together without forming rouleaux, and several black but rather small pigment masses.

Next to Laveran's discovery in importance was the proof by Major Ronald Rose, in 1897, that the anophelinæ, a genus of mosquitoes, when fed on malarial patients were found to contain certain pigmented cells in the stomach wall which Ross recognized as a stage in the development of the parasite outside the human body. Major Ross, stimulated by Manson's suggestion to him, had worked long and persistently on the mosquito theory of malaria before success crowned his efforts, and there is no more fascinating reading than the account he gives in his Nobel Medical Prize Lecture in 1902. The discovery was no lucky accidental finding, but was an example of persistent effort under most discouraging and trying circumstances, and should serve for all time as a stimulus to original research. Ross soon worked out the entire cycle of development in the mosquito, and, working with birds, inoculated them with malaria from mosquitoes which had previously been infected. The Italians Bignami, Bastianelli, and Grassi claimed part of the glory, but unsuccessfully. They did, however, in 1898, produce human estivo-autumnal malaria in cases bitten by a mosquito that had previously fed on a case of this type of fever. Later in the same year this was also accomplished by them with tertian and in 1899 with quartan malaria. Professor Koch was probably the first to verify Ross' work, and he gives Ross due credit.

The mosquito theory of malaria was, of course, as old as the hills, but it took a man of Ross' type to prove it.

My time limit forbids the description of the life cycle of more than a single variety of organism. Let us take, for example, that of tertian malaria and consider in the first place the human cycle, the cycle taking place in the human blood. This is known as the Schizogonic or asexual cycle. Beginning with the introduction by the mosquito of the so-called sporozoites, needle-shaped bodies which move around in the blood until they find red cells to which they attach themselves and later penetrate. Losing their elongate form they become irregular in shape, changing their form by a constant amœboid motion. Growing larger they eat up the hemoglobin in the cells and become pigmented. The full-grown tertian parasite occupies a large portion of the cell, and the cell is swollen and pale. The pigment is fine and moves actively. Just before sporulation occurs the pigment becomes less active and gathers in clumps in the centre. The spores when formed are oval in shape and number from about 14 to 26 and are small in size, they are not arranged in a very regular rosette form. At the time of the chill, which occurs in tertian malaria every forty-eight hours, the sporulating organism ruptures and the spores or merozoites are set free and these move about and attach themselves to red corpuscles and start the cycle over again. The chill is probably due to toxins set free at this time. I have seen under the microscope a segmenting tertian organism rupture and the merozoites start off in various directions, followed by a leukocyte that captured them one after another, going first in one direction and then in another, until all were engulfed. This cycle of schizogony keeps on repeating itself every forty-eight hours, at times, especially early in the disease, anticipating or taking less than forty-eight hours, and later, as the disease begins to disappear, a little longer, and finally tends to die out even without treatment. This is an asexual form of reproduction. If a mosquito were to bite a patient with malaria and withdraw nothing but these so-called schizonts, it could not infect another human being, because the schizonts will not develop within the mosquito, but are digested. A provision of nature to prevent this untimely extinction of the parasite is found in the formation of what are known as gametes. These are sexual forms and appear only after the infection has existed for a few days. The gametes are divided into macrogametes, or female forms, and microgametes, or male forms. Tertian gametes can be distinguished from

adult schizonts by being larger, showing less ameboid movement, and containing more active and abundant pigment. The macrogametes and microgametes can also be readily differentiated by anyone, as Galen would say, who takes pains and has a genuine interest in medicine. These gametes, or sexual forms, finding their way into a mosquito undergo in the midgut certain changes. The male, or microgamete, throws out flagellæ which sever their attachment and start off on the search for the female, or macrogamete. These free swimming male elements are known as microgametocytes. Finding the macrogamete the microgametocyte enters it, impregnates it, and the resulting fertilized body is known as the zygote. The zygote makes its way into the wall of the midgut of the mosquito, becomes encysted, and is now known as an oöcyst. This oöcyst enlarges and contains so-called sporoblasts which divide up into many sporozoites. The oöcyst finally bursts and the sporozoites escape into the body cavity and from there travel to the salivary glands, and when the mosquito again bites another victim the sporozoites are injected into the human blood stream. This exogenous form of development in the mosquito is known as sporogony and takes from eight to sixteen days or longer, depending largely on the temperature. The beginning of sporogony can be seen in a drop of blood under the microscope at times. If the specimen contain many adult gametes it will be observed fifteen or twenty minutes after taking the blood that the microgametes throw out flagellæ which are waved about in a very rapid manner. These flagellæ may even be seen to detach themselves and swim off. MacCallum, in 1897, saw, while watching this stage in the development of Halteridium of birds under the microscope, the free flagellum, or microgametocyte, enter the macrogamete. This fact was known to Ross and helped him in the interpretation of his findings.

In 1902 Schaudinn described still another cycle, the Parthenogenetic. Parthenogenesis, or virgin birth, is a reproduction by unfertilized females. Schaudinn believed that this theory explained the frequently observed relapses occurring, perhaps year after year, in patients who have had malaria and have had no possible reinfection—patients who have moved to non-malarial countries and who, notwithstanding, suffer relapses at times in midwinter when no mosquitoes are about. The theory is that after a typical attack of fever has disappeared and all the schizonts

have vanished, leaving nothing but gametes, that these go into retirement in the spleen or other internal organs and after a time the macrogametes, or female forms undergo parthenogenesis; that is, without any fertilization from a male element they proceed to sporulation much as occurs in schizogonic sporulation, the spores then entering red cells and becoming true schizonts and starting again the cycle of schizogony. This parthenogenetic cycle, however, has not been universally accepted by the authorities. It is an ingenious and interesting theory if true.

Schizogony and sporogony in the quartan form is very similar to that of the tertian just given. The cycle of schizogony, or the cycle taking place in the human blood, however, takes seventy-two hours instead of forty-eight. The schizont shows less ameboid movement, the pigment is coarser and less active, the outline of the organism is not so irregular and tends to stretch in a band across the cell when half grown. The infected red cell does not become paler and swollen, but remains normal or slightly smaller in size and somewhat darker in color. The sporulating or segmenting quartan has the merozoites or spores arranged often in a perfectly regular rosette or marguerite. The number of merozoites usually range from 8 to 12 less than in the tertian.

In both tertian and quartan fever a very perfect series can be obtained by taking spreads of blood at regular intervals of time, beginning at one chill and extending until the next. If schizonts are found in widely varying stages of development the diagnosis can be readily made of a double or triple infection just as the finding of different types of organisms will enable one to make a diagnosis of a mixed infection. Unfortunately schizogony cannot well be studied in the peripheral blood from cases of estivo-autumnal malaria. One usually must study the blood from the spleen to see all the stages of development. In the blood from the finger is found in estivo-autumnal malaria the earliest stage, the little ring-shaped or round hyaline body resembling closely to the uninitiated the tertian and quartan ring bodies. In addition to the young schizonts which have this ring form, certain peculiarly shaped gametes, or sexual forms, are found called crescents, which are absolutely indicative of estivo-autumnal malaria. The young schizonts, or ring forms, and the gametes, or crescents, are in most cases the only stages found in the peripheral blood in both varieties of estivo-autumnal malaria, the malignant tertian as well as the quotidian. Both stages

differ in these two varieties, so that even if one or other stage is absent for the time from the blood, nevertheless the distinction can still be made between a case of malignant tertian and one of quotidian estivo-autumnal malaria. Thus the ring forms of the quotidian type are smaller, and frequently double or triple infection of a single cell may be observed. The malignant tertian ring form is larger and has often a typical signet-ring shape, usually only one organism will be found in a cell. The crescents, or gametes, in malignant tertian are slender crescentic-shaped bodies with pointed extremities, while in the quotidian type the crescents are shorter, plumper, and have rounder extremities. The crescents, as has been said, are the gametes, or sexual forms, and are divided just as the gametes of simple tertian and quartan are into macrogametes, or female forms, and microgametes, or male forms. The microgametes can be seen under the microscope at times to become circular and to throw out flagellæ or microgametocytes just as the microgametes of simple tertian and quartan do. MacCallum saw one of these microgametocytes attach itself to a macrogamete in the blood of a case of estivo-autumnal malaria just as he had earlier observed while studying avian malaria.

If blood from the spleen be studied in cases of estivo-autumnal malaria the other stages of development can be seen, and the young schizonts or ring forms can be traced up to the segmenting forms. In the quotidian type, schizogony or the human asexual cycle takes twenty-four hours. The segments, or merozoites, number from six to eight, and are very minute. In the malignant tertian type, schizogony takes forty-eight hours, just as in the simple, or benign tertian. The merozoites number from about ten to fifteen in this type.

The transfusion from a patient with malaria of blood containing schizonts into a healthy individual will usually inoculate the disease, but if the blood contain nothing but gametes this has not been shown to occur, although if the theory of parthenogenetic reproduction hold true, there is no very good reason why it might not take place.

I wish that I could feel that after this necessarily cursory discussion of the subject that I had impressed upon you all that if you will take pains and have a genuine interest in medicine you can easily differentiate the varieties and stages of development of the plasmodium,

because the variety of organism and stage of development should make a difference both in your prognosis and treatment of this disease.

The other afternoon, having discovered a case of malaria in the wards of the University Hospital, I sat fascinated for over an hour watching through the microscope the enactment of a drama of protozoan life to me more interesting than any modern problem play of the stage.

Having first observed a flagellated microgamete, I saw while watching it a polymorphonuclear leukocyte approaching in its stealthly ameboid way, followed at a distance by another faithful protector of our health. The first leukocyte had already captured, when I saw it, a free flagellum, or microgametocyte, one end of which still struggled vainly to escape in order to continue its search for some fair macrogamete. Nearer and nearer drew the leukocyte to the apparently unsuspecting male gamete until blocked by some intervening red corpuscle it paused. The second leukocyte thereupon overtook it, passed it, brushed the red cells aside, and grasping the gamete in his clutches, soon had torn it asunder into two equal halves. The other leukocyte, the one that had lost the race, thereupon started off at a right angle and captured another free flagellum, and thus the futile and impotent attempts at reproduction of the plasmodium were thwarted by these leukocytes—efforts that could not but be futile outside of a mosquito, prevented unnecessarily by leukocytes even after their removal from the body—both acting true to their instinct even under such discouraging circumstances. The observation of similar occurrences has convinced me that there is no more attractive work than watching these minute bodies play their little parts. Let me heartily recommend it to you.

No. 2.

Extracted from the American Journal of the Medical Sciences,
May, 1912, No. 5, vol. cxliii, p. 716.

ADAMS-STOKES SYNDROME, WITH COMPLETE HEART-BLOCK AND PRACTICALLY NORMAL BUNDLE OF HIS.

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The case herewith described was first reported by Dr. Alfred Stengel and Dr. William Pepper in the Amer. Jour. Med. Sci., October, 1910, under the title, "Heart-block, with an Indication of Genuine Hemisystole." From that time onward the patient was almost constantly under observation, and many more pulse tracings were obtained before his death. An autopsy was held and a careful examination of the heart was made. The full pathological report follows later, but suffice it here to say that the bundle of His was practically normal. In addition to this interesting finding, several other features make us believe this further report on the case is fully justifiable.

Six cases have been previously reported of heart-block without any lesion of the bundle of His, or with but such slight alteration of the bundle, as might be expected in many hearts where no block had occurred.

Krumbhaar,[1] in 1910, in his article on "Adams-Stokes Syndrome, with Complete Heart-block, without Destruction of the Bundle of His," states that he could find but 2 cases similar to his own, and since that time we have been able to find but 3 additional cases.

Deneke and Fahr's[2] Case. This case was one of complete block. The authors admit that they did not succeed in completely examining the bundle which was abnormally long but found no lesion in the parts examined.

[1] Arch. of Int. Med., 1910, v, 583.

[2] Arch. f. klin. Med., 1906, lxxxix, 39. See also Virchow's Arch., 1907, clxxxviii 562.

Nagayo's[3] Case. Here the heart-block was only incomplete. No lesion of the bundle was found, but there was extensive myocarditis, and Nagayo believes that the block occurred through the altered musculature of the ventricle.

Krumbhaar's[4] Case. Here complete heart-block with Adams-Stokes syndrome was known to have existed for five years. At autopsy a chronic myocarditis of the ventricles was found. The bundle of His was traced in unbroken continuity from Tawara's node to beyond the bifurcation, with no abnormality other than a slight increase in connective tissue, no greater than is found in other hearts that have never exhibited heart-block. Fibrous changes were found in the muscle bundle of Keith in the sino-auricular junction.

Griffith and Cohn's[5] Case. This is a case with lengthened *a-c* interval and with attacks of partial and complete heart-block, and showed the bundle of His continuous from the auriculo-ventricular note to the bifurcation, although its diameter was somewhat reduced and the fibers compressed by diffuse fibrosis. Beyond the bifurcation it was interrupted by granulation tissue.

Mallard, Dumas, and Rebattu's[6] Case. This was a case of partial block when tracings were made, but in which probably complete block had occurred at times, as spells of unconsciousness are mentioned. At autopsy there was an extensive fibrous mediastinitis, with involvement of the vagi. The region of the bundle of His was sectioned and but one in twenty of the sections stained. No lesion was demonstrated.

Holst and Monrad-Krohn.[7] The authors report a case of a male, aged fifty-four years, who for eight days had repeated attacks of syncope, with disappearance of the radial pulse. Between the attacks the pulse varied from 40 to 80, Tracings showed at times a partial block. At other times a slowing of both auricle and ventricle. At autopsy no lesion of the a.-v. bundle was found, except that it was slightly more fatty than usual. Both vagi, however, showed evidence of extensive degenerative neuritis. The authors believe the cardiac attacks to be of vagal origin.

AUTHOR'S CASE.

The following chronological list of events will serve graphically to place the patient's life on view:

1871, born in Italy. 1894, syphilis, in Philadelphia Hospital. 1900, married. 1902, wife aborted. 1903, child born, died from

[3] Zeit. f. klin. Med., 1909, lxvii, 495.
[4] Loc. cit.
[5] Quart. Jour. Med., 1909-10, iii, 126.
[6] Arch. des Maladies du cœur, Paris, 1911, iv, 298.
[7] Quat. Jour. Med., 1911, iv, 498.

burns. 1905, child born, living and well. 1906, child born, living and well. 1907, child born, living and well. 1907, patient had attacks of epigastric pain. 1908, child born, living and well. Patient fainted. 1909, severe pains and syncopal attacks. January 25, 1910, pains. February 5, admitted to University Hospital, with complete heart-block. February 8, 1.30 P.M., complete heart-block still persisting; patient given two hypodermics of atropine. 5.30 P.M., incomplete block 3-1 rhythm. February 9, incomplete block 2-1 rhythm. February 10, 2-1 rhythm, alternating with normal rhythm. February 24, left University Hospital, not having had while there a single syncopal attack. March to May, seen at office, either 2-1 rhythm or normal rhythm. In May started to work as cabinetmaker again. May to October, worked. October 22, slight syncopal attack. October 25, syncopal attack, and patient was taken to Jefferson Hospital. October 26, had six to eight syncopal attacks. October 27, had four or five attacks. Refused to stay longer in hospital, and went home. October 28, one attack; admitted again to Jefferson Hospital; discharged next day. October 30, had four syncopal attacks. December 5, the patient came to the University Hospital to see Dr. Pepper, who found him sitting in the waiting room. The man stood up, and told Dr. Pepper how glad he was to see him again. He suddenly grew pale, put his hand to his heart, fell over on a bench, and had a general convulsive attack, with unconsciousness. He had at first stertorous breathing followed by shallow breathing. In about a minute his radial pulse, which had been absent, returned and immediately the color came back to his face and he regained consciousness, and in a few minutes walked up to the ward, having been persuaded to stay for a while in the hospital. This was the first time we had seen him have a syncopal attack. His radial pulse on this second admission to Dr. Stengel's wards was 44, falling shortly to between 24 and 36.

December 6. Tracings showed complete block, or complete dissociation of auricular and ventricular action, and during the patient's stay in the hospital this persisted unfailingly. There were no syncopal attacks on this day. Atropine, which had during his first stay in the hospital apparently interrupted the complete block, changing it first to partial block and then to normal rhythm, had no beneficial effect.

December 7. The following notes were made by the resident physician, Dr. Sledge: Patient's pulse has continued to run at about the same rate. This morning he had an attack, and was watched by one of the nurses. His pulse stopped for a few seconds; respirations at first were slow and noisy; face remained flushed, and consciousness was not lost. Pulse returned rapid and fluttering, respirations came back to normal, and in two minutes pulse had slowed down to 20. Patient was much exhausted. At 11.30

today, while sitting down on patient's bed to take his blood pressure, he looked up at me and said, "I think I am going to have another spell." I reached for his wrist and felt only one beat of his pulse; he became very pallid, there was a general muscular spasm with twitching of his limbs, followed in less than a minute by opisthotonos. (His opisthotonos lasted about a minute, followed by relaxation and intermittent spasms of extremities; absolute relaxation occurred when respirations stopped). No heart sounds were audible from cessation of pulse to its return; after that they were distinct. Breathing became rapid at first and very noisy, beginning to become slower and less noisy at the expiration of one minute, stopping altogether at the end of four minutes from the beginning of his attack. After respiration stopped, the patient slowly became cyanosed (he had been pallid before), and the vessels of the neck were distended, and no venous pulsation could be detected; he lay in this condition for nearly three minutes, and was apparently dead. Artificial respiration was begun, with head hanging over side of bed, and with my finger over his radial artery; at the expiration of one minute, his radial pulse suddenly recurred, soft and running, and his face became very much flushed; artificial respirations were stopped and he continued to breathe, respirations being slow and shallow. In less than a minute he was perfectly conscious, and attempted to move in bed; pulse had slowed down to 20. On being asked how he felt, patient said that he was thoroughly exhausted. Flushing was quickly followed by pallor. I asked the patient why he told me that he thought that an attack was coming on, and he replied that everything had begun to appear black, and his vision was disappearing. One hour after the attack was over the patient expressed a wish to die, and said such an end could not be far off. The thought of these attacks is a great mental strain on him. Patient had another attack tonight at 9.45; pulse stopped for over three minutes; respirations did not cease, and recovery was very rapid.

December 8. Patient had another attack at 12.30 last night, with loss of consciousness, but no cessation of respiration; attention was called to him by his noisy breathing. Four minutes after the nurse reached his side the radial pulse returned. After the attack had subsided, the patient was very hysterical for half an hour, believing that he was near death. He was quieted by a small dose of morphine. Patient continued restless and nervous the rest of the night; this was especially marked at six, and again at eight o'clock this morning; at both of these times he thought an attack was coming on, but pulse did not stop, nor had he respiratory difficulty. At 12.30 today patient was given an injection of "606" by Dr. Longcope. Dr. Pepper made pulse tracings before and after the injection, but no change was noted. At 5.15 tonight patient had some respiratory difficulty for a few seconds; pulse did not stop, nor was consciousness lost.

December 9. Patient had a short attack this morning at two o'clock; another attack came on at 8.30, and lasted over four minutes from the time the nurse reached his side; artificial respiration was required. At 11.30 he had another attack that lasted six minutes; respirations and jugular pulse stopped; eyes showed markedly dilated pupils; after this he was wildly hysterical for a half hour. Another attack occurred at 4.30 and lasted about six minutes; artificial respiration was required for nearly a minute; violent mania was present after return of pulse for fifteen minutes. Patient was taken home in the ambulance at his own request.

He had on arriving at his home another severe attack, while the resident physician who accompanied him was present, and then for seven weeks was entirely free from them, although we had told him that he would undoubtedly have them and that he would die within twenty-four hours after leaving the hospital. He was visited at his home every two or three days for seven weeks, tracings being made on each occasion by Dr. Pepper. On December 13, 1910, he showed an incomplete block with a 3-1 rhythm, and on December 22, 1910, an incomplete block with a 2-1 rhythm; but the rhythm never became normal, and with the exception of these two occasions there was always complete auriculoventricular dissociation. Just before Christmas, 1910, his wife gave birth to a healthy boy; but this excitement in the family brought on no syncopal attacks in our patient. This child showed a negative Wassermann, as did its mother, although the patient on two occasions had given a positive reaction himself. He remained in the house sleeping on a lounge and walking around the first floor, but not venturing on the street, and on February 1, 1911, having been much wrought up over the withdrawal of his weekly income from an insurance company he had three syncopal attacks in one day and died during the last one.

A partial autopsy was performed by Dr. Austin about eight hours after death, and the heart removed. It was found to be greatly distended with dark fluid blood, a large quantity running out on cutting the vessels. The right side was particularly distended, the auricles, although enlarged, were not so extremely distended in proportion to the ventricles. The valves were perfectly healthy, and no macroscopic change in the heart, aside from the dilatation, was found. During life the right border was more displaced than the left, and there had been always noted a long systolic murmur at the apex. The patient's fingers had become quite clubbed, and the nails curved during the last year.

Autopsy Protocol. Subject is a fairly well-developed Italian, male, adult. Little panniculus adiposis is present; no edema; no scars. An incision was made along the middle of the sternum, the skin and muscle reflected, and the sternum removed in the usual

manner. The abdominal viscera were not fully exposed, owing to restrictions under which the autopsy was conducted.

The pleural cavities were free from fluid; the lungs appeared normal. The pericardium was much distended, and on being opened contained 30 c.c. of clear fluid and a greatly distended heart. The cardiac distention was most marked on the right side. On cutting through the vena cavæ the entire mediastinum filled quickly with fluid blood. The heart and four inches of the aorta were removed for study. Sections were taken for histological study from the liver and from the right kidney, which was lobulated and deeply congested.

The heart weighed 420 grams; epicardium was smooth and glistening; moderate amount of subepicardial fat. Blood in the cavities was almost entirely fluid. The myocardium was grossly normal. The left ventricular wall at the base was 14 mm. and the right ventricular wall 3 mm. thick. The endocardium was smooth, no lesion being visible in the region of the bundle of His or of the sino-auricular bundle. The valve orifices measured in circumference: tricuspid, 15 cm.; mitral, 10 cm.; pulmonic, 8 cm.; aortic, 6.5 cm. Valve leaflets were thin and pliable. The region of the sino-auricular and His bundles was preserved intact for serial sectioning. Sections were taken also from other portions of the myocardium. The aorta showed a few nodules apparently beneath an intact intima.

Histological Protocol. Autopsy No. 3559, '99, 12. Report by Dr. Austin. The following tissues were prepared and submitted for histological examination: Heart, aorta, liver, kidney.

Heart: Sections through myocardium and endocardium show ventricular muscle fibers well developed. Nuclei stain normally. Striations are distinct. Throughout the sections there is a moderate diffuse fibrosis, which around some of the larger vessels is quite marked. The arteries show thickening of their adventitia. The endocardium is normal. The vessels are moderately filled with blood.

Sino-auricular bundle of Keith-Flack: Several transverse sections were made through this bundle and stained with hematoxylin and eosin and with Mallory's connective-tissue stain. The muscle fibers of the bundle appear well developed. There is conconsiderable interstitial tissue in the bundle, not, however, an amount that can be considered excessive, the connective tissue forming a considerably less conspicuous feature of the cross-section than does the muscle tissue.

The bundle of His: The bundle was examined by serial longitudinal sections and stained with hematoxylin and eosin and with Mallory's connective-tissue stain. The course of the bundle was followed from Tawara's node to just beyond its bifurcation as an unbroken bundle, about 5 mm. in width. The course of the bundle

is somewhat S-shaped. The muscle fibers of the bundle are slender and very closely approximated, with little interstitial tissue. The nuclei stain well. The cytoplasm shows distinct striation.

Aorta: Section shows nodular fibrous thickening of the deeper portion of the intima and fibrosis of the inner layers of the media. Musculature of the remainder of the media is well preserved.

Liver: Section shows a normal capsule. The lobules are distinctly demarcated. Immediately around the bile ducts there is a moderate fibrosis, with round-cell infiltration. The bile ducts show normal mucosa. The parenchymatous cells are small and well defined. Their nuclei stain well. In a few of these cells one or two small fat vacuoles are seen. Throughout the lobules the cells show a moderate deposit of intracellular, pale yellow, amorphous pigment. The capillaries and the larger vessels contain little blood.

Kidney: Section shows a thin capsule. The glomerular tufts are large filling the capsules, and show moderate congestion; otherwise normal. Tubular epithelium shows well defined finely granular cells; nuclei are well stained. In a few of the convoluted tubules the epithelial cells appear moderately swollen. In some of the lumens is a little granular or homogeneous material. The tubules are in close apposition and there is but little interstitial tissue. Throughout cortex and medulla is marked congestion, without pigmentation. Treponema pallidum could not be demonstrated in the liver, kidney, or aorta by the Levaditi method.

Conclusions. The special points of interest in this case, in addition to those noted in the first report,[3] that we wish to bring out in this paper are:

1. That we have here a progressing typical case of heart-block lasting for three or four years, at first partial block alternating with normal rhythm, with occasional attacks of complete block, and later persistent complete block, and yet at the autopsy apparently no sufficient lesion was discovered in the bundle of His to explain the block.

2. The giving of a dose of "606" to the patient while he had complete heart-block and was having attacks daily of Stokes-Adams syndrome without any noticeable good or bad result.

3. We wish to call attention to the extreme length of several of the syncopal attacks. One attack timed by Dr. Sledge, the resident physician, lasted eight minutes, during the last four minutes of which the auricles apparently stopped beating in addition to the cessation of the ventricles. Apparently the man was dead, but artificial respiration brought him to life. Another attack witnessed by Drs. Edsall, Longcope, Sledge, and Pepper, and a number of medical students, was nearly as long, and very dramatic.

[3] Amer. Jour. Med. Sci., October, 1910.

This lasted six minutes, and for fully three minutes the auricles had apparently stopped beating, as no sounds could be heard over the chest, no pulsation noted in the neck, no respiratory movements occurred, and the patient's color, which at first had been white, became dusky. We all thought the man dead except Dr. Sledge, who, with some assurance on account of his previous success, began artificial respiration, and again restored the man to life and consciousness, which was promptly followed by a violent hysterical outburst, in which the man sang, cried, and shouted, and though none of us understood much Italian, we frequently heard the words "La morte" uttered with the most blood-curdling clearness. That these periods of apparent cessation of auricular action may have been due to auricular fibrillation cannot be denied.

4. In the first report of this case, already referred to, attention was particularly called to what was believed to be a genuine instance, though an isolated single one, of hemisystole. Apparently, as shown by the tracing on a single occasion, the left ventricle did contract normally after the auricular contraction; but the right ventricle did not contract, or possibly contracted very feebly, and the following statement was made in that article: "Probably it would be unwise, from this single observation, to say that in this particular case the division of the bundle of His going to the right ventricle was more involved or damaged than the division going to the left ventricle; but if further similar findings had been noted possibly such a localization could have been made."

The serial sections showed at the bifurcation of the bundle the branch passing to the right ventricle slightly encroached upon by an area of fat and fibrous tissue, while the branch to the left ventricle was normal.

It is, of course, difficult if not really impossible to properly interpret the concurrence of the single hemisystole and the slight lesion of the right branch of the bundle of His, if lesion it was. No other hemisystoles were noted, and the slight encroachment on the right branch of the bundle was not more than is sometimes found in apparently normal hearts.

5. It is interesting to note the occurrence of clubbed fingers in this case without any valvular disease of the heart or pulmonary disease.

6. That although during the man's first stay in the University Hospital he showed for several days constant irregular ventricular action during complete block without syncopal attacks, he never showed this irregularity later, even though he had syncopal attacks and complete block.

No. 3.

Extracted from the American Journal of the Medical Sciences, September, 1911

THE QUANTITATIVE DETERMINATION OF FUNCTIONAL RENAL SUFFICIENCY BY THE DUBOSCQ COLORIMETER; INDIGOCARMIN VERSUS PHENOLSULPHONEPHTHALEIN.[1]

A PRELIMINARY REPORT.

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(From the William Pepper Laboratory of Clinical Medicine.)

It is conceded that the most reliable individual kidney test is one dependent upon a quantitative metabolic study. By reason of the complicated conditions entailed, the requirement of particular physiologico-chemical knowledge, and the consumption of time demanded for the completion of the necessary observation, which even then may not be conclusive as von Noorden has demonstrated, such a procedure is destined never to become popular. Indeed, the mere fact that every four or five years witnesses the announcement of a new method for determining the kidney function suffices to prove (1) that the old tests are inadequate or unsatisfactory, and (2) that it still remains the keen concern not only of the internist, but particularly of the surgeon, to find some test, whereby the health or disease of the kidney may conveniently be determined.

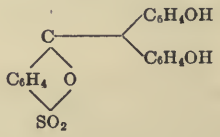
Various drugs and dyes have been utilized in countless attempts to solve this perplexing problem, in the subcutaneous employment of which attention has been directed to the time of onset of their elimination by the kidneys, to the constancy of reaction, and to the intensity, maximum, and duration of their excretion. Attempts to compute the quantitative elimination of these substances have never, until recently, been crowned with notable successes. Formerly, with methylene blue, in order to determine the amount of the dye excreted, the colored urine was measured and placed in a glass container. Precisely the same quantity of water was placed in another glass, and known quantities of methylene blue added

[1] Read before the College of Physicians of Philadelphia, February 1, 1911.

until the two solutions became homogenous in coloration. The percentage of the aniline dye eliminated in the urine was then easily and approximately computed.

During the past year, Rowntree and Geraghty have called attention to the great advantages of phenolsulphonephthalein, a substance first described by Ira Remsen, for a functional renal test, placing especial emphasis upon the quantitative estimation of the percentage of the drug eliminated by the kidney during the first hour or two following its injection subcutaneously.

The structural formula of this phthalein may be represented as follows:



This substance is a bright red, crystalline powder, slightly soluble in water, but more so in alcohol; insoluble in ether; in dilute alkaline solution it is a purer red than phenolphthalein, being purple in strongly alkaline solution.

This phthalein has certain properties not possessed by phenolphthalein which recommends it highly in work on the physiology of the kidneys. It has a stronger avidity as an acid and is much more completely eliminated by the kidney than phenolphthalein. It may be administered by mouth and subcutaneously without ill effect. Employed more accurately by the latter method in doses of 6 mg. to the cubic centimeter, it is absolutely non-irritating, devoid of toxicity, and appears in the urine in normal individuals in about ten minutes. It is also excreted in the bile, only to be reabsorbed, however, in the intestinal tract.

Phenolsulphonephthalein is unquestionably one of the very best substances at our command for purposes of functional renal diagnosis and prognosis. The test is very delicate, and, as is the case with phloridzin, it may prove to be oversensitive. Owing to the small quantity of the drug required for injection, which produces no pain or tenderness, it can be highly recommended to the internist for use in very sick and nervous patients, in whom it is desired to estimate the total renal function, but for unilateral diagnosis, the grave concern of the surgeon, it is extremely doubtful whether phenolsulphonephthalein can ever supplant indigocarmin.[2] The latter, although not eliminated so extensively by the kidney, has

[2] For description and technique for employment of this dye in functional kidney diagnosis, see author's articles, "Chromocystoscopy in Functional Renal Diagnosis, Based upon the Employment of Indigocarmin," Surg., Gyn., and Obstet., April, 1909, or Penna. Med. Jour., September, 1909; "Uber die Chromoureteroskopie in der funktionellen Nierendiagnostik," Zeit. f. Urologie, April, 1911; or "The Value of Chromoureteroscopy in Functional Kidney Diagnosis," Surg., Gyn., and Obstet., May, 1911.

not yet been shown to be inferior to phenolsulphonephthalein as a quantitative functional test. It seems unfortunate that two such meritorious tests must be brought into comparison. It would be better, perhaps, if we were to differentiate the subjects suitable for the application of either, because in unilateral diagnosis indigo will suffice to meet the demand in cases impossible of ureteral catheterization, where the phthalein test must necessarily fail.

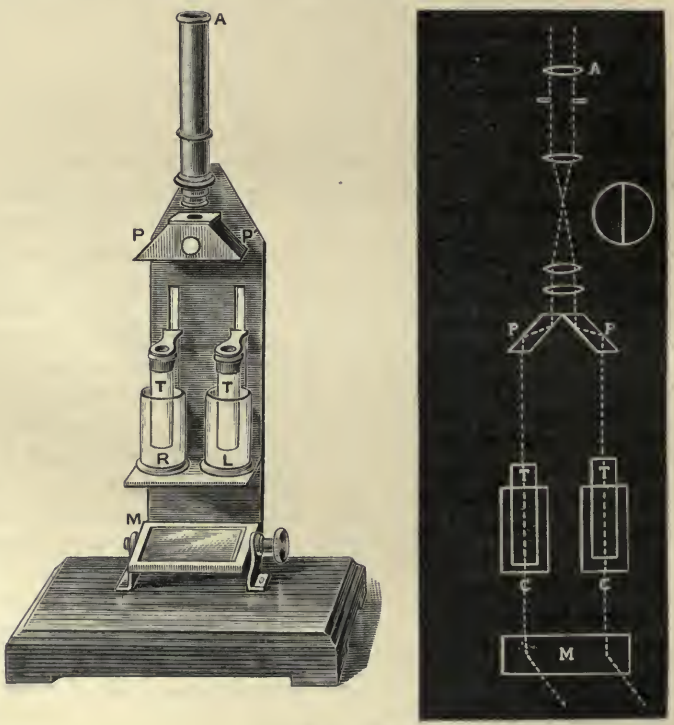
The technique of the phenolsulphonephthalein test is as follows: Fifteen minutes to half an hour before administering the test the patient is requested to drink two glasses of water to insure free renal activity. One c.c. containing 6 mg. of the phthalein is injected subcutaneously.[3] If it is desired simply to learn the total kidney sufficiency, as may be the case in the various forms of nephritis, or the extent of renal drainage due to vis a tergo pressure, atrophy of the parenchyma of the organs, etc., because of obstruction in the lower urinary tract, as by prostatic enlargement, etc., a catheter is introduced into the bladder and the onset of the excretion of the phthalein noted by the beautiful amethyst-red produced when it comes in contact with the drop or two of 25 per cent. sodium hydroxide placed in the bottom of the receptacle used for collection. The quantities of urine for the first and second, or possibly third, hours are then collected. Instead of the procedure by catheterization, at first continuously and then hourly, the patient may be requested to void voluntarily every five minutes until the onset is observed, and then at hourly intervals. Such a procedure is simplicity itself, but not more so than the indigocarmin technique. On the contrary, if it be desired to determine the functional activity of the individual kidneys separately, an act is demanded which may be, and not infrequently is, impossible, namely, catheterization of one or both ureters, in order to convey the phthaleinized acid urine externally to an alkaline medium, so as to study the color reaction. With indigocarmin, catheterization of the ureters is rarely necessary, since the onset of the indigo elimination can be readily seen through the cystoscope as the blue jets of urine are ejected from the ureteral orifices into the bladder. Indeed, from an analysis of over one hundred cases subjected to the indigo test, the time of onset runs a close parallel to the quantity eliminated, and judging alone from the time and character of the elimination, in no instance has a false prognosis relative to renal sufficiency or insufficiency been made.

It has been alleged against indigocarmin that it does not lend itself well to colorimetric determinations. It is claimed that the urinary constituents cause a decomposition of the dye. With this statement I cannot agree, and believe that indigo is as efficient as the phthalein for colorimetric readings.

[3] This substance is prepared by Hynson, Westcott & Co., Baltimore, Md.

It is indisputable that for certain kidney conditions quantitative determinations are most valuable, whether the substance employed be indigocarmin, phenolsulphonephthalein or what not. During the past year or two Oppenheimer and Rowntree and Geraghty have employed the Duboscq colorimeter with gratifying success for the estimation of renal activity, and the last two in particular are very high in their praise of the instrument.

As shown in the figures, the Duboscq colorimeter consists of two glass cylinders, cut with parallel plane surfaces placed on arms capable of vertical movement by the manipulation of set screws on the



Duboscq colorimeter.

posterior surface of the instrument. These crystals may be raised and lowered in glass reservoirs containing solutions, the comparative colors of which are to be determined. In one reservoir, *R*, is placed the standardized solution; in the other, *L*, the solution to be tested. The mirror *M* is adjusted so as to reflect light through the perforated stage, *S*, into the reservoirs supported by the same, thence to the glass parallelopipeds *P* and *P'*, and so through the telescope to the ocular *A*. By manipulation of the set screws the crystals are elevated or lowered until a homogeneous coloration appears at the

ocular. Scales, with Vernier attachments, invisible in the figures, furnish the readings from which the percentage of color in the solution to be tested can be computed.

In the performance of the colorimetric test, $\frac{1}{2}$ c.c., containing 3 mg. of phenolsulphonephthalein[4] is placed in a flask and diluted up to 1000 c.c., adding one drop of 25 per cent. sodium hydroxide to elicit the amethystine coloration. This can be preserved indefinitely for future determinations. The reservoir R is then about one-half filled with this control solution and the plunger lowered until the index on the scale reads 20. This reading is arbitrary, as any other may do. Within a few minutes following the subcutaneous injection of the patient with 1 c.c. or 6 mg. of the phthalein, it begins to be eliminated in the urine, occurring normally in the acid state as an orange yellow. This may be collected by voluntary urinations, by urethral catheterization, or by ureteral catheters if the work of the kidneys individually is desired. The onset of elimination is determined by collecting the urine in the presence of a drop or two of the sodium hydrate solution, noting the occurrence of the pinkish color. Quantitatively, the amounts of the first and second hours are collected separately. Each amount is rendered definitely alkaline by the addition of sodium hydrate and is diluted up to 1000 c.c. A quantity of this is filtered and placed in the reservoir L. The left hand set-screw is then manipulated until a similar coloration is observed on both sides, and the reading taken. If, for example, the reading on the left is found to be 40, and, as previously stated, the control reads 20, it is obvious that the solution to be tested is only half the concentration of the control, which may be graphically represented as follows: $\frac{20}{40} \times 100 = 50$ per cent. Inasmuch as the control is made up of but one-half in the case of phenolsulphonephthalein, and only one-quarter in the case of indigocarmin, of the amount injected into the patient, it is necessary in the former instance to divide the result by two and in the latter by four in order to determine the actual percentage eliminated of the amount injected into the patient.

The Duboscq colorimeter is an ideal instrument for quantitative colorimetric determinations, not only by virtue of the simplicity of its construction, but also because of the accuracy of results obtained by its use. A review of Table I will demonstrate the close parallelism existing between the findings with this instrument as compared with known solutions of indigo and phthalein.

[4] If indigocarmin is used, 5 c.c. of a 0.4 per cent. solution is placed in the flask for the preparation of the control solution. It will be noted that only a fractional part, in the case of the phthalein one-half and with indigo one-fourth, of the amount administered to the patient is employed in the control. This is done because the solutions in these dilutions lend themselves more favorably to colorimetric readings.

TABLE I.—Solutions in distilled water.

No.	Known quantity of indigocarmin.	Amount estimated.	Known quantity of phenolsulphonephthalein.	Amount estimated.
1	0.0014 gram	0.0013 gram	0.0027 gram	0.0028 gram
2	0.0039 "	0.0039 "	0.0023 "	0.0024 "
3	0.0029 "	0.0028 "	0.0037 "	0.0037 "
4	0.0056 "	0.0057 "	0.0042 "	0.0043 "
5	0.0053 "	0.0052 "	0.0055 "	0.0055 "
6	0.0071 "	0.0071 "	0.0074 "	0.0074 "
7	0.0065 "	0.0065 "	0.0089 "	0.0088 "
8	0.0087 "	0.0086 "	0.0087 "	0.0087 "
9	0.0078 "	0.0078 "	0.0095 "	0.0095 "
10	0.0093 "	0.0094 "	0.0098 "	0.0098 "

That quantitative colorimetric determinations of indigocarmin and phenolsulphonephthalein in functional kidney diagnosis are of great value is undisputed. Whether or not the quantitative estimation will in time supersede in value the onset of elimination is a mooted question and to my mind a very doubtful one, save for the determination of the total renal function particularly for the purposes of the internist. There can be little doubt that in certain conditions, as nephritis, and especially in prostatic enlargement, where, as a rule, the kidneys are more or less damaged by a vis a tergo pressure from retained urine, the colorimetric determinations are of exceptional value, and I confidently believe that every surgeon contemplating prostatectomy will not only decrease his mortality, but will also avoid debasing the profession of medicine, by refusing to operate in such cases when the total output for the first hour of indigo and phthalein falls below 10 and 20 per cent. respectively. I have long since been convinced that an onset of the elimination of indigo after twenty minutes bespeaks renal insufficiency and contraindicates operative interference. Again, it must be borne in mind that even though the quantitative elimination is found to be below the figures above stated, operation may be considered, provided the elimination during repeated determinations remains constant, evidencing no tendency to fall, thereby establishing a stable kidney activity. On the contrary, an operation undertaken in the presence of a steadily decreasing functional capacity, as measured most accurately and satisfactorlly by colorimetric determinations, is little short of criminal, as the patient will invariably die.

An important field which should be studied, relative to the quantitative elimination of indigo or phthalein, is that of all operative cases. It is quite possible that by an analysis of several hundred cases thus tested by these substances before operation, data might be derived that would in the future lower the general surgical mortality.

The problem that I hope to solve eventually—as I do not think the results of the cases analyzed to date sufficiently conclusive—is the determination of the relative merits of indigocarmin and phenolsulphonephthalein. In order to accomplish this I have under-

taken comparative studies of the action of both substances on a series of dogs and patients. It was at first attempted to study the onset of elimination of both substances from the ureteral orifices into the bladder of large female dogs by cystoscopy. The procedure was difficult, owing to the fact that the etherized dog seemed able to retain very little fluid in the bladder in the presence of the cystoscope. It was also very evident that the anesthetic (ether) markedly diminished the excretion of urine and, in one case at least, produced anuria. In the dogs subjected to indigocarmin, the fluid used for distending the bladder was water; in those injected with phenolsulphonephthalein, the fluid employed was 25 per cent. sodium hydrate. In no case was indigo or phthalein eliminated from the ureteral orifices for forty-five minutes. At the termination of that period the dogs were allowed to revive from the anesthetic. The two dogs injected with indigo immediately voided a faintly blue-colored urine; one dog having received phthalein voided at the end of an hour, the urine demonstrating the presence of that drug, the fourth dog refused to urinate. The results are tabulated below:

TABLE II.—Dogs Cystoscoped.

No.	Substances used in tests. Indigo.	Phthalein.	Appearance of drug by cystoscopy, under ether.	Onset of secretion subsequently.
1	20 c.c. of 0.4 %		0	45 minutes.
2	20 c.c. of 0.4%		0	45 minutes.
3		1 c.c. =6 mg.	0	60 minutes.
4		1 c.c. =6 mg.	0	

Owing to the poor success of the first attempt, it was decided to perform ureterotomies under ether in the second series, catheterizing the ureters and collecting the respective urines, both for onset of elimination and quantitative study. The results are noted in Table III.

TABLE III.—Dogs Operated Upon.

No.	Substances used in tests.		Onset in minutes.		Quantitive determinations.						Total.
					First hour.		Second hour.		Third hour.		
	Indigo.	Phthalein.	Right ureter.	Left ureter.	Right ureter.	Left ureter.	Right ureter.	Left ureter.	Right ureter.	Left ureter.	
1	40 c.c. of 0.4%		5	5	1.63%	1.03%	1.37%	0.51%	1.48%	0.64%	6.66%
2	40 c.c. of 0.4%		45	45	.....	.....	trace	0.29%	.....	.....	0.29%
3	.....	2 c.c. =12 mg.	0	0	0	0	0	0	.....	.....	Nil
4	.....	2 c.c. =12 mg.	15	15	1.35%	2.41%	trace	1.32%	0	0.35%	5.43%

Judging from the few dogs thus far utilized, there would seem to be slight preference in favor of indigocarmin. By multiplying the number of experiments with dogs, I hope eventually to throw some light on this important problem, although it is not improbable that the human subject will suffice to settle the dispute.

Table IV.—Quantitative Colorimetric Tests on Human Beings.

Normal Cases.

Name.	Diagnosis.	Indigocarmin. Amount injected.	Onset. R | L	Quantity eliminated. 1 hr.	2 hrs.	3 hrs.	4 hrs.	Total.	Phenolsulphonephthalein. Amount injected.	Onset. R | L	Quantity eliminated. 1 hr.	2 hrs.	3 hrs.	4 hrs.	Total.	Urinalysis and Remarks.
L. S.	Normal (ambulatory)	8 mg.	Before 10 min.	11.84%	4.86%	1.75%	0.91%	19.36%	6 mg.	Before 10 min.	37.59%	23.69%	9.62%	..	70.9 %	Negative.
I. G. C.	Normal (ambulatory)	8 mg.	Before 9 min.	10.86%	8.44%	2.06%	1.01%	22.37%	6 mg.	Before 9 min.	58.47%	6.28%	3.62%	1.4%	69.77%	Negative.
S.	Normal (ambulatory)	....	....	...	..	..	..	...	6 mg.	Before 15 min.	33.44%	18.66%	5.62%	4.32%	62.04%	Negative.
B. A. T.	Normal (ambulatory)	8 mg.	5 min.	7.53%	?	2.56%	1.44%	?	6 mg.	Before 9 min.	30.12%	18.38%	13.58%	3.76%	65.84%	Negative.

Abnormal Cases.

R. C.	Nephrolithiasis (Rt.) (bedridden)	8 mg.	10 min.	7 min.	3.35%	5.47%	1.35%	0.51%	10.68%	6 mg.	18 min.	11.00%	6.95%	3.22%	1.89%	23.06%	*Urine:* negative save for few erythrocytes. Calculus 1 cm. in diameter removed.
W. E.	Myocarditis (bedridden)	8 mg.	9½ min.	9½ min.	10.82%	2.07%	..	..	12.89%	6 mg.	?	5.91%	21.36%	...	..	27.27%	*Urine:* Acid, 1012, faint trace of albumin, hyaline and light granular casts, cylindroids, mucus. *Blood pressure* {Sys. =185 Dias. =125
M. K.	Nephritis, interstitial, chronic (bedridden)	8 mg.	13 min. feeble	0 in 30 min.	0.90%	0.86%	..	..	1.76%	...	...	...	...	...	..	...	*Urine:* Acid, 1009, cloud of albumin, hyaline and light granular casts, cylindroids, leukocytes. *Blood pressure* {Sys. =220 Dias. =125
J. S.	Nephritis; tuberculosis, pulmon. (bedridden)	8 mg.	9 min.	9 min.	4.03%	6.49%	..	..	10.52%	6 mg.	No e	liminati	on for a	period	of two	hours.	*Urine:* Acid, 1019, cloud of albumin, many hyaline and light granular casts, few erythrocytes, mucus. *Blood pressure* {Sys. = 125 Dias. = 80
C. O.	Prostatic hypertrophy; cardio-renal disease (bedridden)	8 mg.	15 min.	15 min.	6.25%	1.56%	..	..	7.81%	6 mg.	20 min.	7.22%	9.61%	...	..	16.83%	*Urine:* Acid, 1015, faint trace of albumin, few hyaline and granular casts, few red blood cells and white blood cells. *Blood pressure* {Sys. =165 Dias.=105
H. R.	Nephritis, hemorrhagic? (bedridden)	8 mg.	11 min.	12½ min.	?	?	..	..	2.04%	...	...	....	...	...	....	....	*Ureteral Catheterized Specimens:* Acid, 1015, heavy cloud of albumin, leukocytes and pus cells, erythrocytes, bacteria, no casts.

N. A.	Diabetes mellitus (bedridden)	....	..	..	...	..	..	..	...	6 mg.	15 min.	2.61%	23.07%	trace	..	25.68%	Sugar=6.25%
J. McD.	Endocarditis (bedridden)	....	..	..	...	..	..	..	...	6 mg.	Earlier than40, not at 20 min.	? 3.1 %	? 9.04%	? 10.12%	.. ..	13.87% 22.26%	*Urine:* Acid, 1028, large amount of albumin, pus, hyaline and granular casts, cylindroids. Lost cardiac compensation.
B. O.	Nephritis, parenchymatous, chronic (bedridden)	....	..	..	...	..	..	..	...	6 mg.	12 min.	10.80%	9.35%	2.8 %	..	22.95%	*Urine:* Acid, 1021, albumin =9 pro mille (Esbach), many hyaline, light and dark granular casts, colloid casts, many white blood cells. *Blood pressure* {Sys. =140 Dias. =120
D. W.	Nephritis, interstitial, chronic (bedridden)	....	..	..	...	..	..	..	...	6 mg.	....	1.61% 2.77%	9.34% 11.95%	5.26% 18.56%	.. ..	16.21% 33.28%	*Urine:* Alkaline, 1018, trace of albumin, few hyaline and granular casts, few erythrocytes. *Blood pressure* {Sys. =235 Dias. =150
R. C.	Fracture, compound; amputation of leg; (bedridden)	....	..	..	...	..	..	..	...	6 mg.	?	3.11%	1.28%	0	..	4.39%	*Urine:* Cloud of albumin, few cylindroids, leukocytes in excess.
J. J.	Lymphadenectomy (bedridden)	....	..	..	...	..	..	..	...	6 mg.	11 min.	22.83%	5.18%	trace	..	28.01%	*Urine:* Acid, 1023, faint trace of albumin, hyaline and light granular casts, many cylindroids, mucus, excess of leukocytes.
H. S.	Tuberculosis, hip (bedridden)	....	..	..	...	..	..	..	...	6 mg.	59 min.	21.36%	?	1.06%	..	22.42%	*Urine:* Alkaline, 1008, faint trace of albumin, few polynuclear granular casts, few cylindroids, leukocytes, erythrocytes.
G. P.	Varicocele, post-operative (bedridden)	....	..	..	...	..	..	..	...	6 mg.	30 min.	14.04%	10.57%	1.01%	..	25.62%	*Urine:* Alkaline, 1022, faint trace of albumin, normal leukocytes, epithelium.
L. B.	Tuberculosis, os calcis. (bedridden)	....	..	..	...	..	..	..	...	6 mg.	20 min.	30.03%	1.59%	trace	..	31.62%	*Urine:* Acid, 1021, few hyaline casts, cylindroids, leukocytes in excess, mucus.
F. W.	Wound, gunshot, spine; monoplegia (bedridden)	....	..	..	...	..	..	..	...	6 mg.	12 min.	12.72%	1.12%	...	..	13.84%	*Urine:* Alkaline, 1026, faint trace of albumin, mucus.
H. J.	Tuberculosis, spine; psoas abscesses; amyloid kidneys? (bedridden)	....	..	..	...	..	..	..	...	6 mg.	29 min.	trace	0	...	..	trace	*Urine:* Acid, 1017, trace of albumin, hyaline, dark and light granular casts, normal leukocytes, mucus.

In Table IV are tabulated a number of cases, normal and diseased, that have been subjected to the indigocarmin and phenolsulphonephthalein tests. In a few instances, comparative applications of both methods for as many as four hours have been made.

It will be observed from a review of the tabulated cases, although too few in number for absolute conclusion, that the onset of elimination of both indigo and phthalein runs a close parallelism with the quantitative output; that as substances for quantitative determination in the estimation of renal sufficiency, they are essentially equal in value; that both substances are largely eliminated during the first two hours after injection; that the percentage output of indigo is approximately about one-half that of phthalein, which fact, however, is of no moment in drawing conclusions; that in normal ambulatory cases the onset occurs in about ten minutes, while the total output exceeds 60 per cent. of the amount injected; that in contradistinction to the ambulatory cases, the bedridden patients, even though possessing supposedly normal kidneys, excrete less than one-half of the amount eliminated by the cases at liberty to move about, and, finally, that the output of indigo occurs slightly earlier and continues no longer than the phthalein.

Conclusions. 1. Quantitative colorimetric determinations of indigocarmin and phenolsulphonephthalein are of very great value in the estimation of the total renal function, particularly in such conditions as nephritis and damaged kidneys, incident to prostatic enlargement, etc., causing poor drainage and resulting in vis a tergo pressure. These substances routinely employed by the surgeon as indicators for or against surgical intervention, particularly in contemplated prostatectomies, but likewise in other fields of surgery, will aid materially in the reduction of operative mortality.

2. Although each substance has its particular advantages and indications as a test, indigocarmin, at least for the purposes of the surgeon, especially in the diagnosis and prognosis of unilateral renal disease, seems just as useful and possibly more practical than the new drug phenolsulphonephthalein.

3. Phenolsulphonephthalein in many respects is an ideal substance for employment in studying the pathology and physiology of the kidney. It may possibly be more sensitive than indigocarmin, in fact, may prove to be too delicate. On the other hand, the technique of the test is extremely simple and may be employed painlessly. Preference should be extended to this drug over indigocarmin whenever it is desirable to learn the total or combined efficiency of both kidneys.

In conclusion, I desire to express my grateful appreciation to Drs. Edsall, Frazier, Riesman, and T. T. Thomas for permitting me to use material that made it possible to conduct, in part, these studies.

No. 4

The Modern Diagnosis of Tuberculosis of the Kidney.*

BY B. A. THOMAS, A.M., M.D.,
Philadelphia.

The motives prompting the presentation of an article on this literature-burdened and timeworn subject are trifold; first, to direct the attention of the average general practitioner of medicine to the prevalence of the condition and moreover to emphasize the caution that renal tuberculosis commonly masquerades under remote, unrecognized or misinterpreted urinary symptoms; second, to demonstrate the urgency and, indeed, the necessity of early diagnosis, if it is desired to reap the high percentage of cures possible by nephrectomy; third, to review the more important modern procedures of diagnosis and particularly to discuss the present status of the advisability of ureteral catheterization of the presumably normal side in the presence of tuberculous infection of the bladder.

PATHOLOGY.

Inasmuch as the symptomatology of renal tuberculosis is directly dependent upon the morbid process, a brief résumé of the anatomico-pathological lesions will be in order, as a fundamental knowledge of these is necessary for a thorough conception and explanation of the various symptoms of the disease, subjectively and objectively.

It is to-day universally conceded that tuberculosis of the kidney almost invariably arises as an infection from the blood (hematogenous); exceptionally and rarely the infection may ascend the ureter from the bladder (urogenous) or reach the kidney by contiguity or through the lymphatic system (lymphogenous). Clinically, urogenital tuberculosis is almost invariably primary in the kidney in both sexes, the exceptions being fewer in the female than the male. In eighty per cent. of cases only one kidney is primarily affected, explainable on the basis of a *locus minoris resistentiae* due to traumatism. Shortly before death in untreated or improperly treated cases the sister organ becomes involved, usually through additional hematogenous implantation or by transvesical urogenous ascent of the other ureter. In our series, males have been the victims twice as often as females. Usually patients are afflicted in the second and third decades of life, although eight per cent. occurred prior to the twentieth year and thirty-three per cent. after the age of forty. In the cases of unilateral disease, the right kidney was involved over three times as frequently as the left. A noteworthy fact is that in seven of the twenty-four cases analyzed the disease had become bilateral at the time surgical treatment was sought. Moreover, it is estimated that about one in every three operations on the kidney is for tuberculosis, nor do these figures include those deplorable cases of inoperable bilateral involvement.

The anatomico-pathological processes may assume the following forms:—

A. *Acute Miliary Tuberculosis.* In this form both kidneys are involved and the affection is simply the counterpart of an acute general dissemination of the disease. It is not amenable to surgical intervention.

B. *Tuberculous Nephritis.* This may be either (1) toxic or (2) bacillary. The former is that type of nephritis occurring in tuberculous subjects and encountered also in the "good" kidney of individuals suf-

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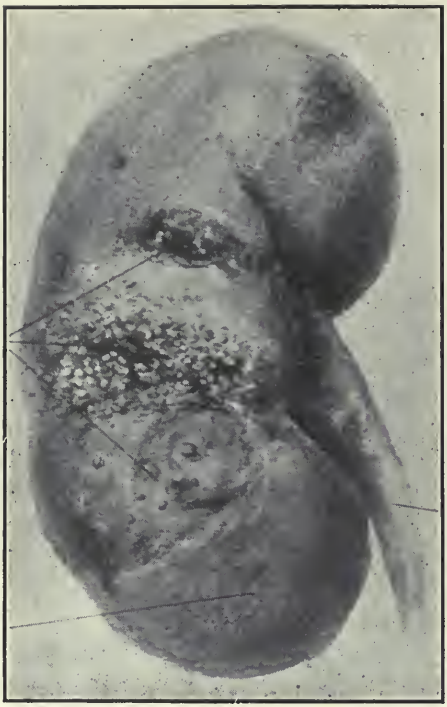

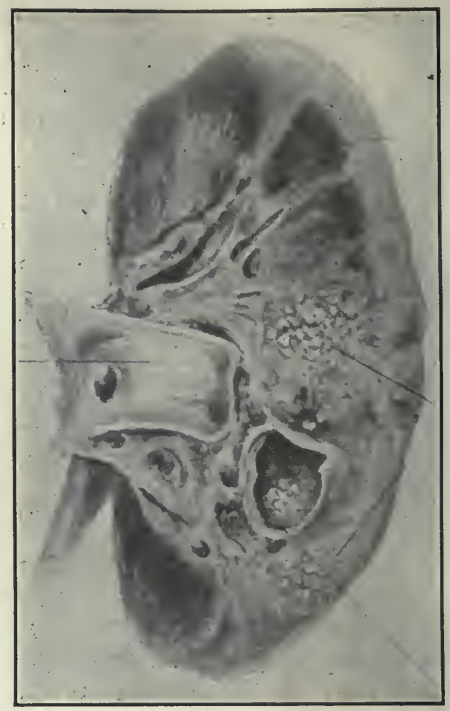

Figure 1. Tuberculosis of kidney. Early stage. Author's Case.

fering from a well-defined tuberculosis of the other side. Under such circumstances nephrectomy often results in the restoration of health to the toxic kidney. In the bacillary type of tuberculous nephritis, tubercle bacilli are eliminated from the kidney in the urine throughout a chronic course, the renal lesions appearing inconspicuous, although a glomerular and tubular nephritis is usually demonstrable.

C. *Chronic Miliary Infiltrating Tuberculosis.* The process is unilateral and the tubercles are larger than in the acute miliary form. Although the tubercles may be disseminated throughout the parenchyma, there is little tendency to caseation, but on the contrary a formation and proliferation of other new connective tissue resulting in fibrous degeneration.

D. *Tuberculous Ulceration of the Papillae.* This type is relatively rare, but explains those cases characterized by initial marked and continuous hematuria. The renal papillæ show small ulcerations with submucous tubercles. Such cases have been erroneously diagnosed and treated as essential hematuria.

E. *Caseous-cavernous Renal Tuberculosis.* In this, the commonest form of the disease, there occurs a deposition of tubercles in the vascular zone between the cortex and medulla of the organ. These foci undergo caseation, become confluent, form cavities and infiltrate in the direction of the pelvis or capsule or both (Figure 1). The latter early shows isolated or conglomerate projecting caseating tubercles; later the capsule becomes thickened and the kidney enlarged, its exterior appearing nodular and irregular. The morbid process may be limited to the parenchyma, occasionally one or more caseous areas with or without the deposition of lime salts, being demonstrable in the poles of the organ, usually the upper (Figure 2), or it may communicate with the pelvis, implanting tubercles

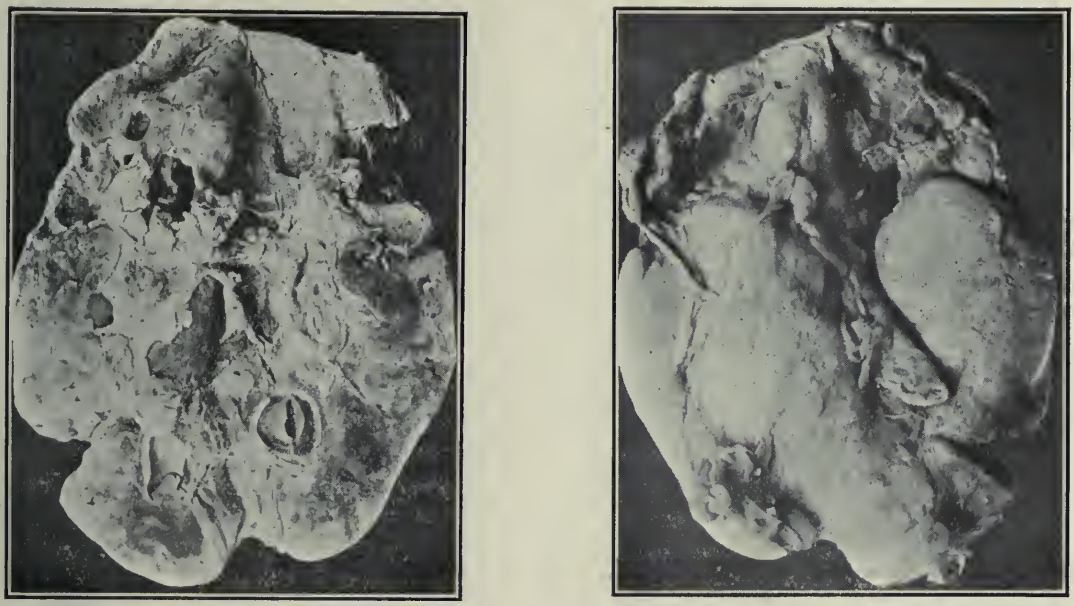

Figure 2. Tuberculosis of kidney. Polar localization. Note that upper pole is simply a sac, before section filled with pus; remainder of organ not involved. Case of Dr. Charles H. Frazier.

and resultant ulceration in that structure. Sooner or later the ureter and bladder become involved. The former at times becomes greatly thickened and capable of abdominal, vaginal or even rectal palpation. Not infrequently its lumen undergoes stricture formation and may be completely occluded. The vesical pathology will be described under cystoscopy.

F. *Tuberculous Pyonephrosis.* This is simply a terminal stage of the previously described pathological process, in which the kidney becomes transformed into an irregular sac of pus or multilocular intercommunicating caverns distended with purulent material, the result of caseation and liquefactive necrosis of the medullary and papillary portions of the parenchyma of the organ (Figure 3). Frequently the purulent content undergoes additional infection by other pyogenic bacteria. Perinephritis, in one form or another, ensues or the ureter becomes plugged or completely stenosed resulting in that condition known as "closed pyonephrosis," associated with that apparently paradoxical state of a normal urine and bladder.

SYMPTOMATOLOGY.

Restriction of time and space prohibits a thorough review of the various subjective, objective, general and local symptoms leading to a diagnosis of the disease. Owing to the fact that renal tuberculosis is prone to masquerade under remote, vague or misleading symptoms, thereby misguiding the practitioner, to institute treatment for "lumbago," "dyspepsia," "indigestion," "cystitis" or a "nervous bladder," the importance of a few clinical symptoms must be emphasized. First, the most prominent, commonest and in ninety per cent. of cases the earliest symptom of tuberculosis of the kidney is irritability of the bladder. Thus in the male, if tuberculous extension from the epididymis, prostate and seminal vesicles can be excluded, and bladder tuberculosis is established, because of the extreme improbability of the latter being primary, it invariably betokens a descending renal infection; in the female, vesical tubercu-

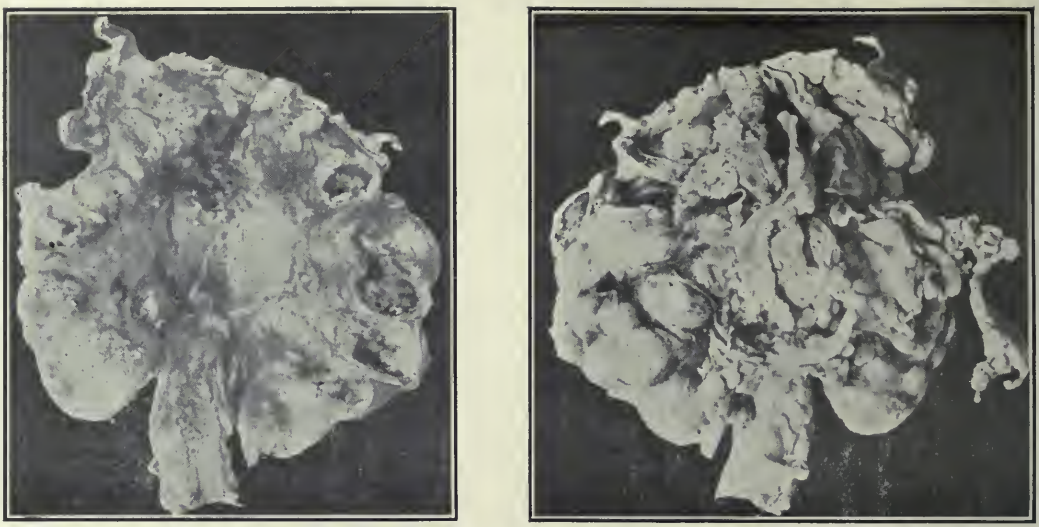

Figure 3. Tuberculosis of kidney. Advanced stage. Parenchyma in state of caseous-cavernous necrosis. Exterior of organ nodular and adherent to surrounding structures. Ureter involved and thickened. Author's case.

losis means almost invariably renal tuberculosis. Therefore, any patient suffering from frequency of urination, dysuria and pyuria or hematuria, particularly by day, over a period of several weeks, for no obvious reason, should be regarded as a tuberculous kidney suspect and submitted to a routine urological examination. This is the golden opportunity in the first six or twelve months of the disease, when, unilateral, nephrectomy promises eighty per cent. of recoveries as contrasted with eighty per cent. of mortality from other methods of treatment. Second, equally misleading are those cases presenting vague or indefinite symptoms, as a slight dull or dragging inconstant lumbar pain, gastrointestinal derangement, loss of weight and strength, a clear urine and a normal bladder. Third, it is undoubtedly true that, at one time or another in the course of renal tuberculosis, tubercle bacilli, blood and pus cells can be demonstrated bacteriologically and microscopically. However, when the patient reaches the surgeon the bacillus tuberculosis is demonstrable in only about eighty per cent. of cases. This occurrence is readily explained by the fact that at this time the ureter is frequently closed and the diseased kidney in a state of so-called "autonephrectomy." The diagnosis may also be difficult in the earliest stage of the infection, when the bacillus may not be demonstrable. Here the dictum of Rovsing, that pyuria in the absence of the common pathogenic bacteria means tuberculosis, is indisputable. It matters not whether polyuria or albuminuria are associated urinary findings.

Pain either renal, ureteral or vesical, tenderness in the costolumbar angle, hematuria, pyuria and palpability of the kidney and ureter are commonly present and not infrequently appear as initial symptoms. The physician who treats his case as one of "weak" or "nervous" bladder and waits for the development of fever, rigors, sweats, loss of strength and weight and a palpable abdominal tumor in order to substantiate his diagnosis is criminally negligible or ignorant.

DIAGNOSIS.

The diagnosis of tuberculosis of the kidney depends not only upon the clinical

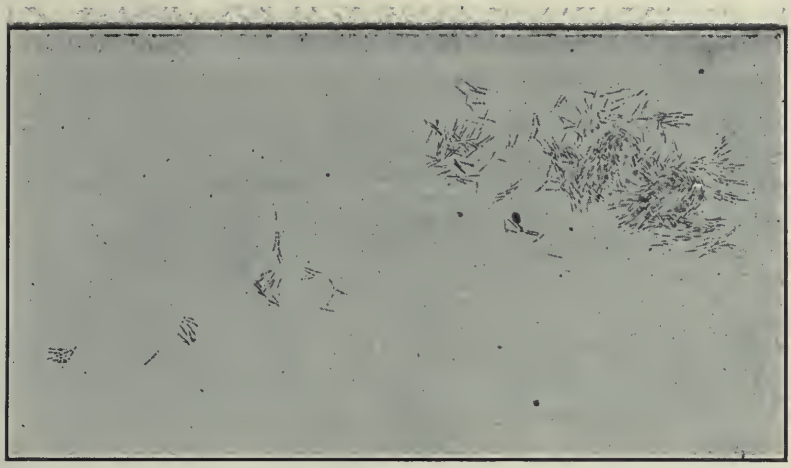

Figure 4. Tubercle bacilli in urine. Stained with carbolfuchsin and Gabbett's solution.

symptomatology, valuable as those observations may be, but particularly on the bacteriological laboratory methods in conjunction with certain modern diagnostic instruments and procedures.

Unquestionably the absolute diagnosis of urogenital tuberculosis rests upon the demonstration of the tubercle bacillus. The localization, degree of involvement and original source of the infection is determined by radiography, pyelography, cystoscopy, functional kidney tests, exploratory lumbar incisions and ureteral catheterization.

Bacteriological Examination. Although an acid urine containing a dirty, grayish sediment of pus in the bottom of a glass is suggestive of renal tuberculosis, it is of course unreliable. The following methods are practiced for demonstrating the tubercle bacillus in the urine: First, the stained *smear.* Gabbet's method is probably most extensively employed and is sufficiently reliable, so far as stained smears go (Figure 4), although the Pappenheim method is recommended because of its alleged value in differentiating the smegma bacillus. If the clinician is obliged, an imaginary state, to depend upon the stained smear, repeated examinations, if necessary, of twenty-four hour specimens of urine should be done, utilizing the sediment from the bottom of the receptacle. Treatment of the urine with antiformin and chloroform will increase the number of positive findings. A staining method which has been often used and can be highly recommended is that of Wehrli and Knoll.[1] It is possible by this method to find the bacillus in fifty to eighty per cent. of positive cases, depending upon the persistence of the microscopist. Aside from the comparative unreliability of this method, much valuable time for the patient may be sacrificed by the often necessary resort to repeated searches. Consequently, guinea-pigs should be inoculated at the time of the first examination, if smears result negatively. Second, guinea-pig inoculations. Various methods have been utilized, namely the intravenous, the intracardiac, the subcutaneous, the intrahepatic, the intraperitoneal and the Bloch. The objection to most of these is (1) that it takes too

[1] A solution of Much's methylviolet B. N. saturated alcoholic (absolute alcohol) solution 10 c.c., 2 per cent. carbolic water 100 c.c. is mixed with a second solution of fuchsin 1 gm., absolute alcohol 10 c.c. and distilled water 100 c.c. immediately before use and filtered. The smear is then covered with the filtrate and heated for four minutes over the flame until bubbles appear in the solution, then iodized for five minutes with Much's potassium iodid-peroxid of hydrogen solution; then differentiated in 2 per cent. hydrochloric acid alcohol until the first bluish clouds change to the red fuchsin. Wash in absolute alcohol and mount. The Ziehl-Neelson bacilli appear stained red; the Much bacilli as dark blue granular lines; the Much granules as dark blue.

Figure 5. Tuberculosis of liver. Intraperitoneal method.

Figure 7. Hagemann's allergic cutaneous reaction. Note the reddish blue center, surrounded by a porcelain white ring, which is circumscribed by an inflammatory areola.

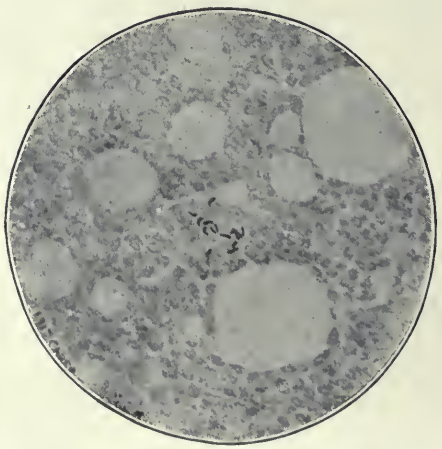

Figure 6. Tubercle bacilli in lymph node. Bloch's method.

long, three to six weeks, to substantiate the diagnosis (Figure 5), (2) that unless the material to be injected is heated, many animals die prematurely of septicemia and (3) that the customarily employed degree of heat will often kill attenuated or relatively nonvirulent bacilli, thereby permitting of erroneous results. The Bloch method is vastly superior to any of the others and should be the one of choice in that it is unnecessary to heat the material and particularly because the required time is reduced from approximately four weeks to seven to ten days. In this technic, after repeated washings by centrifugation of the sedimented twenty-four hour specimen, a cubic centimeter or two of the precipitate suspended in sterile water or salt solution is injected subcutaneously into the guinea-pig just below the inguinal lymph nodes, which are

Figure 8 Tuberculosis of kidney. Observe circumscribed calcareous area in kidney, mistaken for calculus. Cystoscope established diagnosis. Patient of Dr. Charles H. Frazier. Skiagram by Dr. H K.. Pancoast.

Figure 9. Tuberculous pyonephrosis. Observe irregular outline of enlarged kidney. Patient of Dr. John H. Musser. Skiagram by Dr. H. K. Pancoast.

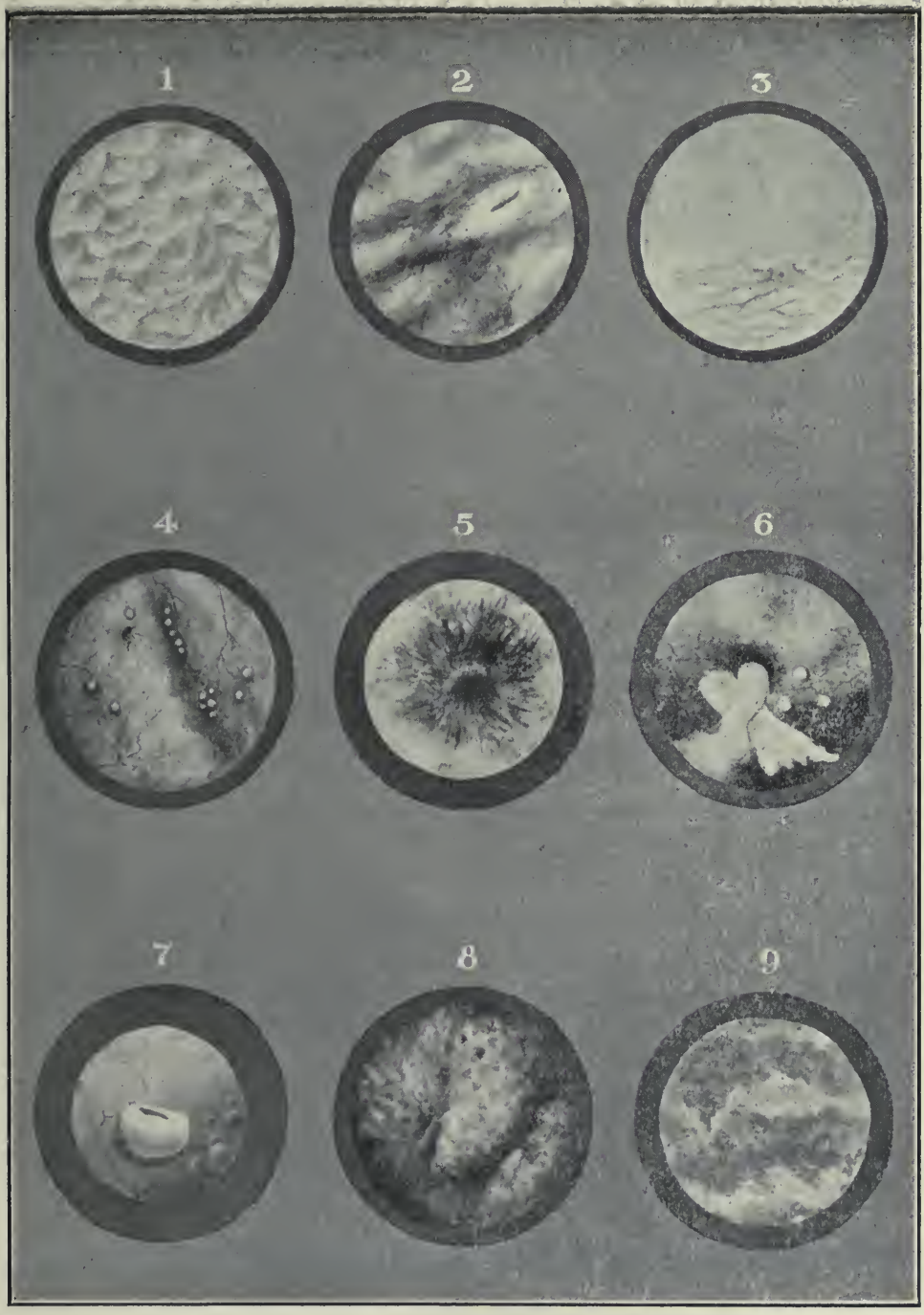

Figure 10. Cystoscopic views of reno-vesical tuberculosis.

1. Bullous edema.
2. Acute cystitis.
3. Closed ureter; bladder normal.
4. Miliary tubercles surround ureteral orifice.
5. Typical retracted enlarged immobile inflamed orifice.
6. Sewer-pipe orifice pouring forth pus.
7. Inspissated shell of pus surmounting orifice.
8. Tuberculous granulomata of bladder.
9. Advanced tuberculous vesical ulceration.

previously bruised between the fingers. A second animal should be used as a control. At the expiration of seven to ten days the guinea-pig is killed and its lymph nodes removed and macerated between two glass slides, stained by carbolfuchsin and Gabbet's methylene blue and examined for the bacillus tuberculosis (Figure 6). If necessary the nodes may be subjected to a routine histopathological examination. The control animal may be killed and examined at the end of five or six weeks. By this method tubercle bacilli are demonstrated in over eighty per cent. of tuberculous cases.

Recently, Hagemann created a sensation by announcing the cutaneous allergic reaction in animals, claiming to have obtained positive responses by this method as often as by any other technic, and in four instances to have found the skin reaction positive when all the other methods resulted negatively. The particular feature of the test consists in the ability to read the reaction in twenty-four to forty-eight hours, thereby markedly facilitating an early diagnosis. The technic, briefly, consists in rendering the guinea-pig tuberculous and then about the second or third week when it is hypersensitive, injecting it intradermically with one tenth of a cubic centimeter of the exudate, pus, etc., when, if the material to be examined is positive, there will occur a typical allergic reaction, as in Noguchi's luetin test, about the inoculated site (Figure 7). We have utilized the test extensively the past few months, but thus far our success has not been so brilliant as Hagemann's.

Radiography and Pyelography. Not infrequently the calcareous deposits in a caseated kidney are capable of casting a shadow in the skiagram. Under such circumstances the value of the *x*-ray in the differential diagnosis of calculus and tuberculosis may be nullified (Figure 8), and at other times it will suffice, when the organ is an enlarged caseated mass, to establish the diagnosis (Figure 9). Although, in the opinion of the author, pyelography is seldom if ever necessary in the diagnosis of renal tuberculosis, it may assist in the interpretation of the living pathology. Skiagraphic shadows, after the injection through the ureteral catheter of certain silver preparations, notably collargol, will define enlargement and irregularity, of the renal pelvis together with single or multiple foci depending upon the presence of cavernous areas in the parenchyma. The ureter also commonly shows dilatations and stenoses.

Cystoscopy. The cystoscope is by far the most important instrument for both the localization of high urogenital tuberculosis and, in the majority of cases, the actual diagnosis of the disease *per se.* The author well remembers that, considerably less than a decade ago, it was taught in a number of the leading medical schools and hospitals of this country, although it was permissible in selected urological cases to employ cystoscopy, to pass a cystoscope into a tuberculous bladder was a homicidal act. Times have changed, for to-day a number of world-renowned urologists do not hesitate but insist that catheterization of a normal ureter in the presence of tuberculous cystitis is a harmless procedure. It frequently happens that the cystoscope reveals an intravesical picture pathognomonic not only of tuberculosis of the bladder but also of the kidney above, primarily affected. Thus we are in a position to determine in most instances (1) whether or not tuberculosis exists, (2) the degree or extent of involvement and (3) whether only one or both kidneys are implicated.

The living pathology of the bladder commonly associated with renal tuberculosis may assume various appearances. During the incipiency of the disease, before suppuration occurs and while the organ is still functionally sufficient, the morbid process consisting simply of a bacillary nephritis,

the bladder lesions by cystoscopy may appear inconspicuous; at other times, and particularly if a mixed infection supervenes, the ureteral orifice appears inflamed and swollen and a general acute cystitis is associated, occasionally almost obliterating the crateriform openings of the ureters (Figure 10, 2). Presumably it is in these types of the disease that Buerger has recommended the excision of a portion of the ureteral orifice mucosa through the cystoscope and careful examination of the same for tubercle bacilli. The recommendation is certainly not without objection and we have failed, in a large series, to discover a case in which the procedure was warranted. Usually as the bacilli descend the ureter, the presence of the disease in the bladder is characterized by inflammatory evidence of the ureteral orifice; it begins to assume rigidity, losing its contractile and expansile properties; ulcerations and not infrequently miliary tubercles can be noted not only in the region of the ureteral orifice of the affected side and trigone (Figure 10, 4), but throughout the mucous membrane, even on the lips of the ureteral orifice of the normal side; bullous edema of the mucosa about the orifices or in the trigone is frequently observed (Figure 10, 1). As the process continues, the orifice becomes markedly infiltrated, enlarged and retracted (Figure 10, 5). Often the appearance is that of an open sewer-pipe pouring a stream of pus into the bladder (Figure 10, 6). In one instance a pure white shell of inspissated pus surmounted the orifice, exhibiting a crevice to accommodate the elimination of renal products (Figure 10, 7). Not uncommonly, the ureter becomes completely occluded by semisolid plugs of pus or undergoes stricture formation resulting in atrophy of the orifice (Figure 10, 3). In such a condition there may be neither pyuria nor tubercle bacilli in the urine. Occasionally, irregular granulomata form in the bladder (Figure 10, 8). In the last stage of the disease all vesical landmarks become effaced and the bladder wall is simply an expanse of confluent deep ulcerations covered with blood coagula and exudate (Figure 10, 9). If it is impossible by cystoscopy or chromoureteroscopy to determine the operability of the respective kidneys, recourse should be had to bilateral exploratory lumbar nephrotomy to determine the relative integrity of the two organs. In a few rare instances by the adoption of such measures, the lesser involved of the two kidneys has been removed with alleged cure. Such a procedure is seldom indicated and when nephrectomy is performed in the case of bilateral infection, the patient invariably dies in less than a year.

Functional Kidney Tests. Those commonly employed are indigocarmin, phenolsulphonephthalein, cryoscopy, urea determination, together with the various bacteriological, physical and chemical examinations. Of the special tests the author utilizes and prefers, for surgical purposes, indigocarmin by the method of chromoureteroscopy.[2] In a series of about 250 observations by this technic, the interpretations with respect to diagnosis and prognosis have been almost infallible. The chief noteworthy point of superiority of indigocarmin over phenolsulphonephthalein is that it renders ureteral catheterization for the purpose of determination of unilateral renal sufficiency unnecessary. Moreover, it aids materially in some cases in the localization of the ureters, the catheterization of which may be a physical impossibility.

Ureteral Catheterization. Allusion has already been made concerning the harmlessness of ureteral catheterization of the normal side in the presence of a tuberculous infected bladder. The question has

[2] "Chromocystoscopy in Functional Renal Diagnosis Based upon the Employment of Indigocarmin," PA. MED. JOUR., Sept., 1909. "The Value of Chromoureteroscopy in Functional Kidney Diagnosis," *Surgery, Gynecology and Obstetrics*, April, 1911.

been discussed and has able advocates pro and con. Personally, we are unreservedly opposed to the practice and see no reason for a reversal of attitude until our opponents are able to prove the harmlessness of the act by furnishing results better or at least as good as those herewith tabulated (See Table). No man can deny that there exists at least a potential danger of implanting tubercle bacilli in the ureter by the act of catheterization in the presence of tuberculous cystitis. Certainly we should not permit the practice upon ourselves and this is a proper consideration to extend our patients. On the other hand, since the danger can not be denied, what is to be accomplished by resort to the practice? Assuredly, the finding of tubercle bacilli in the ureteral catheterized urine does not mean necessarily that a surgical lesion exists in the respective kidney or that it is functionally insufficient or that the bacilluria is doomed to be permanent. Again it is quite possible that the bacilli discovered in the catheterized urine may have been carried into a normal ureter from an infected bladder by the catheter itself. Finally, a negative result does not by any means exclude the possibility of tuberculosis in the suspected kidney, for in the technic usually employed, the catheter is allowed to remain *in situ* but fifteen or thirty minutes, and it is well known that even in a twenty-four hour specimen of urine failure to demonstrate the tubercle bacillus is not unusual.

Thus on one side of the scales our dissenters may have nothing or what is worse, a false result, while on the other there is potential danger. Consequently, ever remindful of its merits in our service, we recommend for the serious consideration of our opponents the harmlessness, unequaled value and practical utility of chromoureteroscopy employing indigocarmin. If one kidney is functionally sufficient and its fellow is insufficient, operation with a view to nephrectomy on the affected side should be done, irrespective of the absolute diagnosis of tuberculosis which is of little moment under the circumstances. If bilateral infection exist, the operator may be justified in removing the more diseased of the two organs with the hope of effecting cure; if the disease has remained unilateral, which will be the case in the vast majority of patients, he will have the satisfaction of knowing that he is not responsible for an implantation infection by catheterizing a healthy ureter.

CLINICAL OBSERVATIONS.

Statistics compiled from patients, who present themselves for treatment of kidney tuberculosis, demonstrate that about seventy per cent. have symptoms dating back more than a year and that only ten per cent. consult the surgeon within six months of the onset of symptoms. The pertinent question therefore arises, Why are these patients prevented so long from receiving surgical aid? The answer seems to be, firstly, that either the patient because of the insidious onset of the disease or the general practitioner misled by the vague or remote symptoms fails to appreciate the urgency of early operation; secondly, there appears to exist in some localities in the minds of physicians, who ought to know better, a prevalent opinion that nephrectomy is not the best treatment for tuberculosis of the kidney. Consequently, many practitioners have stooped to treat these cases by tuberculin, climatotherapy, heliotherapy, etc., at a time when they might have been cured by nephrectomy. Israel, I believe, is the authority for the statement that there exists no authentic record of any case of renal tuberculosis cured by tuberculin. True as this may or may not be, it does not unveil the untold abuse of a therapeutic agent capable at least of symptomatic benefit (See Case 4 in Table). Precise and exact judgment must here be exercised in the treatment of each individual

ANALYSIS OF CASES OF KNOWN RENAL TUBERCULOSIS.

No.	Name.	Age.	Chief Complaint.	Duration.	Chromoureteroscopy. (Indigocarmin). Kidney. Bad	Good	Ureter Catheterized.	Nephrectomy.	Time Since Operation or Cystoscopy.	Present Symptoms.	State of Health.
1.	Frank A.	28	Iliocostal pain	2 yrs.	+ 0	6 min.	—	+	2 yrs.	None	Excellent
2.	Lizzie L.	29	Vesical irritability	5 mos.	+ 0	10 min.	—	+	5 yrs.	None	Excellent
3.	Edward O'M.	56	Vesical irritability	2 yrs.	+ 0	19 min.	—	+	3 yrs.	Lumbar sinus	Good.
4.	Anna M.	22	Vesical irritability	19 mos.	+ 23 min.	21 min.	—	— Tuberculin	2 yrs.	None	Excellent
5.	Jerome L.	16	Vesical irritability	2 mos.	+ 0	0	—	—	1 1-2 yrs.		Died 1 yr. later
6.	William L.	40	Vesical irritability	1 yr.	+ 0	0	—	—	2 1-3 yrs.		Died 3 mos. later
7.	Miss L.	12	Vesical irritability	?	—		—	—	7 yrs.		Died
8.	Mary B.	52	Hematuria	?	+ 20 min.	20 min.	—	—	3 1-2 yrs.		Not traceable
9.	John S.	27	Nephritis	2 yrs.	+ 9 min.	9 min.	—	—	2 2-3 yrs.		Not traceable
10.	David C.	27	Vesical irritability	9 mos.	+ 30 min.	30 min.	—	—	3 1-2 yrs.	Occasional vesical irrit.	Fair
11.	John B.	20	Iliocostal pain	6 mos.	+ 0	7 1-2 min.	—	+ Tuberculin	1-2 yr.	None, save bacilli in urine.	Excellent
12.	Ragna B.	30	Hematuria and vesical irritability	4 yrs.	+ 20 min.	12 min.	—	—	1 yr.	Same as before	Unimproved
13.	Pearl H.	42	Iliocostal pain	3 yrs.	+ 0	12 min.	—	+	2 yrs.	None	Good
14.	Matthew C.	30	Costo-lumbar pain; vesical irritability	10 mos.	+ 0	7 min.	—	—	1-3 yr.		Not traceable
15.	John D.	38	Vesical irritability	2 yrs.	+ 0	16 min.	—	—	5 yrs.		Not traceable
16.	Albert W.	32	Vesical irritability	1 yr.	+ 0	15 min.	—	Nephrotomy	3-4 yr.		Died 5 wks. later of miliary T. B.
17.	Herman R.	24	Hematuria; vesical irrit.	10 mos.	+ 0	20 min.	—	—	3 1-2 yrs.		Died 2 mos. after cystoscopy
18.	Anthony S.	49	Vesical irritability	17 mos.	+ 0	12 min.	—	—	1 1-4 yrs.	Frequency of urination	Fair. Better than before cystoscopy
19.	James S.	29	Vesical irritability	14 mos.	+ 0	17 1-2 min.	—	Nephrectomy	1 2-3 yrs.	Vesical symptoms improved	Bad
20.	Andrew W.	41	Vesical irritability	2 yrs.	+ 0	11 min.	—	+	1 3-4 yrs.	Frequency of urination	Good
21.	J. W. M.	40	Vesical irritability	2 yrs.	+ 0	12 min.	—	+	2 yrs.	None	Excellent
22.	Washington K.	36	Lumbar pains	2 yrs.	+ 0	9 min.	—	+	2 yrs.	Vesical symptoms and T.B. in urine.	Good. Better than before operation.
23.	Mamie R.	32	Renal colic	9 yrs.	+ 0	9 min.	—	+	3 1-2 yrs.	None	Excellent
24.	Mary H.	39	Vesical irritability	8 yrs.	+ 0	12 min.	+	+	7 yrs.	None	Good.

case, namely, whether nephrectomy, the treatment *par excellence,* or tuberculin, the "runner-up," is to be employed. If the diagnosis is made when it should and can be, there will be little use for tuberculin. Tuberculin therapy is permissible only when operation is inadvisable or as a postoperative adjunct to treatment; namely, when (1) bilateral bacilluria exists and tests show both kidneys to be normal and equal in function or by other examinations it is impossible to determine which organ was primarily affected; (2) both organs are extensively involved, insufficient functionally and obviously inoperable; (3) metastatic tuberculous foci are disseminated in peritoneum, prostate, bones, joints, etc.; and (4) postoperatively to assist in the cure of the involved ureter, bladder, etc. Diagnostically, tuberculin is of little value in this condition and then only when a local reaction in the kidney or urine is considered in conjunction with the systemic response.

Wildbolz, in a recent analytical study of 316 cases of tuberculosis of the kidney, treated nonoperatively under the best climateric and heliotherapeutic conditions in Switzerland, finds that seventy per cent. of the patients have died and that only ten per cent. survived longer than five years after the onset of symptoms, and that only one patient was entirely well.

Thus the results in our own series of definite renal tuberculosis comprising twenty-four cases (See Table), warrants the conclusion that the patient's best hope lies in nephrectomy with a prognosis directly proportional to the earliness of operation.

CONCLUSIONS.

1. Tuberculosis of the kidney is a prevalent disease masquerading frequently under remote, indefinite and misinterpreted symptoms.

2. Such cases are commonly treated medically in a palliative manner under the diagnosis of "nervous bladder," "cystitis," "gastrointestinal derangement," "lumbago," etc., until the patient is no longer amenable to surgical intervention.

3. The only treatment worthy of consideration for unilateral renal tuberculosis is nephrectomy and the prognosis is directly proportional to the earliness of operation; of eleven patients nephrectomized from six months to seven years ago, none has died. This result is unusual as the mortality is alleged to be approximately twenty per cent. This incongruity may be explicable on the assumption that dependence on functional kidney tests has not discredited surgery, since there exist a few patients in the series for whom operation was discouraged in view of the advanced state of the disease.

4. Occasionally, even in bilateral involvement, it is justifiable, with the expectation of recovery, to remove the more diseased of the two kidneys, provided the other is functionally sufficient.

5. The use of tuberculin diagnostically is practically valueless; therapeutically, however, it has a definite sphere of usefulness, but is commonly subjected to misuse. It should never supercede nephrectomy when indicated, and at best should be regarded simply as an accessory to nature capable of symptomatic improvement, if not cure.

6. Cystoscopy, and particularly chromoureteroscopy employing indigocarmin, in our hands, has been the diagnostic procedure of merit *par excellence;* indicative of the good offices of indigocarmin, not a single patient nephrectomized has since died.

7. Radiography is especially of value in the differential diagnosis between calculus and tuberculosis; it may deceive, however, if calcareous deposits have occurred in the tuberculous area. Pyelography should seldom, if ever, be practiced in the diagnosis of this disease.

8. The determination of the functional capability of the respective kidneys is of more importance with respect to operative intervention and recovery than the mere demonstration of tubercle bacilli in the urine.

9. Routine catheterization of the normal ureter in the event of unilateral tuberculous infection and cystitis, is an act absolutely unnecessary for the proper treatment of these cases and as yet not proved to be harmless.

Inasmuch as only seven of the twenty-four cases presented in this report are from my own service, I desire to express my indebtedness to Drs. Martin, Deaver, Stengel, Wood, Müller and especially to Dr. Frazier for granting me permission to analyze the cases cystoscoped on their services.

No. 5

A PRELIMINARY REPORT ON THE TECHNIC AND STATISTIC RESULTS OF THE WASSERMAN REACTION.*

By John L. Laird, M.D.,

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(Read before the Section on Medicine, Medical Society of the State of Pennsylvania, Harrisburg Session, September 26, 1911.)

The various modifications of the Wassermann reaction now in use and the influence of technic upon results render it advisable in a treatise on the Wassermann reaction to state the technic employed.

Illustrative of technic, first, I shall give a list of the reagents employed in this work, with their preparation and respective quantities in cubic centimeters and unit equivalents; then describe the performance of a single test and its reading; and finally call attention to certain technical features of importance.

REAGENTS.

1. Hemolytic antigen: A suspension of red blood corpuscles of the sheep, prepared by diluting with normal salt solution the washed corpuscles from 10 c.c. of whole sheep's blood to a total quantity of 96 c.c. Quantity = 1 c.c. = 1 unit. The unit for the entire reaction.

2. Hemolytic amboceptor: The inactivated serum of rabbits previously immunized to the corpuscles of the sheep. Quantity = 1 c.c. diluted to equal 1 unit.

3. Complement: The fresh serum of a normal full-grown guinea pig. Quantity = 0.05 c.c. = 1 unit.

These three reagents comprise the hemolytic system.

4. Syphilitic antigen: An alcoholic or watery extract of syphilitic fetal liver, prepared after the formula of Dr. Fritz Lesser. Quantity = 0.2 c.c. = 7 + units.

5. Patient's serum: Inactivated by heating to between 50° and 55° C. for one-half hour. Quantity = 0.1 or 0.2 c.c. The unknown unit to be determined by the test.

The Performance of a Wassermann Reaction. To perform a Wassermann reaction on a single case, really three tests must be performed, one on a known syphilitic case, one on a known nonsyphilitic and one on the test case.

We place into test tubes, numbered from 1 to 10, the various reagents in the following combinations:

Tube 1. 0.2 c.c. syphilitic antigen; 0.1 c.c. syphilitic patient's serum and 0.05 c.c. complement.

Tube 2. 0.2 c.c. syphilitic antigen; 0.1 c.c. non-syphilitic serum and 0.05 c.c. complement.

Tube 3. 0.2 c.c. syphilitic antigen; 0.1 c. c. test serum and 0.05 c.c. complement.

Tube 4. No antigen; 0.1 c.c. syphilitic serum and 0.05 c.c. complement.

Tube 5. No antigen; 0.1 c.c. non-syphilitic serum and 0.05 c.c. complement.

Tube 6. No antigen; 0.1 c.c. test serum and 0.05 c.c. complement.

Tube 7. 0.2 c.c. syphilitic antigen; no serum and 0.05 c.c. complement.

Tubes 8, 9 and 10. 0.2 c.c. syphilitic antigen; 0.1 c.c. of each of the three sera, but no complement.

Tubes are then incubated at 37.5° C. for three-fourths of an hour.

Into every tube is then placed 1 c.c. of properly diluted hemolytic amboceptor and 1 c.c. of the hemolytic antigen.

The whole incubated at 37.5° C. until complete hemolysis has taken place in tube 7, which should be in one and one-half to two hours.

A preliminary reading is then made and the tubes set in the ice-box or left at room temperature, if not too warm, for from twelve to twenty-four hours, when the final reading is made.

Final Reading of Reaction. With tube 7 showing complete hemolysis, the other tubes should be grouped for the final reading as follows and should show:

1. Tube 1. No hemolysis.....
   Tube 4. Complete hemolysis....
   Tube 8. No hemolysis..... = Positive reaction.

2. Tube 2. Complete hemolysis....
   Tube 5. Complete hemolysis....
   Tube 9. No hemolysis..... = Negative reaction.

3. Tube 3. Partial or no hemolysis
   Tube 6. Complete hemolysis....
   Tube 10. No hemolysis..... = Positive reaction.

or

3. Tube 3. Complete hemolysis....
   Tube 6. Complete hemolysis....
   Tube 10. No hemolysis..... = Negative reaction.

Variations in the above reading and their significance are as follows:

Tube 1. Hemolysis = antigenic power of syphilitic antigen weakened or lost. Repeat test with proper antigen.

Tubes 4, 5 or 6. Partial hemolysis = nonspecific deviation of complement; if this nonspecific deviation equals the deviation in tubes 1, 2 or 3 the test must be read negative.

Tubes 8, 9 or 10. Hemolysis = incomplete inactivation of the patient's serum, and, if the test is negative, the serum must be completely inactivated and the test repeated.

Tube 7. Incompleted hemolysis = weakness of one of the reagents of the hemolytic system or, less likely, an increase in the complement adsorptive power of the syphilitic antigen. The values of the various reagents should be tested by titration and the test repeated with these corrected values.

A positive reading should not be made where the difference in the degree of hemolysis between a test tube and its control is less than one-fourth of a unit.

The comparison of the test tubes with their controls, in the reading of results, is of great importance to differentiate the specific from the nonspecific deviations of the complement.

Technical Features of Importance. As the hemolytic antigen determines the unit for the entire reaction, constancy of value in this reagent is naturally of importance. We have found that the above method of preparation produces the most constant suspension of corpuscles, 1 c.c. containing approximately one billion corpuscles.

The use of one unit of hemolytic amboceptor instead of two, as in the original Wassermann technic, is of some importance. An excess of hemolytic amboceptor has the power to set free complement which has been adsorbed, and may, therefore, render weakly positive cases negative or doubtful, by liberating the complement adsorbed by the organic antigen. Contrary to some beliefs, however, an excess of even twenty or thirty units has no power to liberate complement which has once been fixed by a specific reaction. And, on the other hand, if one unit of amboceptor is sufficient to cause complete hemolysis of the hemolytic antigen, as the term implies, why use two units?

The syphilitic antigen should be a syphilitic antigen and not the nonspecific, which is considered by some authorities to be of equal value. The probable reason for the nonspecific antigen giving positive results in syphilis at all—that the real antigen probably exists in the serum of many syphilitic patients and acts through the nonspecific antigen as a medium—is sufficient proof of its unreliability in all cases of syphilis.

The patient's serum should be inactivated to destroy any possible complement it may contain and thus eliminate an error due to an excess, or at least to the ignorance of the amount, of complement acting in the reaction; which brings me to probably the most important point in the technic for the attainment of accurate results.

The use of one unit of complement instead of two, as in the original Wassermann technic: Again I ask, If one unit of complement is sufficient to produce complete hemolysis of the hemolytic antigen, why use two? It has been considered dangerous to use one unit of complement because there are present, in some normal sera, substances which have the power of deviating a certain amount of complement and, with this accurate amount used in the test, a negative case might be read positive. That such substances do exist in normal sera is true, but by a comparison of the test tubes with their controls, as described above, we can readily see that such deviation is nonspecific and should not, and could not, properly be read positive. On the other hand, with the use of but one unit of complement or just enough to produce complete hemolysis, nothing can happen to the least part of that complement which will not be evident in the result, in the incomplete hemolysis; and, moreover, there are many cases of known syphilis which, in the earlier stages and in the later stages as a result of treatment, show only one or less than one unit of syphilitic amboceptor, which if two units of complement were used would show a negative reaction, but with one unit show a markedly positive result.

STATISTIC REPORT.

The statistic report is upon 2672 reactions on 1555 cases, the work covering about two years. The numerical compilation is based upon the reactions, as repeated reactions have been performed on the same

cases in different stages of the disease.

Tables 1, 2, 3 and 5 are based upon reactions on known syphilitic cases. The diagnosis has been made by reputable syphilologists, on sufficient clinical evidence through a typical course of the disease, or in a few early cases, upon the finding of the treponema pallidum in the secretion from typical lesions.

Table 1 represents reactions on known syphilitic cases having manifest lesions.

The percentage of positive reactions in the primary stage can be seen to steadily increase from 40 per cent. in the first, to 100 per cent. in the fourth week; and from this time on in the cases which have received little or no treatment, to remain at 100 per cent. The negative and doubtful reactions obtained in the fifth week, with the patient under treatment, both showed positive at a later date in spite of treatment, showing, however, that the positive reaction may be delayed until after the fourth week by treatment. The negative obtained in the sixth month was on a patient with fading lesions after numerous injections of soamin. In those on the patients after one and a half and two years' treatment (3 negative), the lesions were distinctly retrogressive, and, in one case, rather doubtful as to their syphilitic nature. The one negative in the fifteenth year after three years' treatment was obtained during a prolonged severe treatment for a profuse deep-seated tertiary eruption on the back; the lesions were slowly healing. Including these cases, however, we have, after the fourth week in cases showing lesions, regardless of treatment, over 99 per cent. positive.

TABLE 1. SYPHILIS, WITH LESIONS.

Primary, without treatment.	REACTION	POSITIVE	NEGATIVE	DOUBTFUL	%POSITIVE
First week	33	13	20	0	40
Second week	42	31	8	3	74
Third week	31	28	3	0	90
Fourth week	37	37	0	0	100
Fifth week	16	16	0	0	100
Primary, with treatment.					
Fourth week	2	2	0	0	100
Fifth week	5	3	1	1	60
Secondary, with little or no treatment.					
Three months	238	238	0	0	100
Six months	44	44	0	0	100
One year	22	22	0	0	100
One and one-half years	12	12	0	0	100
Two years	17	17	0	0	100
Secondary, with fairly continuous treatment.					
Three months	79	79	0	0	100
Six months	50	49	1	0	98
One year	24	24	0	0	100
One and one-half years	3	2	1	0	66
Two years	13	11	2	0	85
Tertiary, with treatment less than three years.					
Third year	23	23	0	0	100
Fourth year	9	9	0	0	100
Fifth year	24	24	0	0	100
Tenth year	10	10	0	0	100
Fifteenth year	12	12	0	0	100
Twentieth year	2	2	0	0	100
Twenty-fifth year and over	2	2	0	0	100
Tertiary, with treatment of three years or more.					
Third year	9	9	0	0	100
Fourth year	10	10	0	0	100

Fifth year	4	4	0	0	100
Tenth year	19	19	0	0	100
Fifteenth year	4	3	1	0	75
Twentieth year	7	7	0	0	100
Twenty-fifth year or over	5	5	0	0	100
Total	808	767	37	4	95

Table 2 represents reactions upon known cases of syphilis showing no lesions.

We have had only one opportunity of performing the test on a patient during the incubation period; this was doubtful ten days before and positive five days before the appearance of the chancre.

The gradual decrease in the percentage of positive reactions in the secondary stage from 100 per cent. to 53 per cent. at the end of two years shows the effect of continuous mercurial treatment, but, in these cases, showing no lesions, still a little more than half show a positive reaction. This result is due almost entirely to the use of but one unit of complement in the technic, as shown by reports of some other serologists who, with the use of two units of complement, put the average time for the disappearance of the positive reaction at from four to eight months. In the cases under the heading tertiary syphilis, with less than three years' treatment, the three giving negative reactions had all received over two years' treatment. The increase in positive percentage from 40 per cent. in the third year to 53 per cent. in the fifth year, after three years' treatment, is due to a certain number of returns of the positive reaction after the cessation of treatment at the end of the third year. For the remaining periods, up to the thirty-fifth year, the percentage remains about 40 per cent., including cases on interval yearly treatment; this, I think, shows that three years' treatment is not sufficient, and that some cases have been practically incurable.

TABLE 2. SYPHILIS, WITHOUT LESIONS.

Incubation period.	REACTION	POSITIVE	NEGATIVE	DOUBTFUL	%POSITIVE
First week	1	0	0	1	0
Second week	1	1	0	0	100
Secondary, with little treatment.					
Three months	2	2	0	0	100
One year	3	3	0	0	100
One and one-half years	2	2	0	0	100
Two years	3	3	0	0	100
Secondary, with fairly continuous treatment.					
Three months	9	8	1	0	89
Six months	24	22	2	0	92
One year	46	32	14	0	70
One and one-half years	32	21	11	0	65
Two years	76	44	31	1	53
Tertiary, with treatment less than three years.					
Third year	5	5	0	0	100
Fourth year	9	9	0	0	100
Fifth year	19	19	0	0	100
Tenth year	12	9	3	0	75
Fifteenth year	7	7	0	0	100
Twentieth year	1	1	0	0	100
Twenty-fifth year and over	3	3	0	0	100
Tertiary, with treatment of three years or more.					
Third year	40	16	24	0	40
Fourth year	33	16	17	0	48
Fifth year	32	17	15	0	53
Tenth year	29	12	14	3	42

Fifteenth year	25	10	15	0	40
Twentieth year	24	8	16	0	33
Twenty-fifth year and over	20	8	11	1	40
Total	458	278	174	6	61

Table 3 represents the statistics on known cases of syphilis in which the exact stage of the disease and the amount and manner of treatment could not be ascertained. Most of these cases have received some treatment.

TABLE 3. SYPHILIS, MISCELLANEOUS.

Stage and amount of treatment uncertain	REACTION	POSITIVE	NEGATIVE	DOUBTFUL	% POSITIVE
	116	101	12	3	87

In table 4 the various conditions represented were generally held to be due most probably to syphilis.

TABLE 4. PARASYPHILITIC CONDITIONS, AND CONDITIONS SUPPOSEDLY DUE TO SYPHILIS, REGARDLESS OF TREATMENT.

	REACTION	POSITIVE	NEGATIVE	DOUBTFUL	% POSITIVE
Paresis	65	55	10	0	85
Tabes dorsalis	37	30	7	0	81
Dementia præcox (chronic)	4	4	0	0	100
Cerebrospinal syphilis	12	11	1	0	92
Spastic paraplegia	1	1	0	0	100
Arteriosclerosis	3	3	0	0	100
Aneurysm	7	5	2	0	71
Interstitial nephritis	3	3	0	0	100
Hepatic cirrhosis	3	2	1	0	66
Eye conditions	30	29	1	0	97
Osseous conditions	2	2	0	0	100
Hereditary conditions	56	32	23	1	60
Total	223	177	45	1	79

The varying percentage of positive results in the above conditions are variously explainable on the consideration of the effects of treatment, on certain conditions being sequelæ rather than complications of syphilis, and lastly on the word "supposedly" due to syphilis. This last explains certainly the low percentage, 60 per cent., of positive reactions in the supposed hereditary conditions, which, together with developmental and functional abnormalities, supposedly due to syphilis in the parents, included an array of conditions from active congenital lues to "red feet." In all cases of active congenital lues, except one on prolonged treatment, a positive reaction was obtained.

Table 5 gives the results of the reaction on known cases of syphilis after the administration of salvarsan. These cases have not been followed over a long enough period of time to make a detailed and conclusive report advisable at this time. The reactions were performed from one week to six months after the injections of salvarsan, most of which were intravenous.

TABLE 5. SYPHILIS, AFTER TREATMENT WITH SALVARSAN.

Salvarsan.	REACTION	POSITIVE	NEGATIVE	DOUBTFUL	% POSITIVE
One injection	81	67	9	5	82
Two injections	12	9	3	0	75

Three injections	2	1	1	0	50
Salvarsan, followed by mercury	38	14	21	3	38
Mercury, followed by salvarsan	44	30	9	5	70
Total	177	121	43	13	69

Four cases out of the nine negative reactions obtained after one injection of salvarsan received the injections before the second week of the chancre, the Wassermann reactions in two being negative before the injection, the diagnosis having been made on the finding of the treponema pallidum. Two of these cases have since shown positive reactions.

The maximum effect of salvarsan, as shown by the Wassermann reaction is shown at from the fourth to the sixth week after the injection. In the majority of cases the Wassermann reaction is, at this time, markedly reduced in degree of positiveness. The best results seem to be shown by the use of salvarsan followed by mercury in which we have obtained 38 per cent. of positive reactions.

Table 6 represents the reactions on doubtful cases, in which syphilis could not be absolutely excluded. This includes suspected cases showing general symptoms and having indefinite histories; doubtful lesions in which the manifest lesions are still undiagnosed; and conditions thought possibly due to syphilis, although no syphilitic history was obtained.

TABLE 6. DOUBTFUL CASES IN WHICH SYPHILIS COULD NOT BE EXCLUDED AS A CAUSE.

	REACTION	POSITIVE	NEGATIVE	DOUBTFUL	% POSITIVE
Suspected cases	157	58	86	13	37
Doubtful lesions	126	14	98	14	11
Cases in which there was no specific history.					
Valvular conditions	7	2	5	0	30
Miscarriage and sterility	13	0	13	0	0
Nervous cases	20	0	20	0	0
Osseous lesions	5	0	5	0	0
Eye conditions	60	11	48	1	18
Epilepsy	1	0	1	0	0
Multiple sclerosis	6	0	6	0	0
Arteriosclerosis	3	0	3	0	0
Apoplexy	2	0	2	0	0
Neurasthenia	7	0	7	0	0
No lesions	1	1	0	0	100
Total	408	86	294	28	21

Table 7 represents reactions on nonsyphilitic cases, in which syphilis could be excluded with a fair degree of certainty.

TABLE 7. NON-SYPHILITIC CASES IN WHICH SYPHILIS COULD BE EXCLUDED WITH A FAIR DEGREE OF CERTAINTY.

	REACTION	POSITIVE	NEGATIVE	DOUBTFUL	% NEGATIVE
Normal	100	0	100	0	100
Syphilophobia	13	0	13	0	100
Chancroid	140	0	140	0	100
Gonorrhea	44	0	44	0	100
Herpes	6	0	6	0	100
Carcinoma	25	0	25	0	100
Sarcoma	6	0	6	0	100

Tuberculosis	35	0	35	0	100
Hodgkin's disease	5	0	5	0	100
Brain tumor	21	0	21	0	100
Dementia præcox (toxic)	2	0	2	0	100
Amyotrophic lateral sclerosis	3	0	3	0	100
Myelitis	1	0	1	0	100
Thrombosis	3	0	3	0	100
Epilepsy	2	0	2	0	100
Sciatica	4	0	4	0	100
Alcoholic neuritis	2	0	2	0	100
Diabetes	5	0	5	0	100
Anemia	10	0	10	0	100
Leukemia	2	0	2	0	100
Hepatic cirrhosis	1	0	1	0	100
Nephritis	1	0	1	0	100
Typhoid fever	5	0	5	0	100
Tonsillitis	1	0	1	0	100
Arthritis	6	0	6	0	100
Stomatitis	1	0	1	0	100
Scabies	5	0	5	0	100
Acne	6	0	6	0	100
Eczema	5	0	5	0	100
Psoriasis	7	0	7	0	100
Ichthiosis	1	0	1	0	100
Tænia versicolar	4	0	4	0	100
Copaiba eruption	2	0	2	0	100
Varicose ulcers	5	0	5	0	100
Miscellaneous	3	0	3	0	100
Total	482	0	482	0	100

This table speaks for itself, nothing more need be added.

In the compilation of the above statistics, I have been as just, both to the Wassermann reaction and to the clinical side of the question, as was possible, asking the advice of others when my judgment failed me as to how to classify a case.

I wish, finally, to thank those of the profession who have been instrumental in affording me material for this work.

No. 6.

A New Method of Using Fehling's Solution

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A NEW METHOD OF USING FEHLING'S SOLUTION*

JOHN W. HUNTER, M.D., PHILADELPHIA

The principal difficulty which obtains in the use of Fehling's solution is the determination of the end-point of the reaction, that is, the point at which the copper has been just completely reduced. Heretofore, we have usually relied on our judgment as to whether the blue color had entirely disappeared from the more or less clear supernatant fluid after the precipitate had settled.

This, I contend, is not a reliable method of determining the end-point. We can often demonstrate the presence of unreduced copper in what is apparently a colorless or rather "blueless" supernatant fluid. Several means of fixing the end-point have been suggested, but they are all more or less cumbersome or time-consuming, especially for ordinary clinical work.

The method which I propose has proved very satisfactory and has the merit of being simple, rapid and fairly accurate. The principle on which it depends is that of separating the more or less clear supernatant fluid into two adjacent layers by heating the upper portion and then comparing these two layers after the reducing substance has been added to the upper hot layer. If there is reducible copper in the fluid the upper layer will show a reddish tinge whose density will depend on the amount of copper reduced.

The technic is as follows: The urine is diluted five times if the specific gravity is 1.030 or below and ten times if above 1.030.

Into a long, comparatively narrow test-tube is put 1 c.c. of Fehling's solution and a small amount of very finely powdered talcum or pumice. This is diluted with 3 or 4 c.c. of distilled water so that we have in the tube a fairly long column of fluid. After boiling, a few tenths of a cubic centimeter of the diluted urine are added and the contents

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of the tube brought to the boiling point. The precipitate is allowed to settle. This settling takes place rapidly if the pumice or talcum is very finely powdered. After the precipitate settles the tube is cooled by holding it in a running stream of cold water for a moment or two; it is then wiped with a towel and the upper portion of the supernatant fluid heated to or near to the boiling point. One-tenth cubic centimeter of the diluted urine is then carefully added and after a moment or two the appearance of the two layers (cold and hot) is noted. If there is a reddish tinge, due to suspended cuprous oxid, in the upper layer, the contents of the tube are again boiled, the precipitate allowed to settle and the foregoing procedure again carried out. This process is repeated until on the addition of 0.1 or, to be more accurate, 0.01 c.c. of diluted urine to the upper hot layer, no reddish tinge is discernible in that layer. This means that there was no copper to be reduced, it all having been reduced by the previous additions of diluted urine.

The amount of diluted urine added less the last instalment is the amount required to reduce 1 c.c. of Fehling's solution and from this the percentage calculation is made.

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MALIGNANCY OF INTESTINE

Diagnosed by Passage per Rectum of Piece of Intestinal Wall, Probably an Intussuscipiens.

No. 7.

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and
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The following case deserves to be put on record because of the passage per rectum of a mass of intestinal wall about six weeks after an attack of intestinal obstruction and localized peritonitis. This seems to have been an intussusception in which the peritoneal surfaces united without disturbing the continuity of the lumen for any long period, this all in the presence of malignancy at or near the site of the obstruction.

The patient, Mrs. K., 64 years old, had suffered more or less with constipation since early last fall, with gradual loss of flesh up to the time of the onset of the acute symptoms, which occurred about March 1st last, upon which date she was compelled to go to bed. For several weeks previous to the development of the acute symptoms she complained of a severe stabbing pain while at stool, which radiated from the left iliac region. This pain was attributed to the different purgatives she had been freely using, on her own volition, from the beginning of the constipation in the Fall.

Examination on the first day of her confinement to bed revealed marked emaciation, dry and dark coated tongue, weak pulse of 110 and temperature 102.5 deg. On palpation of the abdomen there were found rigid muscles and an enlargement in the left iliac region, with marked rigidity and sensitivity of the muscles in this locality. There was a slight nausea but no vomiting. Localized peritonitis was diagnosed but of uncertain origin, as the tense muscles and extreme tenderness at this time precluded any satisfactory examination. The pulse rate and temperature continued about the same for several days and then gradually fell to nearly normal. The treatment consisted of warm applications externally (they seemed more comfortable to the patient than cold) in conjunction with opiates. This treatment was continued for about two weeks and then stopped. Then followed an obstinate flatulence which periodically caused severe pain in the region of the descending colon, continuing until the gas would pass from the bowel, which it would do in a great volume and then immediate relief followed. A slow but continued improvement in the patient's condition followed and nothing unusual occurred until the passage from the bowel of the small fleshy mass accompanied by a slight hemorrhage, which specimen was examined. From the date of its passage she had free bowel movements daily but with some distress at the time and for a short time after. The stools were extremely dark and continued so.

During the late spring and early summer the bowels moved daily, but there was much flatulence with tenderness and some enlargement in the left iliac region. Early in July there appeared a pus collection in the left iliac region requiring incision and drainage. The patient died ten days later. The only post-mortem permitted was an enlargement of the abdominal drainage wound. This revealed a large new growth of the colon at the sigmoid flexure. There was no constriction of gut visible, and, furthermore, she had had a daily free and painless stool. No metastases were found before or after death.

The specimen first examined was passed at stool as above mentioned, about six weeks after the original attack of intestinal obstruction. It was immediately hardened in alcohol but had degenerated before passage. The whole gut wall could be traced in a perfect ring but one side was thin and soft, the other wide and dense. Under the microscope a partly decomposed tissue was found which in the thin, soft part seemed like normal mucous and muscular intestinal layers, while the denser portion contained a malignant growth, probably an adeno-carcinoma. This occupied two-thirds of the cross section. Serous surfaces could not be found, and in nearly all places there seemed to have been a round cell infiltrate. While it cannot be proven, the obstruction is taken as an intussusception, with sloughing off of the intussuscipiens, healing together of the peritoneal surfaces and reestablishment of continuity of lumen. At no time does there seem to have been any long continued obstruction.

The pieces removed post-mortem consisted of cross section of colon thickened and irregular in course. The thickening was diffuse and nodular, more of the former on the mucous surfaces, the serous side showing some firm masses covered by a few old adhesions. The microscopical sections made from three representative areas revealed an adeno-carcinoma. The nodules are due to a penetration of the submucous and muscular layers with considerable fibrous tissue overgrowth. The part of the mucosa not involved by tumor shows a low grade of atrophic colitis. There is an excess of round cells scattered throughout all the sections. One or two small abscesses are found.

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No. 8

THE PREPARATION AND EMPLOYMENT, IN A SERIES OF CASES, OF A POTENT POLYVALENT ANTISTAPHYLOCOCCIC SERUM *

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Impressed by the questionable propriety of the employment of active immunity with bacterins in the acute stage of an infection, whether localized or generalized, as in furuncle or septicemia — conditions in which, it seems logical to infer, the human organism is already sufficiently stimulated by viable bacteria to the production of specific antibodies, if not completely overwhelmed as in the latter instance, rendering the employment of an inactivated specific antigen inadvisable if not positively harmful — I attempted in September, 1909, the preparation of an antiserum which I hoped might prove useful in antagonizing the diverse lesions of that commonest of all pyogenic cocci, the *Micrococcus pyogenes aureus.*

Various attempts have been made on horses and other animals to produce a potent antistaphylococcic serum, but they have almost invariably resulted unsuccessfully, although Doyen and Paltchikowsky assert that they have succeeded partially. Emery states that "it is possible by special methods to produce a serum which has a slight bactericidal effect, but normal human serum or the serum of a patient who has been submitted to a course of antistaphylococcic vaccination is quite powerless in this respect." Schorer asserts that "the value of the serum . . . is inconsiderable, and its injection in the treatment of staphylococcus infections is seldom or never warranted." Such noted authorities as Ehrlich, Bordet and Citron omit even to mention, in their works

* From the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania. Read before the Philadelphia Pathological Society, Dec. 12, 1912.

on immunity, the existence of antistaphylococcic serum. It is therefore with considerable trepidation that I venture to assert the successful preparation of a potent polyvalent antistaphylococcic serum.

The experiments of Neisser and Wechsberg show the production of a typical hemolysin by the *Micrococcus albus* and *M. aureus* in culture growth. Following the subsequent discovery by these two serologists that human and certain other animal serums normally contain antistaphylolysin, although less than is the case with immune serums, Bruck, Michaelis and Schulze have attempted to employ the presence of antistaphylolysin in blood-serum as evidence of the existence of staphylococcic infection.

Furthermore, agglutination tests have shown that the staphylococci in pus may be agglutinated by the serum of the patient, while those from the air, skin, etc., are not so influenced, thus demonstrating, as is the case with many other bacteria, the variability or diversity of the strains of the bacterium in question. I feel convinced that one of the most important factors conducive to the successful production of our serum has been the utilization of staphylococcus cultures from many sources, thereby effecting a polyvalency of unmistakable potency.

PREPARATION OF THE SERUM

Eighteen strains of the *Micrococcus aureus* were isotated in pure culture from as many various sources, as follows:

Furunculosis	8
Carbunculosis	4
Subcutaneous abscesses (scalp)	3
Thoracic empyema	1
Axillary abscess	1
Septicemia (osteomyelitic)	1
Total	18

Each of these strains was cultured on agar-agar tube-slants at 37 C. (98.6 F.) for twenty-four hours, and the resultant growths washed off by the addition of 5 c.c. of bouillon to each of the cultures. The combined 90 c.c. were then shaken mechanically for fifteen minutes; 0.25 c.c. of this bacterial suspension was then flooded on the agar-agar surface of each of two Petri dishes,

presenting, respectively, an area of 12½ square inches. The growths from the two Petri dish cultures, after an incubation at 37 C. for twenty-four hours, were washed off in 30 c.c. of sterile 0.85 per cent. sodium chlorid solution. This bacterial suspension was standardized so that each cubic centimeter was estimated to contain 32,400,000,000 cocci and then sterilized by submersion in a water-bath at 60 C. (140 F.) for one hour.

From the same polyvalent staphylococcic suspension, agar-agar tube-slant cultures were made preparatory to the determination of the opsonic indices[1]— the controls

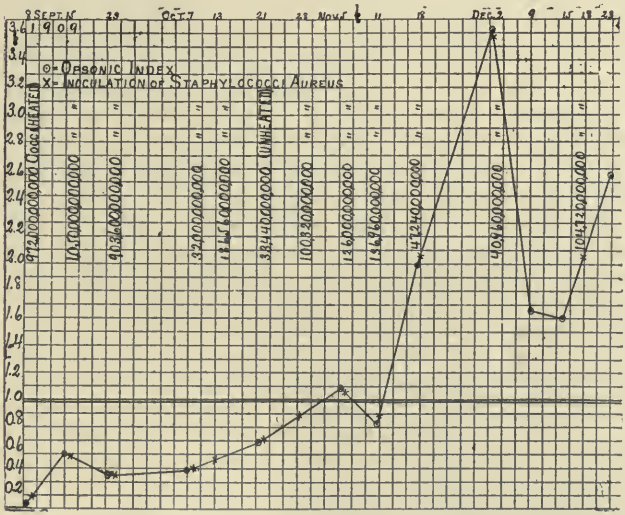

Chart showing inoculations and curve of opsonic indices illustrating immunity of ram.

by which the degree of immunity of the animal to be inoculated was governed.

The animal selected for the purpose of immunization was a full-grown, 3-year old, vigorous, uncastrated ram weighing 165 pounds. His opsonic index, Sept. 9, 1909, prior to inoculation, for the polyvalent staphylococcic culture was determined to be 0.12. The curve of the indices during the course of inoculations can best be studied by reference to the accompanying chart. On the

1. In all determinations of the opsonic index of the animal, washed human leukocytes were employed for the purpose of phagocytosis.

same date the ram received intraperitoneally the initial dose of 972,000,000,000 dead staphylococci. September 15, the ram's opsonic index was found to be 0.51, and he was inoculated with the growth from three Petri dishes, containing 1,050,000,000,000 cocci heated at 60 C. for one hour. September 23, the animal's index had fallen to 0.27, and he received intraperitoneally, 903,600,000,000 staphylococci heated at 60 C. for half an hour.

October 7, the index was 0.35 and the ram was injected with the agar-agar growth from six Petri dishes, estimated to contain 32,000,000,000 cocci heated for half an hour at 60 C. October 13, the animal received his fifth inoculation of 186,560,000,000 cocci, heated at 60 C. for only fifteen minutes. October 21, the eighteen various strains of staphylococci were again subcultured and fresh polyvalent suspensions prepared for Petri dish growths and culture for the determination of the opsonic index. The ram's index on this date was found still to be in the negative phase, namely 0.64, and his health appeared to be excellent. Accordingly it was decided to continue the inoculation with living bacteria, and 33,440,000,000 unheated staphylococci were injected intraperitoneally. One week later another injection of 100,320,000,000 living cocci was made. November 5 the opsonic index had risen to 1.02 and the ram received intraperitoneally the bacterial suspension from four Petri dishes, containing approximately 126,000,000,000 unheated staphylococci. Six days later the index had fallen to 0.8 and the ram was inoculated with 136,960,000,000 unheated cocci. November 18 the opsonic index was found to be considerably higher, reaching 2.0; the animal was inoculated with 47,240,000,000 unheated bacteria. December 2, the ram's immunity apparently reached its highest point, 3.55; nevertheless in an attempt to carry it still higher an injection of 40,960,000,000 unheated cocci was given. The indices determined seven and thirteen days later were found to have fallen to 1.65 and 1.6, respectively. December 18, the seventh inoculation (104,720,000,000 unheated cocci), with living bacteria, and the twelfth injection in all of staphylococci, was given. Five days later, December 23, the opsonic index was found to have risen to 2.6 and on the following day the ram was bled to death aseptically from the carotid artery. The serum

was separated from the clot and hermetically sealed in glass ampules containing 1 and 2 c.c. each.

The employment, practically, of this product of which I still possess a few sample ampules, I shall describe in twenty-eight case reports largely from the Surgical Outpatient Service of the Hospital of the University of Pennsylvania.

EMPLOYMENT, CLINICALLY, OF ANTISTAPHYLOCOCCIC SERUM

Although it was my desire to test the value of this serum practically in cases of staphylococcic bacteriemia, I have been disappointed in that respect, since there has been but a single case of septicemia, in which the blood-culture demonstrated the *Micrococcus aureus*. Consequently I have been obliged to apply it therapeutically, during its period of probable potency in a series of carbuncles and furuncles, many of which have been of an aggravated type.

Case 1.—Feb. 8, 1910. S. S., aged 15. An acute osteomyelitis of tibia, following osteotomy, developed a typical septicemia. Cultures both from the medullary cavity of the tibia at operation and subsequently from the blood demonstrated *M. aureus*. Later a suppurative arthritis of knee, demanding arthrotomy, and a suppurative inflammation of the wrist supervened. Bacterin therapy, instead of improving, probably aggravated the patient's condition. A course of hypodermic medication with mercury succinimid, strychnin sulphate, iron citrate and arsenic trioxid converted a most desperate condition into a curable case. After an illness of ten months, inoculations with antistaphylococcic serum were instituted. During a period of four weeks, five subcutaneous brachial injections of 1, 2, 2, 1.5 and 2 c.c., respectively, of the serum were administered. The patient exhibited marked improvement, his wrist healed immediately, he was able to go about on crutches, he gained greatly in weight, all sinuses about the knee, with a single exception, healed and he was discharged from the hospital precisely one month later.

Case 2.—March 11, 1910: J. P., aged 20. A virulent furunculosis of the head, face, neck, shoulders and arms had existed for two or three weeks. The infection was advancing rapidly, several new boils appearing daily; patient was feverish and obviously suffering from marked toxemia. Two c.c. of antistaphylococcic serum were injected subscapularly; two days later 2 c.c. more; four days later, 3 c.c. additional, and after another interval of four days a final 3 c.c. dose. After the first inoculation no new furuncles developed, and within two weeks

the patient was entirely well, even the old boils having disappeared.

CASE 3.—Dec. 3, 1910: V. C., aged 19. A recurrent furunculosis of four weeks' duration began in the axilla and recently manifested itself on the back of the neck. Patient was given 2 c.c. of antistaphylococcic serum subcutaneously. Two days later patient stated that boils on neck and in axilla were practically painless; he felt much better generally than for four weeks, no new boils developed and six days after the inoculation he was discharged cured.

CASE 4.—Dec. 3, 1910: D. E. M., aged 23. Patient had suffered for a week, with no improvement, from a large carbuncle of the back, measuring 2 inches in diameter. One inoculation of 2 c.c. of antistaphylococcic serum caused a marked subsidence in the inflammatory phenomena, and the lesion progressed to a rapid healing.

CASE 5.—Dec. 19, 1910: W. W., aged 24. Patient has a predisposition to furunculosis and recently lost considerable weight. During the past five days he has been the victim of an extensive outbreak of boils, no less than twenty appearing on the leg, thigh, hip and back, all on the left side. He was given an inoculation in the hip of 4 c.c. of antistaphylococcic serum. When seen six days later he felt much improved generally, and had noted the development of but two new boils. A second inoculation of a similar quantity of serum, increased to 6 c.c. one week later, caused the rapid disappearance of all furuncles.

CASE 6.—Jan. 4, 1911: E. B., aged 34. Patient had a carbuncle, similar to the present one, on his wrist eight months ago; the present carbuncle is located on the upper forearm, is about 2 inches in diameter, and has six or eight openings. One injection of 4 c.c. of antistaphylococcic serum caused the lesion to undergo rapid resolution.

CASE 7.—Jan. 10, 1911: L. G., aged 21. A relapsing furunculosis of the back, confined to an area 6 inches in diameter, had existed for two weeks, with little or no tendency toward improvement. An inoculation of 4 c.c. of the serum was given; the result cannot be noted, as the patient never returned to the dispensary.

CASE 8.—Jan. 23, 1911: M. T., aged 21. Three days previously carbuncle developed on neck; this was followed by the formation of three additional associated boils. An inoculation of 4 c.c. of antistaphylococcic serum was given in the scapular region, followed three days later by a second similar inoculation. The effect was marked and the patient proceeded to a speedy convalescence.

CASE 9.—Feb. 14, 1911: P. A., aged 36. Patient was the victim of furunculosis one year ago; during the past three weeks two or three furuncles formed on the neck, followed, the pres-

ent week, by two additional ones. Two inoculations of 4 c.c. each of the serum, with an interval of three days, caused the rapid disappearance of all old boils and prevented the development of any new ones. Three days later when the patient was discharged he was given a prophylactic dose of 6 c.c. of the serum.

Case 10.—Feb. 18, 1911: L. P., aged 20. For two weeks, patient has been the victim of a virulent furunculosis; at present he has more than a hundred boils distributed over the hips and lower extremities. An inoculation of 4 c.c. of antistaphylococcic serum in the thigh produced a most pronounced benefit, although during the following four days three new boils appeared. A second inoculation of 6 c.c. of the serum sufficed to produce a complete cure.

Case 11.—March 20, 1911: H. H., aged 11. Patient was the victim of acute furunculosis. A single inoculation of 15 minims of the serum resulted in immediate cure.

Case 12.—April 1, 1911: E. McL., aged 17. Patient had acute furunculosis. An inoculation of 2 c.c. of antistaphylococcic serum resulted in rapid convalescence.

Case 13.—May 21, 1911: G. H. B., aged 21. Patient suffered from an acute boil. An injection of 4 c.c. of antistaphylococcic serum caused the furuncle to heal unusually rapidly with no recurrence.

Case 14.—May 29, 1911: G. W. L., aged 19. A large, indolent, painful furuncle of the buttock had existed for several days. On the second day after the administration of 4 c.c. of the serum, the condition was markedly improved and the "core" of the boil was readily removed. Healing followed rapidly.

Case 15.—May 31, 1911: L. A. E., aged 21. Acute furunculosis of the buttock. An injection of 4 c.c. of antistaphylococcic serum remarkably improved the lesion, and convalescence was rapid.

Case 16.—June 12, 1911: W. W. C., aged 20. Acute relapsing furunculosis of the buttocks. An inoculation of 4 c.c. of antistaphylococcic serum beneficially influenced the lesions, although convalescence was more protracted than is usually the case, owing to the fact that the patient was an oarsman and obliged to row daily.

Case 17.—June 17, 1911: F. E., aged 20. Furunculosis of the forearm. Inoculations of the serum of 4 and 6 c.c., respectively, with an interval of two days, caused the furuncle to heal rapidly. About one month later a new boil appeared on the leg; this also rapidly disappeared after an injection of 4 c.c. of the serum. No recurrences subsequently were observed.

Case 18.—June 20, 1911: J. N., aged 21. Furunculosis. June 20: Inoculation with *Micrococcus aureus* bacterin, 4 minims. June 23: antistaphylococcic serum, 2 c.c. June 26: antistaphyl-

ococcic serum 1 c.c., no new furuncles. June 29: antistaphylococcic serum, 1 c.c., two small abortive furuncles observed. July 3: *Micrococcus aureus* bacterin, 0.25 c.c. July 6: *Micrococcus aureus* bacterin, 0.5 c.c. July 10: *Micrococcus aureus* bacterin, 1.0 c.c. July 14: *Micrococcus aureus* bacterin, 1.6 c.c. July 17: *Micrococcus aureus* bacterin, 3.5 c.c. July 21: *Micrococcus aureus* bacterin, 2.0 c.c. July 28: *Micrococcus aureus* bacterin, 2.0 c.c. August 15: Has had but one boil on buttock since last visit, but none now for two weeks.

Case 19.—July 7, 1911: N. B. M., aged 31. Furuncle of forearm. Patient was not observed after the initial inoculation of 20 minims of antistaphylococcic serum.

Case 20.—Nov. 9, 1911: G. T., aged 25. Carbunculosis of neck. An inoculation of 2 c.c. of antistaphylococcic serum produced a notable improvement in the condition of the carbuncle. Two days later a second injection of 4 c.c. of the serum was given. The lesion healed rapidly and four days later the patient was discharged cured. One year later patient returned with recurrent boils of the neck. This time the treatment consisted of simple incision and drainage and tincture of ferric chlorid internally. No serum was employed, and it is of interest to note that the convalescence this time required just twice the time consumed on the former occasion.

Case 21.—Nov. 14, 1911: W. M., aged 42; acute furunculosis. Patient has had an attack of recurrent furuncles of seven days' duration; at present three boils are active on the forearm. An inoculation of 3 c.c. of antistaphylococcic serum, repeated three days later by one inoculation of 4 c.c., caused the furuncle to heal very rapidly without recurrence.

Case 22.—Dec. 8, 1911: G. W. S., aged 61. Furunculosis of the scalp. An inoculation of antistaphylococcic serum of 2 c.c., repeated in eight days by a similar injection, caused the prompt disappearance of all active lesions. At a subsequent visit it was learned that no furuncles had reformed.

Case 23.—Dec. 9, 1911: C. P. K., aged 22. The present condition, a recurrent furunculosis, seemed to be the exponent of a bacteriemia, originating from an infected wound of the knee associated with inguinofemoral lymphadenitis, fever and other symptoms of a general toxemia, occurring two weeks ago. At present patient suffers from several furuncles on the neck, accompanied with bilateral superficial cervical lymphadenitis. Four c.c. of antistaphylococcic serum were injected on each side below the furuncle-bearing area. Although the patient was greatly improved, two of the three boils having completely healed, two days later 2 c.c. of the serum were again administered on each side. On the fourth day following the patient's first visit, the neck was entirely healed, although some lymphadenitis still persisted. No more furuncles developed, but in order to increase the duration of the immunity,

inoculation of autogenous bacterins was utilized. Accordingly an injection of 150,000,000 dead *Micrococci aurei* was given followed after two weeks by a second inoculation of 300,000,000 staphylococci, when the patient was discharged cured.

CASE 24.—Dec. 12, 1911: J. J. R., aged 22; acute furunculosis of neck. An inoculation of 2 c.c. of the antistaphylococcic serum caused the furuncle to heal rapidly. Patient seen one month later was in excellent condition and had had no recurrence.

CASE 25.—March 22, 1912: F. F., aged 23; recurrent furunculosis. During past two months patient has been the victim of repeated boils of thigh and neck. The present offender is quite large and painful. Five days after the inoculation of 4 c.c. of the serum, the furuncle was completely healed. There has been no recurrence to date.

CASE 26.—March 25, 1912: E. G. W., aged 42; carbuncle of neck. Present trouble began one week ago as a small papule; it steadily increased to its present dimensions, 2 inches in diameter, and is extremely painful, preventing all sleep last night; pain extends to base of neck and even into arms. Patient has had a headache for two days and is nauseated. Four c.c. of antistaphylococcic serum were injected on each side in the suprascapular regions. Two days later, patient declared himself to be 50 per cent. better; said that most of the pain had disappeared. The inflammatory phenomena were remarkably decreased and circumscribed, and the "core" was removed. Two days later the patient was given a second inoculation of 8 c.c. of the serum and discharged.

CASE 27.—April 12, 1912: T. C. N., aged 26; recurrent furunculosis. During the past six months, patient has suffered from recurrent boils in the nose. An inoculation of 4 c.c. of antistaphylococcic serum was administered. The final result is not noted in this case as the patient did not return.

CASE 28.—May 20, 1912: P. Z., aged 12; acute recurrent furunculosis. During the past week at least a dozen metastatic boils have made their appearance, principally on the neck. One inoculation of 2 c.c. of the antistaphylococcic serum was given. This immediately prevented further recurrence and caused the present lesions to heal spontaneously.

SUMMARY AND CONCLUSIONS

1. The antistaphylococcic serum as herein prepared and described possessed unquestionable therapeutic efficiency in a series of conditions, both general and local, due to infections by the *Micrococcus aureus.*

2. Biologic therapy by a potent polyvalent antistaphylococcic serum is more effective in the presence of a staphylococcic bacteriemia than is the corresponding autogenous bacterin.

3. By virtue of the more immediate and decisive effects of the antiserum, it deserves first choice over the bacterin in the treatment of furunculosis and carbunculosis; on the other hand, a more intensive and lasting immunity can be conferred on the individual by supplementing the serum with two or three inoculations of the autogenous bacterin.

4. It is to be regretted that no attempt was made to standardize this antiserum with respect to standard units, since it must be conceded that the therapeutic failure or inefficiency of many serums is referable to the deficiency of the immune body content of that particular serum; or in other words to an improper or incomplete immunization of the animal utilized for the production of the antiserum.

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No. 9.

Concerning the Presence of Hemolysins in Stool Extracts

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CONCERNING THE PRESENCE OF HEMOLYSINS IN STOOL EXTRACTS*

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The following experiments were undertaken with the aim of ascertaining, if possible, whether extracts of stools from cases of essential progressive pernicious anemia contain hemolysing substances not found in normal stools, nor in stools from other diseases. If repeatedly present, this could be regarded as of some use as a further step in the clinical diagnosis of a disease the etiology of which is still uncertain and the classification of which is hardly satisfactory.

In reviewing the literature on hemolysins in the gastro-intestinal tract, I find that while much has been done with tumor and organ extracts, little attention has been paid to the stools.

Korschun and Morganroth,[1] in their work on the hemolytic action of organ extracts, describe a hemolysin thus derived, which is active against the blood-cells of the same species and possesses the following characteristics: coctostabile, soluble in alcohol, not complex, and inactive in causing antibody formation.

Later Külbs,[2] in a series of experiments on stool filtrates, showed the presence of hemolysins in cases of intercurrent and chronic intestinal disorders as well as in progressive pernicious anemia. In chronic nephritis and diabetes agglutinins were frequently encountered. The hemolytic action of the filtrates was not affected by type of diet, heating or age of filtrate, nor did the reaction play any part in the result. He found no relation existing between the indoxyl content of the urine and the hemolytic effect of the stool. The solubility of the stool and apparent quick passage through the bowel seemed to be more important factors. Thin stools taken directly from cecum and ileum were very hemolytic. He does not refer to the chemical constituents. Injections into animals of 2 to 6 c.c. produced no anemia.

In the following year Tallqvist[3] reported his results on the study of the blood changes produced by the *Bothriocephalus latus,* results which

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*Read before the Pathological Society of Philadelphia, Oct. 24, 1912.

1. Korschun and Morgenroth: Berl. klin. Wchnschr., 1902, p. 870.
2. Külbs: Arch. f. exper. Path. u. Pharm., 1906, No. 55, p. 73.
3. Tallqvist: Ztschr. f. klin. Med., 1907, No. 61.

were most striking and of material value in explaining the etiology of such anemias. He demonstrated the presence of a lipoid substance in the proglottides of the worm, which was extremely hemolytic, could not be dissected out, formed no antibodies, was coctostabile, resistant to proteolytic ferments and showed a great affinity for red blood-cells. On subcutaneous injections into animals a definite anemia was produced, the autopsy findings being similar to those of a progressive pernicious anemia.

He refers to substances possessing similar hemolysing qualities in normal organ extracts, especially in mucous membranes of certain sections of the gastro-intestinal tract. He suggests that in many cryptogenic forms of progressive pernicious anemia there may be qualitative or quantitative anomalies in fat metabolism calling forth a pathological separation and a consequent hemolytic lipoid substance. Such substances have been described in various tumors and secretions. Thus Kullmann[4] found them in mammary and uterine carcinomata. Grafe and Röhmer[5] (whose work was later partially confirmed by Fabian[6]), in a series of over 100 cases, demonstrated hemolyzing lipoids repeatedly in the gastric contents of gastric carcinoma, while Neuberg and Reicher[7] showed that normal neutralized gastric contents of dogs were hemolytic to cells of the same animal. Later Bloch[8] claimed that if the enterogenous theory of progressive pernicious anemia was correct, the toxic agent should be found in the stools. He found hemolytic substances in the stools of six cases of progressive pernicious anemia, in chronic enteritis, (one case), and in tabes, but the most marked hemolysis occurred with normal stools. He concluded that the normally formed lipoid in the intestinal contents was rendered inert by cholesterol, and, when this failed, hemolysis occurred. The fact, however, of the strongest hemolysis having occurred in normal stools, in his own experiments, does not satisfactorily support this theory. He used alcoholic and ethereal extracts only. The substance was not activated by lecithin. Wohlgemuth found hemolysins in pancreatic juice, which were activated by lecithin.

Next Faust and Tallqvist,[9] after further studies in the chemistry of *Bothriocephalus* proglottides showed the lipoid substance to be soluble in warm alcohol and ether, but not in water, and only capable of producing hemolysis when the sheath was disintegrated. The fatty material contained phosphorus from lecithin. Cholesterol was crystallized out, but was not hemolytic. Free fatty acids were found to be intensely hemo-

4. Kullmann: Berl. klin. Wchnschr., 1904, No. 8.
5. Grafe and Röhmer, D.: Arch. f. klin. Med., 1908, No. 93, p. 159.
6. Fabian, D.: Arch. f. klin. Med., 1908
7. Neuberg and Reicher: Biochem. Ztschr., 1907, No. 4, p. 28.
8. Bloch: Biochem. Ztschr., 1908, No. 9, p. 498.
9. Faust and Tallqvist: Arch. f. exper. Path. u. Pharm., 1907, No. 57, p. 367.

lytic, but on further analysis palmitic and stearic acids (saturated) produced no hemolysis, while the unsaturated oleic acid was intensely active. When in combination with a cholesterin ester, it was most effective, although the cholesterin ester of fatty acids is present in most normal blood-serum in small amounts.

Cholesterol in feces is in part enterogenous, while part comes from bile and some from soap in chyle. Faust and Tallqvist hold that stimulation of the intestinal mucous membrane leads to a greater formation and secretion of this substance, and, with it, an increase of soap in chyle and in blood. Thus, through abnormal stimulation of a normal process, blood destruction could be produced.

By feeding *Bothriocephalus* lipoid to dogs large amounts of very hemolytic chyle were recovered in a few hours. Chyle showed neutral fat and free fatty acids, the latter being intensely hemolytic, due to oleic acid. No cholesterol was found in chyle.

Goodman and Robinson,[10] in an unpublished work, demonstrated the presence of hemolytic lipoid substances in ethereal extracts of stools from anemias and uterine carcinoma as well as in normal stools.

TECHNIC

To weighed stool an equal volume of .85 per cent. NaCl was added, the mixture ground up in a mortar, filtered through gauze and allowed to extract over night in an ice-chest. On the following day the mixture was shaken, centrifuged and the supernatant fluid passed through a Berkefeld filter, the filtrate being used as the extract, 1 c.c. representing 1 unit. To a series of tubes containing 1 c.c. of a 5 per cent. emulsion of washed red blood cells from the patient whose stool was being examined, was added a varying amount of the stool extract, from 2 c.c. down to .1 c.c. Sufficient physiological salt solution was added so that the mixture in each tube was brought up to 3 c.c. Similar amounts of the stool extract were added to a 5 per cent. emulsion of foreign human washed red blood-cells. The protocol is as follows:

Protocol of Experiment with Stool Extract

Diagnosis	Amount of Stool Extr. 1 c.c.=1 Unit c.c.	5 Per cent. Emulsion R.B.C. of Same Case, c.c.	5 Per cent. Emulsion Foreign R.B.C. of Normal Resistance, c.c.	Hemolysis
.....	2.0	1	—	.....
.....	1.0	1	—	.....
.....	0.5	1	—	.....
.....	0.1	1	—	.....
.....	2.0	—	1	.....
.....	1.0	—	1	.....
.....	0.5	—	1	.....
.....	0.1	—	1	.....

10. Goodman and Robinson: Unpublished work.

Case		
Gastric—		
1	Hyperchlorhydria and dilatation	Positive-Negative
2-6	Neurosis (5 cases in all)	Negative
7	Carcinoma	Negative-Positive
8-9	Toxic gastritis (?) (2 cases)	Negative
10	Chr. gastritis (2 times)	Positive
11	Gastroptosis and enteroptosis	Positive
12	Undiagnosed	Positive
13	Undiagnosed	Negative
Intestinal—		
14-15	Chr. constipation (2 cases)	Negative
16	Chr. appendicitis, *Ascaris lumbricoides*, and trichocephaliasis	Positive
17	Neoplasm of jejunum	Negative
Kidney—		
18	Uremia	Positive
19	Amyloid nephritis	Positive-Negative
20	Renal calculus and erysipelas	Positive
21	Acute parenchymatous nephritis	Positive
22	Acute parenchymatous nephritis	Negative
23-25	Chr. parenchymatous nephritis (3 cases)	Negative
26	Chr. parenchymatous nephritis with chorea	Positive-Negative
27	Chr. parenchymatous nephritis	Positive-Negative
Metabolic—		
28-33	Diabetes (6 cases of varying severity)	Negative in all
34	Gall-stones	Positive
35	Arthritis, neisserian	Negative
36	Arthritis deformans	Positive
37	Chr. family jaundice	Positive-Negative
38	Chr. family jaundice	Strong
39	Chr. family jaundice	Negative
40	Hepatic cirrhosis	Strong
41	Multiple serositis	Negative
Blood—		
42-45	Progressive pernicious anemia (4 cases)	Negative
46	Progressive pernicious anemia	Very faint negative
47	Progressive pernicious anemia	Positive
48	Lymphatic leukemia	Positive
49	Myelogenous leukemia	Negative
50	Splenomegaly	Positive
Heart—		
51-52	Myocarditis (2 cases)	Negative
53	Chr. in. myocarditis	Negative
54	Chr. in. myocarditis	Negative
55	Mitral regurgitation	Negative
56-66	Normal cases (11 stools from 3 cases)	All Negative

NOTES OF CASES

CASE 1.—Hypertrophy and dilatation of stomach. This was positive to blood of that case while it did not hemolyze foreign blood-cells.

CASE 7.—Gastric carcinoma was at first negative, but a few days later proved to be positive.

CASE 19.—Amyloid nephritis. This was positive at first but later negative as were Cases 26 and 27, both chronic nephritis.

CASE 38.—Chronic family jaundice. This was at first strongly hemolytic and later negative while Case 40, hepatic cirrhosis, was strong but not complete.

CASE 47.—Progressive pernicious anemia. This was complete with same cells but only very slight with normal foreign cells.

To avoid any influence that might be brought about by the increased fragility of individual cases, red blood-cells from a normal case, that is, red blood-cells capable of resisting hemolysis in hypotonic salt solutions down to .44 per cent. NaCl, were used.

It will be observed from the above table that normal stools repeatedly, and stools from progressive pernicious anemia and gastro-intestinal diseases frequently, were negative, and that the results are not analogous to those of Külbs, whose procedure was quite similar to the above. Nor is there any analogy to the results of Bloch, who, in his series of ten cases, used an ethereal and not a salt solution extract.

The properties of the filtrates were as follows: Light to dark brown, of fecal odor and of variable reaction, the latter playing no part in the hemolytic action of the stool. Nor was there any association between the type of food or use of purgatives and hemolysis. No acholic stools were examined. The presence of indoxyl in the urine, and of the phenol and amido groups in the filtrates had no relation to hemolysis, nor did the action of heat (58 C. for one hour) play any part. On prolonged standing the action became weakened and finally lost after seven days.

Fatty acids and neutral fats were present alike in active and inactive stools, as were precipitations of phosphates occasionally.

The results were so inconstant in my series of cases that I feel that no dependence should rest on this test, either as an aid to clinical diagnosis or as a means of enlightenment etiologically.

In conclusion I wish to extend my thanks to Dr. Stengel for the suggestion of this work and the use of the patients in his wards in the University hospital as well as to Drs. E. H. Goodman and Herbert Fox for their kind assistance.

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1913

No. 10.

Reprinted from the New York Medical Journal for March 8, 1913.

CULTIVATION OF *BACILLUS TYPHOSUS* FROM A SPOT IN A LUETIC TYPHOID SUBJECT AFTER THE FASTIGIUM.

By P. G. Skillern, Jr., M. D.,
Philadelphia.
(From the William Pepper Laboratory of Clinical Medicine.)

The following case is of interest because of the infrequency of this examination clinically, and because of its diagnostic value in atypical roseolar rash and in cases modified by some intercurrent disease as, in this case, syphilis. It occurred in the service of Dr. Alfred Stengel at the University Hospital:

Case. N. N., male, aged forty years, white, seaman, native of Finland, admitted October 7, 1912, complaining of weakness, having left a boat which had just arrived from Bombay.

On September 12th, nearly a month previously, while in the Mediterranean Sea, was overtaken with fever, malaise, diarrhea, loss of appetite, headache, and abdominal soreness. On admission he no longer complained of these symptoms, but said he felt very weak. An eruption appeared on body two weeks after onset of illness, and remained.

He contracted a chancre ten years previously. Was a heavy drinker and smoker. Had a fever which lasted four weeks after leaving Bombay. The water, which was taken on board ship at Bombay, was filthy.

Examination revealed a very profuse maculopapular eruption over most of trunk, arms, and legs, as well as on hands and feet and left cheek. This rash had the boiled ham appearance and symmetrical distribution of syphilitic roseola, and did not disappear on pressure. Lymph nodes palpable were the anterior and posterior superficial cervical, supraclavicular, left epitrochlear, and inguinals. An ancient scar, circinate in outline, was found posteriorly over neck and upper part of back.

Clinical examination of the blood gave a positive Widal reaction and a weakly positive (one quarter unit) Wassermann. Smears from the spots examined under dark field illumination showed no spirochete. Culture from the

spots made by writer on October 15th gave a pure growth of typhoid bacilli, which agglutinated with serum from a known case of typhoid. Blood culture taken day after admission proved sterile.

On October 14th, a week after admission, the eruption was fading, leaving a grayish discoloration, and on November 8th, the day of discharge, the spots on abdomen were very faint indeed. At no time during stay in hospital was the temperature over 100° F.

241 South Thirteenth Street.

No. 11.

Extracted from the American Journal of the Medical Sciences,
June, 1913, No. 6, vol. cxlv, p. 879.

ACUTE POLYMYOSITIS.

By Herbert Fox, M.D.,

DIRECTOR OF THE WILLIAM PEPPER LABORATORY OF CLINICAL MEDICINE, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA.

The following observations were made upon a microörganism obtained by blood culture in this laboratory, upon a case of acute polymyositis, in the service of Dr. Charles K. Mills, in the University Hospital. The case itself will be put on record later.

A. G.; acute polymyositis. Material at hand consisted of scrapings and exuded serum from a papular skin eruption. Save for Micrococcus epidermitis albus in the scrapings, these were sterile. Two days later a blood culture was taken (3 c.c. blood to 200 c.c. +1 per cent. glucose bouillon). Specimens were again taken from the then rapidly drying cutaneous eruption, with the same result as above. From the blood culture a coccus was obtained corresponding to the one described by Martinotti[1] in a kidney abscess and named by him Micrococcus polymyositis. The organism in the fifth generation was agglutinated quite promptly, by the patient's serum. This coccus is in many ways identical with Micrococccus pyogenes albus and aureus, and for purposes of comparison a recently isolated strain of the latter was run through with the "polymyositis" organism from this case. The technical work is appended, and from it we conclude that the organism in question does not deserve a separate species name, the proper course being to speak of it as Micrococcus pyogenes var. polymyositis.

Biologically the culture which we shall call No. 5308 in this report differs from the usual forms of Micrococcus pyogenes only as follows: Upon plates and slants both of agar and gelatin it grows as a grayish-blue colony, and only in older cultures may one see a faint grayish-yellow color. It is never dense white or bluish-white. Liquefaction of gelatin is not so rapid in 5308 as in the Micrococcus pyogenes. No constant difference, such as in

[1] Zentralblatt f. Bakteriologie, etc., 1898, xxiii, 877.

stratiform or infundibuliform liquefaction, could be noted. Upon potato there is neither the intense orange of the "aureus" nor the porcelain appearance of the "albus." The medium is not colored. On milk the coagulation does not appear completed until the third or fourth day, after which the clot is so firmly contracted that a suggestion of digestion arises. This last, however, does not occur. It seems worthy of note that no growth was to be seen in the blood bouillon flasks until the end of the third day. These differences were observed in the third and tenth generations. The Micrococcus aureus used for comparison was also tried with the patient's serum. It was lightly agglutinated in the same time as a firm agglutination was observed with 5308.

The Micrococcus aureus and 5308 gave almost identical results when injected intraperitoneally into guinea-pigs.

In rabbits a subcutaneous injection of Micrococcus aureus gave a sharply outlined local abscess. Injections of 5308 produced a diffuse, boggy, tender swelling, with a slight reddening of the skin, all of which subsided in three days without rupture or general involvement.

Both cultures under consideration were injected into the circulation of rabbits, using a completely emulsified twenty-four hour agar growth. The height of the symptoms in the 5308 animal was apparently reached at the end of the second day, and on the third morning both were chloroformed. Martinotti describes a peculiar lameness and gait, and emphasizes the extreme sensitiveness of the animal. Both our animals on the second day were huddled down in the corner of the cage and refused to move, crying piteously if they were forced to do so. No particular differences could be noted other than the greater sickness of the Micrococcus aureus animal. Intravenous injections of Micrococcus aureus gave pyemia, with numerous sharply outlined muscular abscesses very irregularly distributed, abscesses of the lungs, heart muscle, liver, kidney, and a mild seropurulent peritonitis. Injections of 5308 intravenously resulted in a few abscesses in the liver and kidney, and a very slight early plastic peritonitis. In the muscles of the extremities, along the vertebræ and in the pectorals, there were found enormous numbers of minute diffuse, pale gray areas surrounded by a zone of hemorrhage and edema of varying width. In the other muscles these were less numerous. The lesions are more severe in the Micrococcus aureus animal, and while this may suggest a lower virulence of 5308, there is surely some degree of specificity or predilection for the musculature. The virulence of 5308 is less than that outlined by Martinotti for his culture.

Unfortunately for the continuance of the work no rabbits were available when these animals were killed, and when a week later we could obtain the animals the 5308 culture had lost its virulence entirely and would not infect them. Indeed, 5 c.c. were required to kill a small guinea-pig, and it did not seem worth while to sacrifice

animals further, since no increase was noted after two passages. It seems sufficiently proved that this 5308 is one of the Micrococcus pyogenes group, and should not be designated by a separate species name.

In a recent communication Schmitz[2] closely corroborates the above findings. There are a few noteworthy differences, however. His culture produced an intensely yellow growth especially upon ascites agar. My culture, now about nine months old, gave more pigment, a delicate yellow, upon serum agar than upon plain medium; at no time was the color intense. The virulence of his culture rapidly declined upon cultivation, and he noted a decline in virulence for rabbits in a re-isolation of the coccus from an abscess upon a guinea-pig isolated subcutaneously. He could, however, raise the virulence for rabbits by repeatedly inoculating them. His culture gave an infection picture close to that of Martinotti, but his control with Micrococcus aureus failed to show muscular involvement. Mayesima[3] reports a case in which he isolated a Micrococcus pyogenes albus from the blood. He believes the infection atrium may be the tonsil.

This blood-culture finding is interesting from two standpoints: (1) It is another case to support the view that acute polymyositis is a bacteremia due to a micrococcus, with curious and rather uniform predilection for the musculature by which a subspecies or variety of a pus former can produce a definite clinical picture, with lesions more or less characteristic, and quite different from those produced by the most conspicuous member of the group to which it belongs. What determines the bacterium in its behavior is of course wholly unknown, and there is nothing in the clinical knowledge to help us. The infection atrium and the receptivity of the host probably play parts. It is suggested that the infection assumes the form of polymyositis when a Micrococcus pyogenes bacteremia occurs in a person whose condition favors rheumatism. There is of course no pathologic basis for this, as the lesions are different. F. Gottstein[4] however, reports a case which was closely associated with one of acute articular rheumatism. Some attention is being given by the writer to the infections, particularly the subacute, in which pathogenic mutations are noted, and this case is but an example.

[2] Zentralbl. f. Bakt. u. Par., Original, Band 65, 259.
[3] Deutsch. Zeitsch. f. Chir., 1910.
[4] Deutsch. Arch. f. klin. Med., August, 1907.

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Ein Beitrag zum Studium der experimentellen Endocarditis.

Von Dr. med. **Herbert Fox.**
(Wm. Pepper-Laboratorium für klinische Medizin, Universität von Pennsylvanien.)

Das Studium des Ursprungs der vegetativen und ulzerativen Prozesse, die die Herzklappen affizieren, geht bis auf die Zeit zurück, in der man zuerst erkannt hatte, daß dieses Verhältnis bakterieller Natur sei. Vor ungefähr 25 Jahren schienen die Untersuchungen von Orth, Wyssokowitsch und Ribbert das Faktum festzustellen, daß Bakterien sich auf den Klappen vom Blutstrome her an einem Punkte von geschwächter Widerstandsfähigkeit festsetzten. Diesem wurde indessen nicht von Köster zugestimmt, welcher behauptete, daß die Bakterien auf dem Wege der Koronararterien und der Basis der Klappen eindringen, insofern Luschka sich dahin ausgesprochen hatte, daß die Klappen immer mit Blutgefäßen versehen wären. Orth und Ribbert behaupteten, daß diese letztere Annahme nicht richtig sei und daß eine darauf gegründete Theorie irrig ist.

Die früheren Versuche, die in der Absicht vorgenommen wurden, künstlich Endocarditis hervorzurufen, geschahen in der Weise, daß man die Klappen lädierte entweder durch operatives Verfahren oder durch Partikelchen — Kohle, Kartoffelschabsel — die zugleich mit Mikroorganismen in einer Flüssigkeit suspendiert waren. Prudden machte ebenfalls Versuche mit Toxinen in Lösung. Später haben italienische, französische und vor kurzem englische und amerikanische Autoren wiederholt berichtet über die Hervorbringung von Endocarditis durch die einfache intravenöse Injektion von Bakterien, die ganz frei von suspendierten groben Partikelchen oder bakteriellen Produkten war. Auf diese Weise sind alle Formen von Endocarditis hervorgerufen worden. Ribbert und kürzlich Lissauer haben behauptet, daß spontane menschliche Endocarditis fast nur an dem Schließungsrand der Klappen vorkommt, während experimentelle Läsionen auf den freien Rand und die Mitte der aurikularen Oberfläche beschränkt sind, der benachbarten Oberfläche und den Chordae entlang sich ausdehnend. Diese Autoren geben auch an, daß nicht ein zu großer Nachdruck auf die Aehnlichkeit der spontanen und künstlichen Läsionen gelegt werden sollte, weil bei den letztern die Aortenklappe so selten affiziert wird, wofern nicht eine mechanische Verletzung des Endocardiums stattgefunden hat. Es ist indessen zu beachten, daß einer der Fälle, mit denen Roux zu tun hatte, Mitral- und Aorten-Läsionen zeigte. J. M. Beattie, welcher einige Versuche an Kaninchen mit Streptococcus rheumaticus anstellte, nahm Endocarditis mitralis und aortica wahr. Andere englische Autoren haben ähnliche Resultate erhalten. Hinwiederum berichtet Cole, der mit

diesem Organismus und mit Streptococcus pyogenes arbeitete: „Beide Segmente der Mitralklappe zeigten eine beträchtliche Verdickung längs der freien Ränder. Ein Segment wurde entfernt, und es wurden Schnitte gemacht. Diese zeigen eine typische Endocarditis.“ Bei einem andern Herzen zeigte die Mitralis Randauswüchse, an denen ein Thrombus adhärent war.

Rosenow nimmt wahr, daß experimentelle Endocarditis bei Kaninchen auf beiden Seiten hervorgerufen wird, obgleich die Läsionen bei den Tieren rechts reichlicher sind, als in den Fällen vom Menschen, von denen er seine Bakterien erhielt. Er hatte auch einen Fall einer doppelseitigen menschlichen Herzerkrankung und zwei Fälle doppelseitiger experimenteller Erkrankung. Es fanden sich 33 % mehr an menschlichen als an tierischen, ausschließlich linksseitigen Läsionen vor. Die Atrioventrikularklappen litten mehr an embolischen Läsionen, was, wie Rosenow glaubt, davon herrührt, daß sie Kapillaren besitzen. Er ist der Ansicht, daß oxydiertes Blut im linken Herzen, welches die Kokken in Stand setzt, sich besser in Klümpchen anzusammeln und an Oberflächen zu adhärieren, die größere Häufigkeit der Erkrankung beim Menschen nach der Geburt auf jener Seite erklärt. „Embolischer Ursprung kann die größere Häufigkeit von Endocarditis an der Mitralis und an den dreizipfligen Klappen im Vergleich zu der Endocarditis aortica und pulmonalis erklären.“ Tatsächlich zieht er die Myokardveränderungen gar nicht in Betracht. Bei einigen seiner Autopsien wird die Gegenwart einer Myocarditis angenommen. Er hebt auch nicht die Oertlichkeit der Excrescenzen hervor, ob sie gewöhnlicher auf dem Rande oder in der Mitte sind.

Das Interesse des Verfs. für diesen Gegenstand datiert von 1905, als es ihm gestattet war, ein Herz mit vegetierender Endocarditis im Laboratorium von Prof. Albrecht bei der Poliklinik in Wien zu studieren. Die eine Klappenvegetation, beinahe von der Größe der Spitze des fünften Fingers bei einem Erwachsenen, zeigte auf dem Schnitt starke Anschwellung, Infiltration, Massen von Bakterien und Fibrinablagerungen in den beiden äußeren Dritteln, während das innere Drittel Oedem und Massen von Bakterien zeigte, die sich bis tief in das Myocardium erstreckten. Die Muskelfasern waren geschieden durch runde und mehrkernige Zellen und Bakterienhaufen. Das Lumen und die Wand vieler, wenn nicht aller Gefäße an und nahe bei der Basis der Klappe und in der Muskulatur waren von Bakterien infiziert. Eine hatte sogar einen Thrombus, der so sehr mit Kokken angefüllt war, daß er nach einer Färbung nach Gram-Weigert nicht entfärbt werden konnte.

Seit dieser Zeit hat Verf. verschiedene Versuche, Endocarditis hervorzurufen, angestellt, jedoch mit wenig Erfolg. Im vergangenen Winter standen ihm indessen ungewöhnlich gute Kulturen von Streptococcus zur Verfügung und wurden sofort bei Kaninchen injiziert. Die Versuche geschahen an fünf Kaninchen im Ganzen; bei dreien von diesen wurde eine deutliche Endocarditis hervorgerufen, welche einen derartigen Charakter hatte, daß eine Diskussion der Punkte, die in den oben gegebenen historischen Bemerkungen vorgebracht sind, möglich wird.

Fall 1. Mädchen, 15 Jahre. Rheumatische Angina, begleitet von unbestimmten Schmerzen in den Gelenken, intermittierendes Fieber und einige Prostration. Die Tonsillen wurden entfernt, und aus einem dreimal gewaschenen Stück aus dem Zentrum der exzidierten Drüse wurde ein Streptococcus pyogenes

(vielleicht rheumaticus) erlangt. Dieser wurde auf Blutagar zum Wachstum gebracht, mit Salzlösung abgewaschen, die Suspension zentrifugiert, um Klümpchen von Bakterien oder des Mediums zu vermeiden, und direkt in die Ohrvene eines völlig ausgewachsenen Kaninchens injiziert. Alle die Tiere wogen zwischen $3^1/_2$ und $5^1/_2$ (engl.) Pfund. Dies geschah viermal innerhalb 20 Tagen, und die Tiere starben 24 Tage nach der ersten Injektion. Es war fibrinöse Peritonitis und Arthritis des Knies vorhanden. Es war eine blumenkohlartige Masse auf beiden Mitralsegeln, von 3×5 mm Größe, welche die ganze Klappe ersetzte und sich bis zur Basis des Aortensegels der Mitralis auszudehnen begann, welches an sich frei war. Der Raum zwischen den Vegetationen ließ vor dem Oeffnen des Ventrikels nicht die Einführung einer Bleistiftspitze zu. Die Chordae waren nicht mit ergriffen. Eine leichte Anschwellung der Basis des Aortensegels der Mitralis ohne Vegetation fand sich vor. Als ein Stück der Mitralklappe zum Studium entfernt werden sollte, brach die Vegetation ab. Sie wurde besonders geschnitten und bestand aus Fibrin, Kokken und Zelldetritus. Das Hauptbasalstück zeigte den Herzmuskel als Ganzes gekörnt, mit einer Neigung zu fibrillärer Degeneration an einigen Stellen. Die Interstitien enthalten eine leichte Vermehrung von Polynukleären, besonders in der Nähe der Blutgefäße. Der Muskel ist ödematös und erscheint reich an Kernen. Die Basis der Klappe ist von polynukleärer Infiltration durchsetzt und nekrotisch. Zwischen dieser und dem besser erhaltenen Muskel ist ein polynukleäres Infiltrat mit stark hervortretender fibrillärer Degeneration des Muskels vorhanden. Bei Gram-Weigert zeigt sich die größte Intensität an der Klappenbasis, aber Kolonien von Bakterien sind auch innerhalb der Muskulatur ohne umgebende Infiltration vorhanden. Um eine kleine Arterie nahe der Klappenbasis sind zahlreiche, sehr kleine Kokkenkolonien vorhanden, und einzelne oder Diplokokken waren durch die Gefäßwände zerstreut, besonders in den innersten Schichten, zu finden. Zur Zeit des Todes dieses Kaninchens war kein Blutagar zur Hand. Kulturen wurden von der Herzklappe, welche viele Kokkenhaufen zeigte, auf einfachem Agar angelegt und Weiterimpfungen auf Blutagar am nächsten Tage vorgenommen. Leider wurde kein Wachstum erzielt.

Ehe ich zu dem nächsten Falle übergehe, erscheint es angemessen, zu erwähnen, was zur Vorbereitung auf denselben ausgeführt wurde. Um das Myokard und die Klappenbasis und vielleicht die Koronargefäße verletzbar zu machen ohne irgend eine mechanische oder toxische Einwirkung auf das Endothel, wurde das von R. M. Pearce ausfindig gemachte Verfahren, als er mit der Einwirkung des Adrenalins arbeitete, benutzt. Bei angemessenen Quantitäten (2 ccm einer 1—1000 Lösung) pflegt schon nach sehr wenigen Applikationen eine granuläre Degeneration und ein granuläres Oedem des Herzmuskels einzutreten. Ich injizierte daher abwechselnd subkutan und intravenös eine sehr kleine Quantität (0,000 001 Gramm) zehnmal bei jedem der beiden Kaninchen. Das eine wurde zu histologischen Untersuchungen getötet und das andere zur Injektion mit Streptokokken verwandt. Das Herz des getöteten Kaninchens zeigte den Muskel als Ganzes etwas granulär; die Faseranordnung war nicht verändert. Die Querstreifung ist zum größten Teil wohl erhalten; wo aber die granuläre Veränderung am stärksten ist, scheinen die Querlinien aus Granula zu bestehen. Das Auffälligste im Schnitt ist der große perinukleäre Hof, welcher am besten im Querschnitt, aber auch im Längsschnitt hervortritt. Um die Blutgefäße ist eine Rarefaktion des Gewebes, wie durch ein Oedem bewirkt, vorhanden. Dies ist gut zu sehen an und nahe bei der Klappenbasis, aber die Klappe selbst ist nicht geschwollen.

Fall 2. Puerperalsepsis. Streptococcus pyogenes durch Blutkultur. Kulturen in kleinen Quantitäten töten Mäuse in 24 Stunden. Injektionen wurden bei Kaninchen ausgeführt, wobei man mit kleinen Dosen begann und sie sodann steigerte, so daß die Tiere bei den acht Injektionen zehn schräge Blutagarkulturen in die Vene erhielten. Ein Tier, welches mit Adrenalin und eins, welches nicht damit behandelt war, wurden verwandt. Trotz der Vorbereitung dieses Adrenalintieres zeigte dasselbe keine Endocarditis, als es getötet wurde, einen Monat, nachdem die Injektionen eingestellt waren, drei Monate, nachdem die erste Dosis gegeben war. Das andere, nicht mit Adrenalin behandelte Tier starb drei Tage nach der achten Injektion. Das Myokard war dunkel rotbraun gesprenkelt und die Papillarmuskeln waren bleich und glanzlos. Nur die Mitralis war affiziert. Sie zeigte an beiden Segeln eine Vegetation von ungefähr 2×3 mm auf beiden Oberflächen der Klappe mit dem sehr schwach verdickten

Gebr. Gotthelft, Kgl. Hofbuchdrucker, Cassel.

Rande am äußersten Ende derselben. Es war eine geringe Einsenkung zwischen ihr und der Klappenbasis vorhanden, aber diese und der Muskel, an welchem die Klappe entspringt, waren an dieser Stelle bleich und anscheinend infiltriert. Es war eine Arthritis im Kniegelenk vorhanden. Nach der Sektion fand sich, daß diesem bleichen Bezirk an der Basis eine polynukleäre Infiltration mit Oedem und reichlicher Einlagerung von Diplo- und Streptokokken entsprach. Die Kokken waren natürlich zahlreich in der Vegetation, aber nicht in dem freien, nur leicht ödematösen Klappenrande neben derselben; tatsächlich wurden nur einige wenige Paare dort gefunden. Sie waren zahlreich um die Gefäße des Myokard nahe der Klappeninsertion. Die Streptokokken wurden aus diesem Herzen isoliert und bei zwei weiteren Kaninchen injiziert, so daß das eine Adrenalindosen in die Vene des anderen Ohres erhielt zu derselben Zeit, als es die Kokken erhielt, das andere nur die Bakterien bekam. Das mit Adrenalin behandelte Tier zeigte wiederum keine Klappenveränderung, während das nicht mit Adrenalin behandelte eine schwache Läsion längs dem Schließungsrand der Mitralklappe zeigte. Leider mußte zu dieser Zeit die Arbeit unterbrochen werden, weil keine virulente Kultur zu erzielen war; daher wurde es für das Beste gehalten, einen Streptococcus von einem rheumatischen Falle her abzuwarten.

Die histologische Untersuchung des Herzens dieser und anderer Kaninchen hat keine Blutgefäße in der Mitralis und den Aortenklappen außerhalb etwa eines Fünftels der Entfernung von der Basis bis zur Spitze gezeigt. Die Injektionen virulenter Streptokokken, die im Stande waren, eine Valvulitis hervorzurufen, haben auch eine lokalisierte Myocarditis verursacht. Diese Myocarditis hat denselben Charakter, wie der Prozeß, der in den Klappen sich abspielt. In allen unsern experimentellen Fällen sind die Läsionen an der Klappenbasis ebenso alt, wie die Läsionen auf der Klappe selbst. Der Rand der Klappe war zweimal affiziert und die Mitte (sowohl die aurikulare als auch die ventrikulare Oberfläche) einmal. Nur die Mitralis war in meinen Fällen affiziert. Jedoch war die Aortenbasis, einmal im Falle 1, beteiligt. Die Anwendung von Adrenalin als eines Mittels, ein Oedem oder eine Myokarddegeneration zu verursachen, begünstigte nicht den Eintritt einer Valvulitis. Dieselbe blieb aus, wenn das Mittel gegeben wurde, aber sie trat ein bei den Kontrolltieren. Vielleicht wurden unzureichende Dosen angewandt, aber ein zu starker Angriff auf das Myocardium sollte vermieden werden. Es dürfte aus den Beobachtungen des Verfs. und denjenigen der neuern amerikanischen und englischen Autoren hervorgehen, daß experimentelle Klappenentzündung irgendwo auf den Klappen, gewöhnlich der Mitralis, und etwas häufiger auf dem Schließungsrande, als sonstwo, vorkommen kann. Warum die Aortenklappen bei Kaninchen weniger oft affiziert werden, ist schwer zu ersehen, da sie stärkeren Einwirkungen des Blutdrucks ausgesetzt sind und die Voraussetzung sauerstoffreichen Blutes erfüllen, welche von Rosenow verlangt wird. Während die Infektion aller Klappen am häufigsten von dem freien Blutstrom her eintreten kann — Verf. ist noch immer vorurteilslos hiergegen — scheint es, daß die Infektion von der Basis aus nicht zu unterschätzen ist.

Es ist nicht unmöglich, daß eine gewisse Form der Myocarditis allen Klappenentzündungen vorangeht. Wenn nur ein Oedem des Myokard an oder nahe bei einer Klappeninsertion vorkam, wurde die Ansiedlung von Bakterien begünstigt. Warum die Zahl der Fälle von Endocarditis bei Pneumonie mit ihrer starken Bakteriämie und dem veränderten Blutdruck, beim Typhus mit seiner starken Bakteriämie und anderen solchen Infektionen im Vergleich zu der Gesamtzahl der Fälle so gering ist, ist auf Grund der Theorie einer Infektion vom

freien Blutstrom aus schwer zu erklären. Möglicherweise ist eine gewisse chemische oder physikalische Veränderung im Blute vorhanden, wie solches vor einiger Zeit von Thorel vermutet wurde.

Gerade als Verf. die vorliegende Arbeit fertiggestellt hatte, gelangte der letzte Artikel von Rosenow in seine Hände. Dieser Experimentator hat Resultate erzielt, die in vielfacher Hinsicht die oben angegebenen bekräftigen. Er glaubt, daß Pneumokokken von geringer oder veränderter Virulenz die Endocarditis verursachen und daß die Läsionen bei Menschen und die experimentell an Kaninchen erzeugten vergleichbar sind. Die Herzklappen besitzen in einer frühen Periode des Lebens Kapillaren, die die Entwicklung von Endocarditis begünstigen. Da die meisten Läsionen in den mittleren Perioden des Lebens akute Prozesse sind, die sich auf chronisch verdickten Klappen entwickelt haben, so sagt Rosenow, daß die Vaskularisation, die dem Narbengewebe eigen ist, embolische Läsionen begünstigt. Daher können denn sowohl in der Jugend als auch im vorgerückten Alter die Erkrankungen entstehen und entstehen aller Wahrscheinlichkeit nach durch die Blutzufuhr und nicht durch Oberflächenansiedlung von Bakterien.

Rosenow zeigt ferner, daß durch Injektion virulenter Kokken eine Klappenhämorrhagie hervorgerufen wird, welche die Ansiedlung von Bakterien begünstigt. In der Klappe sind die Kokken nicht den Angriffen der Leukocyten ausgesetzt, weil dort wenig Blut und wenig farblose Blutzellen vorhanden sind. Er hebt in seinen Schlußsätzen nachdrücklich hervor, was er in seiner vorliegenden und in früheren Arbeiten vermutet hat, und wiederholt es noch einmal, daß, wenn die Kokken sich nicht in Klümpchen an einander schließen, sie nicht an dem unverletzten Endokard haften können. Diese Arbeit geht nicht auf die Veränderungen des Myokard ein, wie ich dieselben in meiner Darstellung hervorgehoben habe, aber sie zeigt wirklich die große Bedeutung der Läsionen der Klappenbasis für die Entstehung der vegetativen Endocarditis. Ich halte es für wahrscheinlich, daß in allen Fällen experimenteller Endocarditis eine Myocarditis zu finden ist. Die Läsionen mögen gering sein, aber jede Infiltration der Basis wird die Klappe ödematös machen. Ich habe keine deutlichen Hämorrhagien bei irgend einem meiner Tiere gefunden.

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A hitherto undescribed Bacterium associated with a cryptogenic Infection.

[From the Wm. Pepper Laboratory of Clinical Medicine University of Pennsylvania.]

By **Herbert Fox,** M. D.

With 1 Plate.

The case here put on record occurred in the private service of Dr. Alfred Stengel, at the Hospital of the University of Pennsylvania, who asked the author to make the bacteriological studies. The nature of all the conditions from which the patient suffered was never cleared up. While at the University Hospital he was diagnosed and treated as having chronic Endocarditis and a cryptogenic infection probably a continuing endocarditis.

H. J. R., 50, M. native American.
Diagnosis: Chronic endocarditis. Result: Unimproved.
Chief complaint: Fever and weakness.

History of present illness: On last January 2nd and 9th the patient had acute pain directly under the ensiform cartilage. accompanied by nausea and vomiting; no blood; after this pain was worse, requiring a hypodermic injection of morphia; he has had similar attacks off and on for the past 5 or 6 years. After 5 days in bed in the second attack, he went back to work, but found he was running a temperature, which has been diagnosed successively, cholecystitis, hepatic fever, starvation fever, infectious endocarditis. 8 or 10 blood counts by two independent examiners showed nothing but low hemoglobin; Wassermann test negative. 4 blood cultures negative. Teeth were examined thoroughly and found negative, culture of sputum negative

Pertinent medical history: At 8 years of age the patient was expected to die of malaria, contracted at Charleston. He was distinctly puny up to 12 years of age, but family history was first rate. In early adult life he abused digestion badly by going 12 to 14 hours without food, and then eating tremendous meals. At 22 years he had an attack of rheumatism, no arthritic involvement, but lost 25 lbs. in 11 days. Seven years ago he had 13 abscesses, in rapid succession, probably a direct infection from a protracted surgical case he was attending. Six years ago he applied for treatment for stubborn indigestion, with nausea which could not be overcome; he has been subject for many years to bilious attacks. Diagnosis at this time was mitral regurgitation, with angina; angina soon after eliminated by an eminent physician. In the 6 years between then and now he has had 20 attacks of acute vomiting, with more or less pain but only 2 required morphia.

The patient has systematically starved himself of proteid food according to his own story, living chiefly upon carbohydrates and some milk, the latter in variable quantities.

Physicial examination May 8, 12: Patient is of medium stature but heavily built with large coarsely made hands and feet, and heavily formed face and head. Neck is short and thick; skin on face and neck coarse and rough; on arms and hands dry stretched and atrophic.

The Lungs are resonant throughout, expiration is everywhere soft, inspiration is somewhat harsh. No rales are heard on coughing: other signs are normal.

The Heart extends from the top of the third rib to the fifth interspace, and $1^1/_2$ in. to the right in the third interspace, and 4 in. to the left in the fifth interspace. A loud rough systolic murmur is heard at the apex, well transmitted to the axilla and back; posteriorly it is best heard at the angle of the scapula. The pulmonary second is accentuated.

The Liver extends from the 3rd interspace to the costal margin, in the right midclavicular line; posteriorly it extends up as far as the 7th dorsal vertebra, the lower margin is indistinctly palpable on inspiration.

Stomach on auscultatory percussion is somewhat dilated, the upper border being at the ensiform, and the lower curvature at the umbilicus.

The Spleen is enlarged to percussion, but is not palpable.

Colon is distended and felt on both sides in the iliac fossae.

Physicial signs did not change during his stay in the Hospital and the patient asserted that the heart percussion outline was the same as several years before. The fever ranged from 99° to 102° F. with a pulse slightly more rapid than would be expected. Respiration continued rapid 24 to 32 short and sometimes labored, particularly before and after an attack of coughing.

Blood Pressure: Left arm; Systolic 115, Diastolic 60.
Left leg; „ 115, „ 65.

May 2, 1912 Urinanalysis: amber; light flocculent sediment; Sp. Gr. 1023; alkaline; a trace of albumen; no sugar. Scope shows no casts, cylindroids or red blood cells; some mucus; some leucocytes; some epithelium; no crystals.

Blood: Haemoglobin 55%; Red blood cells 4200000; White blood cells 7040.

Differential blood count: Polymorphonuclears 73%
Lymphocytes 21%
Large Mononuclear 6%

Feces: Grayish black, alkaline, occult blood negative, no free bile. Microscopically negative.

Eye examination by Dr. de Schweinitz: The eyes grounds are perfectly healthy, the light reactions normal, the pupils react normally for accommodation, no external muscular paralysis.

May 18, 1912. For the last three days some small petechia have been present on the anterior portion of the right leg. Patient states that 5 years ago much larger purpuric spots appeared on the legs.

May 20. Spots are still present on the legs.
„ 21. 8 c. c. human serum injected intragluteally.
„ 23. 10 c. c. „ „ „ in skin of abdomen.
„ 25. 12 c. c. „ „ „ beneath skin of right side of abdomen.

Blood: Haemoglobin 70%; Red blood cells 4800000; White blood cells 6400.

June 5. Patient was discharged today unimproved.

From 5—18 for two and a half months after leaving here the patient received injections of bacterins made from the organism isolated from his blood. He was started upon 50000 and gradually increased at five days intervals until he was taking one billion. He objected to frequent blood taking so that immunity reactions were not tested. There seemed no improvement in his condition that could be attributed to bacterin therapy. The temperature did not rise or decline during bacterin treatment but the patient wrote to the writer that he felt somewhat stronger, 2 months after leaving Hospital. Late in the summer he was operated upon for cholecystitis or gall stones. He died shortly after the operation. The autopsy revealed only old valvular thickening and some form of nephritis. The autopsy was not made at the University of Pennsylvania and all the reports available were unsatisfactory.

Bacteriological Observation at the University Hospital.

Although blood culture upon case had been made upon previous occasions it was tried again because the course of the fever continued high, higher indeed than before, and because a bacteremia seemed the only rational explanation of the condition. Three blood cultures were made during as many weeks. In the first five flasks, only one showed a growth of the bacterium to be described. Thinking this a contamination the second was made. Upon this occasion three of five flasks contained the organism. When drawing blood for agglutination and other tests, some was planted in bouillon and the rods obtained the third time. There seemed no doubt of its presence in the blood. The second blood culture was carefully controlled. One flask was taken to the bedside and opened but not seeded with blood. It remained sterile. Bouillon was drawn up into the syringe before and after the technic. On both occasions this broth was found sterile upon incubation. When plated

out two types of colony presented themselves and for a few generations it was thought that we had two organisms. It was finally proven by direct microscopic examination of the plate that we dealt with a single species. The two types of colonies and the morphology of the bacteria are as follows: If the plates are examined when 24 hours old no growth is visible to the naked eye but under the microscope one sees faint branching bacterial groups in far from regular colony form. There are long rods and threads from which bacillary forms are springing. In 48—72 hours the two types of colony have become clear. One is a rapidly growing flat 1 mm. pale gray, slightly raised colony with wavy branching margins. From these come the branching rods described as feathers, "test tube brushes" further on. The other colony is pale gray smooth, 1 mm. or slightly larger, round flat and with entire edge. From these come the scattered bacilli almost all about the same length and of the diphtheroid type. Metachromatic granules are more common from the first colony. As will be seen the two types probably represent two phases in development, the more compact kind being those growing from a cluster of single organisms while the looser and paler ones come from a long threat or a feather group. Plates from either type will give both kinds of colony. In very much later generations, the smooth flat entire colony is most numerous, and the irregular type seems to be disappearing.

The following is a detailed description of the organism. At the risk of being considered prolix the author will describe the morphology of the bacterium extensively since it is its most interesting and important feature. The most numerous individual bacteria are moderately long, slender, 1.5—3.3 × .67—1.3 micra, slightly spindly rods with pointed or thin rounded ends. There is much granular protoplasm and irregularity of staining. There is strong suggestion of the beaded diphtheria bacillus but never has a typical Klebs-Loeffler form been seen. These rods seem to grow out into thin irregularly and faintly staining straight or wavy threads which may or may not have a clubbed end. I have not determined as yet under what conditions these clubs appear further than that they are more numerous in agar cultures older than 72 hours but may appear richly in gelatin in the 37 deg. incubator in 48 hours. These threads average 7 micra in length and 1.3 micra in width. The largest seen was 35. micra. The clubbed ends stain more deeply than the thread. These are 1.3—1.6 micra wide and of varying length. The simple rods first mentioned and, to a less extent, the threads contain metachromatic granules. In the former they are usually situated one third away from an end. A third appearance noted was a distinct cuneate form of quite constant size, 2. × .67 micra and staining very uniformly. These observations were made upon Loeffler's staining. Dilute carbolfuchsin or simple basic fuchsin stained the bacteria very uniformly. Gram stain is negative. They are not acid fast. Carbol thionin and diluted thionin gave results similar to but not precisely like Loeffler's stain. When examining cultures 12 to 24 hours old one finds the rods singly or arranged radially about a center in which a bit of stain may collect but in which no structure can be seen. In 48 hour growths these radiating growths have largely disappeared and one finds double rows of parallel rods seeming to branch from an axis although no structure can be surely discerned between the rows (see illustration).

Occasionally there is a streak of stain along this axis suggesting a thread but it is not possible to intensify this by any means of staining. The picture is that of a feather or cross section of a test tube brush with the stalk removed. From this featherlike appearance I have given the species name. After the third day these forms separate and grow as individuals, only unsuccessful attempts at new feathers being observed. The thread form then dominates the field. They remain in fresh cultures, from transferred material. Shadow forms are present in old cultures. A few degeneration forms, cocci or short solid rods, may be thus found in all tubes. The threads are so arranged at times that one thinks there is branching. It probably does not occur however; at least I have never been satisfied that it did. No gonidia are seen although quite definite stain clumps may be found in some threads. While there is a suggestion of a sheath about the threads it cannot be stained by capsule or cellulose methods. No flagella could be stained. The organisms are not motile.

Agar slant. 24 hours. Narrow, raised, translucent, irregular, uneven, pale creamy white, glistening, filiform streak; in 48 hours the growth becomes more luxuriant but at no time is the growth as good as that of a colon bacillus.

Blood serum. Barely visible, slightly raised streak, apparently made up of isolated colonies.

Potato. No growth visible; some forms by scraping.

Litmus milk. Very faint acidity if any change, no sediment, no coagulation.

Bouillon. Faint turbidity, beginning at top and rapidly going downward, at first made up of clouds, then fine granules and at 15 days showing a heavy granular sediment (this sediment is chiefly debris and long broken filiments). No indol.

Gelatin. 37 deg. C. Fine turbidity, apparently made up of minute granules, chiefly at surface and spreading slowly down. No liquefaction in 3 weeks.

Litmus sugars. All showed a filiform growth with a pink limited but luxuriant growth on surface, suggesting nail growth. No gas in any. Dextrose, acid; lactose, no change; saccharose, acid; maltose, acid; mannit, acid; glycerin, no change;

Nitrates not reduced to nitrites. No odor on any medium. Optimum temperature 37 deg. C. Preceptible growth at room temperature after 4 days.

Thermal death point. It is killed at 50 deg. C. in 20 minutes; at 60 deg. C. in 1 minute.

Pathogenic powers. When 2 weeks old, 4th generation, 1, c. c. of a thick emulsion from a 48 hour agar culture was injected into two guinea pigs intraperitoneally and a rabbit subcutaneously. These animals showed nothing and lived indefinitely. When the third isolation was in its third generation two rabbits were injected subcutaneously and intravenously, two guinea pigs intraperitoneally and subcutaneously and a rat subcutaneously. One guinea pig died on the third day apparently from injury and failed to show any pathological change. Cultures from site of inoculation and organs remained sterile. The other animals lived on without evidence of illness.

The following tests were made with this organism and the patients blood.

The serum in a dilution of 1—50 completely agglutinated an emulsion from a 24 hour culture in 15 minutes. The same emulsion and serum were tried for bactericidal substances as follows:

1. 2 parts Pat. Serum + 1 part G. P. complement + 1 part suspension bacteria
2. 2 parts Control „ + „ „ + „ „ „
3. Control of suspension of bacteria.

Plated after 5 minutes
1. 2500
2. 1500
3. 2900

Plated after 30 minutes
1. 3400
2. 1300
3. 2800

Plated after 60 minutes
1. 1400
2. 3200
3. 4000

This indicates a slight restaining power on the part of the patients serum, not however manifest until it had acted upon the bacteria for an hour.

Opsonin test, made with equal parts. .01 c. c. of all three substances

No. 1 Bact. Susp. + Pat. Serum + Normal Leucocytes (7.87) } Index 1.7
No. 2 „ „ + Normal „ + „ „ (4.64) }
No. 3 „ „ + Pat. „ + Pat. „ (4.53) } Index 1.6
No. 4 „ „ + Normal „ + „ „ (2.9) }

It is easily seen that the serum index is fairly high. However if we compare No. 1 and No. 3 the behaviour of the patient's serum with his own and normal leucocytes, we find that the patient can activate for his own leucocytes as 7.87 is to 4.53 or .57. Again if we compare No. 2 and No. 4 or the behaviour of normal serum against normal and the patient's white cells, it is evident that the normal serum can activate for the patient's leucocytes and normal ones only as 2.9 is to 4.64 or .57. This indicates that while the opsonins are above 1 the leucocytes are lacking in phagocytic power. This has been shown before to be the case when bacteria in part immunized themselves against the powers of the leucocytes. This adaptation is common in experimental endocarditis using bacteria isolated from human vegetative endocarditis cases (Rosenow, Journ. Inf. Dis. Vol. 6. 1909. p. 245).

The activity of the serum thus being shown in three receptors we naturally expected to find a complement binding body, as was the case.

The antigen was a suspension of bacteria in .9% NaCl solution kept four hours in the ice box and then shaken for half an hour. By preliminary tests it was found that this antigen in quantity of .1 c. c. did not adsorb any appreciable amount of complement from the haemolytic series, as seen in control below. Nevertheless a slight excess of complement was used in all tests.

The organism upon which this report is made is a wholly new one to the writer and indeed to several bacteriologists with whom he has discussed it. While it is undesireable to add new species to an already unwieldly number it seems that this organism should go on record because nothing like it can be found in the literature and it seems to have something to do with the patients infection. Following Lehmann and Neumann's classification it belongs to the genus Mycobacterium. It is similar in many ways to the diphtheria bacillus but the feathery growth immediately takes it from this group. In using Chester's

book as a guide one would put it after the diphtheria group and before Mycobacterium hastilis. It has by no means such pronounced characters as this last. It certainly may be taken as of importance in the infection from which the patient was suffering when one considers the serologic tests. The author proposes the name **Mycobacterium plumosum** (n. sp.).

Control .05 c. c. G. P. complement + 1 c. c. = diluted amboceptor + 1 billion RBc. = CH.

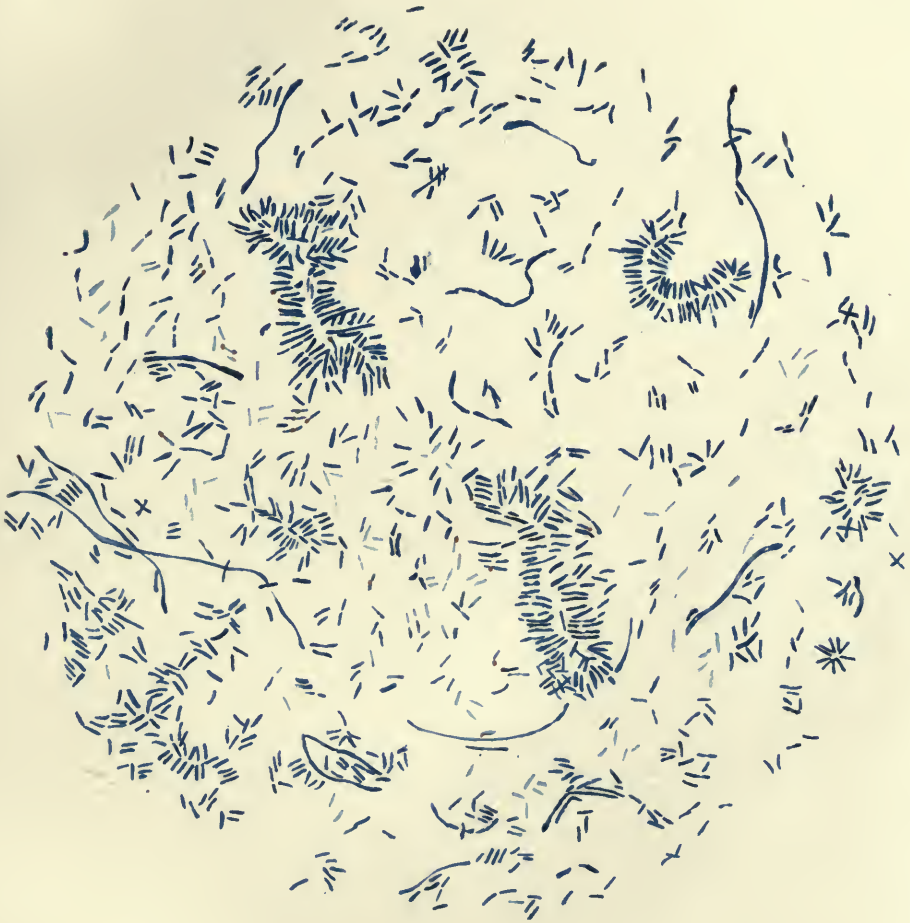
1. .1 cc. ant. + .05 G.P. compl. + .2 pat. serum + 1 cc. amboc. + 1 bill. RBc. = O.
2. .1 cc. „ + .05 G.P. „ + .2 cont. „ + 1 cc. „ + 1 „ RBc. = CH.
3. 0 .05 G.P. „ + .2 pat. „ + 1 cc. „ + 1 „ RBc. = CH.
4. 0 .05 G.P. „ + .2 cont. „ + 1 cc. „ + 1 „ RBc. = CH.
5. .1 cc. ant. + .05 G.P. „ + 0 „ + 1 cc. „ + 1 „ RBc. = CH.

The test of the existence of complement in the patient's own serum was performed as follows:

1. .05 cc. G.P. complement + 1 cc. Hem. amboceptor + 1 billion RBc. = ++++ CH.
2. .1 cc. patient's serum + 1 cc. „ „ + 1 „ RBc. = ++
3. .1 cc. control norm. ser. + 1 cc. „ „ + 1 „ RBb. = +++

There is therefore some complement in the patient's serum.

The same man was the source of the control serum and leucocytes in all these tests.



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THE DIAGNOSIS OF TUBERCULOSIS OF THE KIDNEY.[1]

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It is only within comparatively recent years that tuberculosis of the kidney has become recognized as a distinct pathological entity amenable to treatment offering excellent chances for cure. While formerly looked upon as merely a terminal manifestation of a general tuberculous infection or as a rare disease difficult to diagnosticate, modern methods of examination, together with an accurate knowledge of its mode of infection, pathology, and clinical course, have proved quite the reverse to be true.

Without going into a discussion of the possible avenues of invasion, we can say that it is now a generally accepted fact that the tubercle bacilli reach the kidney by way of the blood stream, and that this infection is primarily unilateral in the great majority of cases. Probably because of the intimate vascular connection a specific infection of the opposite kidney originates more frequently from its sister organ than from any other focus in the body. The course of the disease is progressive, passing from bad to worse, and, according to Israel, there is no authentic case on record which has been cured by other than surgical measures.

While primary tuberculous cystitis must be considered a pathological rarity, the bladder is commonly the seat of disease secondary to a renal infection. In the presence of a tuberculous cystitis, with its changes promoting incomplete evacuation as well as stenosis of the orifice of the sound ureter, an ascending infection of the second kidney, with the ureter as the avenue of entry, is likely to

[1] Presented before the Philadelphia Pathological Society, December 12, 1912.

follow, as the experimental work of both Albarran and Baumgarten has demonstrated. Starting as a single or multiple focus of infection the further progress of the disease may produce changes altering not only the pathological but the clinical picture as well. Excluding miliary tuberculosis, which is merely the local manifestation of a general miliary tuberculosis, four types may be differentiated. 1. Following an eruption of tubercles scattered more or less diffusely throughout the kidney, there may be little or no tendency to caseation, but rather to connective-tissue proliferation, transforming the kidney into a dense, irregular mass, at times impossible to differentiate from a neoplasm. Should areas of softening be present they frequently are surrounded by firm fibrous tissue impregnated with lime salts. The fibrous as well as the fatty 2. capsule participates in this general tissue proliferation. A second type much rarer than the others is characterized by ulceration of the renal papillæ, so-called tuberculous papillitis which was first described by Israel, and due, in his opinion, to the passage of the bacilli through the tubules of the kidney, lodging at the papillæ, where they exert their destructive action. The type most commonly 3. encountered is that presenting one or more cavities at the junction of the cortex and medulla, and not infrequently located at one or the other poles. These cavities vary in size, and may or may not communicate with the pelvis of the kidney. There is generally a chronic interstitial nephritis affecting the remainder of the renal parenchyma. In all these types, especially during the earlier stages of development, the kidney may present no gross enlargement; on the contrary, when extensive interstitial changes with fibrosis are present the kidney may be smaller than normal. It is important to remember that the enlarged kidney may be the healthy one, the increase being due to compensatory hypertrophy incident to the added work which it must assume when the function of the 4. opposite side is seriously impaired. The terminal stage of these various forms is seen in the tuberculous pyonephrosis, with almost or quite complete destruction of the renal parenchyma; a mixed infection may be engrafted on the tuberculous, transforming the kidney into an enormous pus sac. The ureter and bladder likewise participate in the tuberculous process, the infection being secondary to the primary renal focus, and of urogenic origin in most instances. Primary tuberculosis of the bladder is so rarely seen that its demonstration in the female sex means almost invariably that we have to deal with a primary focus in one or both kidneys. Changes in the ureter may be entirely absent, but, as a rule, some evidences of the tuberculous infection are to be found. These may consist merely of discrete tubercles scattered here and there along the mucous membrane, or, in cases of longer standing, there may be extensive thickening of the ureteral walls, with ulceration, connective-tissue proliferation, and contraction, forming one or more

strictures which may completely occlude the ureter, resulting in the closed pyonephrosis. While this is but a cursory review of the pathology of tuberculosis of the kidney it represents the chief manifestations found clinically.

In spite of an enormous amount of literature and discussion on this subject, the diagnosis of renal tuberculosis is seldom made or even suspected by the general practitioner. The obsolete term irritable or nervous bladder remains a favorite expression of ignorance, dividing honors equally with false conceptions concerning the importance of uterine deviations in regard to the production of vesical irritability. An uncomplicated retroflexion seldom if ever produces urinary symptoms, and in the light of our present knowledge, acquired largely through the extensive use of the cystoscope, the term nervous bladder should be stricken from medical nomenclature. As a safe working rule, proven only by the rare exception, every bladder presenting symptoms should be considered the site of some organic lesion, and no effort should be spared to determine the exact nature of this lesion. We do not mean to imply that every irritable bladder is the seat of tuberculosis, but we do claim that only by following this rule can we hope to make the diagnosis sufficiently early to permit the application of proper surgical treatment.

We have reviewed the histories of twenty-five cases of tuberculosis of the kidney which have been admitted to Dr. Clark's service in the University Hospital, and the symptomatology which we shall give represents largely the summary of these cases.

In studying these histories, one is immediately impressed by the rarity with which the symptoms are directly referable to the kidney itself; even in cases of enormous pyonephrosis or complete occlusion of the ureter the most the patient may experience is a dull, indefinite aching sensation in the lumbar region, and even this may be entirely absent. On the other hand, the pain may be so severe as to warrant the diagnosis of calculus; these acute attacks of colic may be due to sudden obstruction of the ureter by a thick plug of pus or may be associated with an extensive perinephritis, and especially periureteritis, upon which is engrafted an acute congestion following exposure to cold or at the time of the menstrual period. This pain is doubtless due to two factors, namely, increased tension upon the thickened, inelastic capsule of the kidney and partial or complete occlusion of the ureter incident to the swelling induced by increased vascularity.

The first and by far the most prominent symptoms of renal tuberculosis during the entire course of the disease are those referable to some abnormality in the function of the bladder. Starting, as a rule, with painless polyuria all degrees of dysuria are met, including the most intense strangury and even complete incontinence. Such vesical disturbances are not necessarily due to an

organic lesion of the bladder itself; even in the earliest stages of renal involvement, with no extension of the process beyond the renal parenchyma, the vesical irritability may be intense. This is difficult to explain, but is supposed to be of reflex origin, due to the intimate nerve connections between the kidney, ureter, and bladder or to the irritating action of toxins eliminated in the urine. In our series of cases there were only two in whom some evidences of bladder involvement were not present, and in both there was increased frequency of urination but no dysuria. As a rule, by the time the patient presents herself for examination the bladder has become involved and the urinary disturbances are in more or less direct proportion to the degree of extension. These symptoms may be decidedly intermittent in their severity, with intervals of comparative comfort between the acute exacerbations. It is doubtless true that many cases reported as improved under tuberculin treatment are merely manifestations of the natural course of the disease.

In addition to these local symptoms are those commonly present in any chronic infection. While in the early stages the general health is but little if any affected, sooner or later indefinite gastro-intestinal symptoms, especially nausea and vomiting, present themselves. There is a progressive loss in weight, and the patient tires easily on slight exertion. Contrary to the opinion so commonly expressed the temperature is normal, or at most shows only a slight evening elevation. Irregular fever, with chills and sweats, are evidences of a mixed infection or a more generally disseminated tuberculous process.

With the exception of the earliest cases, limited to a small abscess in the renal cortex, some degree of pyuria is the rule. The quantity of pus will naturally vary with the extent of involvement of the urinary tract; the greater the bladder involvement the greater the pyuria. In the presence of a mixed infection the urine is often loaded with pus, while in even advanced cases of pure tuberculous infection the pus is found in comparatively small amounts, may be entirely absent, or may be abundant at one examination, with only a moderate amount at the next. These variations are easily explained when we refer to the pathology. In the early cases there is no connection whatever between the tuberculous focus and the pelvis of the kidney. Or in the more advanced case, where are we dealing with a closed pyonephrosis, there is no communication with the bladder, and the pus cannot escape. The rapid change in the quantity of pus is more or less characteristic of renal tuberculosis, and is due either to the rupture of a cortical abscess into the pelvis of the kidney or to some factor promoting better drainage of an abscess which already communicates with the pelvis. While the discussion of the bacterial content of the urine is not in our province, we may be permitted to state

the general working rule that pyuria without demonstrable bacteria, either by smear or culture, strongly suggests the possibility of a tubercular infection. Macroscopic hematuria is rarely seen even in the advanced stages of renal tuberculosis, and when present is usually of vesical rather than renal origin. In one form of the disease—namely, the tuberculous papillitis—profuse hematuria is characteristic. Albuminuria is usually present, but small in amount compared to the degree of renal involvement.

The objective findings must of necessity depend upon the type of disease as well as upon the degree to which the infection has extended to the bladder and ureter. In the early cases, in which the disease is limited to the renal cortex, physical examination will show no abnormalities whatever; usually, however, an enlargement of the kidney is manifest. Under normal conditions the right kidney can be palpated in 75 per cent. of women, consequently disease on this side is more easily demonstrated than when the left kidney is involved. Even a considerable enlargement on the left side may escape detection; this is especially true in those cases in which perinephritic thickening anchors the kidney in its high position. While the kidney may be diminished in size in the sclerotic type of the disease, this is rarely demonstrable clinically; the kidney either escapes detection entirely or a fixed mass can be palpated, consisting not only of kidney but its infiltrated fatty capsule. It must be remembered that a demonstrable enlargement does not necessarily indicate the diseased side, since this increase in size may be due to compensatory hypertrophy of the sound kidney. Tenderness, especially at the costovertebral angle, is rarely absent, and is directly proportionate to the extent of perinephritis. When the disease has extended down the ureter there may be tenderness along its course, but even in those cases with marked thickening and complete stenosis we have never been able to say definitely that the ureter could be palpated by abdominal examination. Thickening of the vaginal portion of the ureter can be readily palpated, and its demonstration may be of importance in determining the side affected. While many authors lay great stress upon this point, and some even go so far as to state that it is pathognomonic of tuberculosis, too great dependence upon its significance will lead one astray, for we have been able to demonstrate ureteral thickening in several cases of pyelocystitis in which tuberculosis could be absolutely ruled out. Its presence is suggestive, but by no means characteristic.

The tuberculin reaction is of doubtful value; we have employed only the subcutaneous injection, and its results were considered significant only in the presence of increased kidney or bladder symptoms.

Lastly and by far the most important factor in the diagnosis is the cystoscope. It is often the only means at our command to

determine the nature of the infection, and only by its use can we estimate the extent of involvement of the urinary tract, which is of the most vital importance in its bearing upon the advisability of surgical intervention. In women the diagnosis of vesical tuberculosis means almost invariably that the primary focus is in one or both kidneys; while in the majority of cases the changes in the bladder are characteristic, we occasionally meet instances of renal involvement, with a normal bladder picture, or, on the contrary, there may be such extensive disease of the bladder that the typical lesions of tuberculosis are masked. In the former the cystoscope is still a valuable aid in that it determines definitely the source of the pyuria; further, in the presence of vesical symptoms, with insufficient evidence in the bladder or its surrounding organs to account for these symptoms, tuberculosis of the kidney is a strong possiblity. While simple cystoscopy may not suffice in the far-advanced changes to warrant a diagnosis of tuberculosis, it at least shows that we are dealing with a severe infection which demands more detailed examinations to determine its exact nature. These include microscopic and bacteriological studies of the urine, ureteral catheterzation, and one or more of the functional kidney tests. In referring to the pathological changes in the bladder we follow the classification of Caspar, designating them as tuberculosis of the bladder and tuberculous cystitis; the former is a distinctly localized process, the latter general, involving not only the mucosa but often the muscularis as well. At the beginning of the disease the bladder as a whole shows little or no change from the normal; the characteristic picture is presented at the ureteral orifice and the mucosa surrounding it. Due to ureteral thickening the orifice is no longer linear but round, with edema, reddening, and superficial, irregular ulceration of its edges and adjacent mucosa. Further ulceration transforms the ureteral ostium into a large, crater-like opening, with rigid, unyielding walls covered with indolent granulation tissue. Contraction of the scar tissue of the ureter produces not only marked irregularity of the orifice, but actual retraction of the entire ureteral region, so that this portion of the bladder assumes a funnel-shape, with the larger opening directed downward. The orifice may also be surrounded by the so-called edema bullosum, or the picture may closely resemble a neoplasm when papillary alterations are present. Miliary tubercles are likewise found, usually situated in the trigone, but may be located in the fundus or sides of the bladder. In our series of cases tubercles were found only twice, which leads us to the conclusion that they are rather unusual manifestations. With further extension of the disease or in the presence of a mixed infection, which is so often present in the advanced cases, a more or less general cystitis ensues, with irregular ulcers and ecchymoses scattered here and there over the universally inflamed mucosa. Because of its long duration the infection

involves not only the mucosa but extends into the musculature, thereby diminishing the size of the bladder until its capacity may be reduced to a few centimeters. Under these circumstances the ureteral orifices may be completely obscured and the typical pathology so masked that a diagnosis of tuberculosis is possible only by animal inoculation.

Having determined the nature of the infection, it is of equal importance to ascertain whether one or both kidneys are involved. In our opinion this can be accomplished only by catheterization of the supposedly sound side with a careful chemical, microscopic, and bacteriological study of the collected urine; a normal ureteral orifice does not prove that the corresponding kidney is not diseased, since the infection may not have extended beyond its original renal focus, nor is a normal functional test sufficient because the disease may not involve enough of the renal parenchyma to seriously interfere with its working capacity. Theoretically, catheterization of the sound ureter is objectionable because of the danger of promoting an ascending infection from an already diseased bladder; practically, however, this objection does not hold when care is exercised in passing the catheter directly into the ureter without touching the mucosa of the bladder and inserting the catheter only a few centimeters rather than into the pelvis of the kidney. The diagnosis is not complete until we have demonstrated not only anatomical but also functional integrity of the opposite kidney; the fact that the patient is free from the symptoms of renal insufficiency is certainly of the greatest value, but this must be further substantiated by the employment of one or more of the functional tests. For this purpose we prefer indigocarmine, not only because of its simplicity and adaptability in even the most extensive forms of bladder infection, but also, and what is even more important, it affords a reliable index of the relative functional capacity of the sound kidney.

When these various examinations have failed to reveal the site of disease, the skiagraph may offer valuable information, as was our experience in one case which baffled the diagnosis by every other means. The cortical abscesses may cast a shadow when a deposit of lime salts is also present, but this is unusual, and therefore of but little practical importance. By means of ureteral catheterization and the injection of collargol the skiagraph may reveal not only strictures of the ureter, but also one or more cortical abscesses in communication with the pelvis of the kidney. In the one case referred to the small abscess was clearly shown and proved at operation.

This *resume* includes the more important clinical factors concerned in the diagnosis of tuberculosis of the kidney. While in many cases the diagnosis is plainly evident from the clinical

findings, in others the nature of the infection is obscure and can be recognized only with the assistance of the laboratory. This phase of the subject will be covered by Dr. Laird.

The diagnosis of renal tuberculosis in the female is practically determined by positive tuberculous findings in guinea-pigs inoculated with the urinary sediment. In the male, however, the close relationship between the urinary and genital tracts renders the differentiation of the two, as the possible source of infection, necessary, and this lies principally in the clinical field. If by the clinical methods already described the kidneys have been proven normal, a thorough examination of the genital tract should be made to determine a possible involvement of the epididymides, vasa deferentia, seminal vesicles, prostate, or testes. As the kidneys are usually the primary foci of infection in the urinary tract, so the epididymides are the primary source of tuberculous infection in the genital tract. Primary tuberculous prostatitis, seminal vesiculitis, and orchitis have been reported, but these conditions were probably due to an indeterminate focus in the epididymis. As such foci may be clinically indeterminable, the secondary involvement of the other genital organs is naturally of importance in the diagnosis. The physical examination may reveal an enlarged, non-tender, non-painful, hard, nodular epididymis; thickening and induration of the vas deferens; a slightly nodular, non-tender, markedly indurated, and fixed prostate or seminal vesicle. The symptoms of tuberculosis of the genital tract are referable to disturbance of the genital function—namely, sexual erethism, discomfort or pain on ejaculation (which may, in acute cases, be slightly bloody), general weakness and nervousness after intercourse, leading finally to neurasthenia, usually sterility and later impotence. Pus may be absent from the urine in incipient cases of tuberculous epididymitis, but is usually present in cases with secondary involvement. The chronicity of the process is an important diagnostic point. The final diagnosis depends upon the finding of the tubercle bacilli in the seminal fluid or prostatic secretion.

Laboratory Method.—Until the last few years the laboratory diagnosis of renal tuberculosis has depended upon the intraperitoneal or subcutaneous method of inoculation of rabbits or guinea-pigs. These methods consume about six weeks time or the time required for general tuberculosis to develop in the inoculated animals. To save this valuable time, Bloch, in 1907, advocated the inguinal method of inoculation, which requires only ten days for a positive diagnosis. Much has already been written upon this subject, but the advantage of the Bloch method as a time-saver over the old method, and the fact that the older, slower method is still quite generally used, especially in this country, were thought sufficient reasons for touching upon it once more in this comparative study.

The technique is as follows: A twenty-four hour specimen of urine is collected from the suspected case in a large sterile bottle, without the addition of a preservative. About 10 c.c. of urine from the lower portion of the specimen are placed into each of two centrifuge tubes and centrifugalized for from two to four hours, dependent upon the speed of the centrifuge, when the supernatant urine may be poured off, leaving the sediment in the bottom of the tubes.

From the sediment in one of the tubes, slide smears are made, which are then fixed, stained and examined microscopically for pus, blood, and bacteria, especially acid-fast bacilli. (Gabbett's method of staining the tubercle bacilli was employed in this work.) Pus is nearly always present in the urine in renal tuberculosis, varying greatly in amount, not only in the various stages of the disease, but also from time to time even in the late stages. This pus has, moreover, often a characteristic appearance both macroscopically and microscopically. The pus in tuberculous urine is grayish and granular, giving the urine when held to the light a ground-glass appearance in contrast to the soft yellowish appearance given by the pus in other conditions. The presence of blood, although occasional in renal tuberculosis, is more indicative of other pathological conditions of the genito-urinary tract. Acid-fast bacilli are nearly always present in the sediment in renal tuberculosis, but are frequently seen in the non-tuberculous conditions. The differentiation of the tubercle bacillus from the other acid-fast organisms, in spite of unceasing efforts at differential staining, is microscopically impossible. Although here as macroscopically the appearance of the pus and the bacilli is sufficiently characteristic to arouse a suspicion which will afterward be proven a surety in a large percentage of cases. In contrast to the more or less discrete leukocytes comprising the pus seen in non-tuberculous genito-urinary affections, there are present large clumps of degenerated leukocytes, about the periphery of which will be found the typical slender, slightly curved, beaded rods, arranged in semiparallel groups, and giving one the impression that these organisms had a distinct part in bringing about the degeneration, whereas the other acid-fast organisms appear to have been accidentally dropped into a field of pus cells. The final diagnosis, therefore, must always depend upon animal inoculation.

For this purpose a suspension of the sediment in the second tube is prepared by shaking with 5 c.c. of sterile water. Two healthy, normal guinea-pigs are inoculated. The inguinal glands of the pigs are first slightly injured and thus rendered more susceptible to the attack of the tubercle bacillus, by pressing and rolling them between the forefinger and thumb for a few moments prior to the inoculation. $2\frac{1}{2}$ c.c. of the prepared suspension, unheated, are then injected into each of the two pigs, subcutaneously, in the inguinal

region directly below the glands. Pressure is again applied for a short time and repeated on the two days following the injection.

Ten days after the inoculation one of the two pigs is chloroformed and the inguinal glands on the injected side removed. These may be either sectioned, stained, and examined for tubercle bacilli, or, more simply and quite as reliably, finely macerated and pressed out between two microscopic slides, and fixed, stained and examined immediately.

In the majority of positive cases the microscopic examination of the inguinal glands results in the discovery of the tubercle bacilli in a few minutes. In some cases, however, in which the tubercle bacilli have been probably few in number or of low virulence the resultant inguinal involvement is so slight that the bacilli may escape detection by a cursory examination, and therefore a thorough search of every portion of the inguinal tissue should be made before a negative diagnosis is given.

In order to control the Bloch method of inoculation the second pig was allowed to live the required six weeks and then examined for general tuberculosis.

STATISTIC TABLE.

	Positive.	Negative.	Doubtful.
Clinical diagnosis	22	29	7
Bloch method	17	40	1
Subcutaneous method	17	40	1

Total, 58 cases. Positive by laboratory methods, 77.3 per cent.

There were 58 cases of suspected renal tuberculosis examined by the combined clinical, Bloch, and subcutaneous laboratory methods. Twenty-two cases were proven, 7 by operation and 15 by subsequent clinical course, to have tuberculosis of the genito-urinary tract; 29 were proven, 3 by operation and 26 by subsequent clinical course, to be non-tuberculous; 7 cases were still clinically doubtful. By the Bloch method of inoculation, 17 cases were positive, 40 negative, and 1 doubtful, due to the premature death of the pig. By the subcutaneous method 17 were positive, 40 negative, and 1 doubtful, due to the same cause. In the clinically proven cases of tuberculosis, therefore, 77.3 per cent were positive by both laboratory methods. The 7 clinically doubful cases gave negative results by both methods. Of the clinically proven negative cases all but 1 gave negative results, and this was positive by both the Bloch and subcutaneous methods. This case was brought to operation on account of the positive laboratory findings; the apparently affected kidney was exposed and split, and showed, macroscopically, an interstitial nephritis and no evidence of tuberculous involvement. Two of the proven positive cases which gave negative laboratory results were closed cases, the ureter of the affected side being obstructed: 1 had advanced bilateral renal

involvement, which shortly caused death; the other 2 were frank cases of unilateral renal tuberculosis. There were 2 clinically positive cases, each giving negative results by each of the two laboratory methods and positive by the other. Another positive case showed numerous tubercle bacilli in the inguinal glands of the pig at the expiration of ten days, and only one small focus of infection in the spleen of the other pig at the end of six weeks.

NOTE.—The Oppenheim method of hepatic inoculation was tried in a few instances resulting in every instance in the premature death of the pigs from septicemia.

CONCULSIONS.—1. The kidney is the primary site of disease in tuberculosis of the female urinary tract; as a rule the infection originates from a focus in some other organ and gains entrance to the kidney by way of the blood stream.

2. The pathology varies greatly in kind as well as in degree, but a definite type usually predominates, altering both the pathological and clinical pictures.

3. Subjective symptoms referable to the kidney disease are by no means characteristic; they are often entirely lacking, may be expressed by a dull, aching sensation in the lumbar region or by attacks of colic resembling calculus.

4. The most prominent symptoms are those referable to deranged bladder function; starting with painless polyuria, all degrees of dysuria are met, including the most intense strangury and even incontinence. These symptoms may be decidedly intermittent in their severity, with intervals of comparative comfort. A cystitis which does not readily yield to the usual appropriate measures should arouse the suspicion of renal tuberculosis.

5. Some degree of pyuria is the rule; hematuria the exception. Intermittent pyuria suggests tuberculosis of the kidney. Pyuria without demonstrable bacteria by smear or culture in a catheterized specimen is likewise suggestive. Albuminuria is usually present, but small in amount compared to the degree of renal involvement

6. In the absence of mixed infection the temperature is normal or shows only a slight evening elevation; irregular fever with chills and sweats is evidence of a mixed infection or a more generally disseminated tuberculous process.

7. The palpatory findings are dependent upon the type and extent of the pathological changes. While enlargement of the diseased kidney is usually manifest, it is important to remember that compensatory hypertrophy of the kidney may be given erroneous conclusions in determining the diseased organ. Thickening of the vaginal portion of the ureter is of value in diagnosis, but by no means characteristic of tuberculous infection.

8. The tuberculin reaction is of doubtful value; the subcutaneous injection should be employed and its results are significant only in the presence of increased kidney or bladder symptoms.

9. By far the most important agent in determining the diagnosis is the cystoscope, which in the majority of cases shows a picture so characteristic that the nature of the infection is at once recognized. Only by its use can we decide the extent of disease as well as the condition of the opposite kidney as regards both its anatomical and functional integrity.

10. The diagnosis of renal tuberculosis should be made in every suspected case by the combined clinical and laboratory examination.

11. The Bloch method of inoculation of guinea-pigs should be used, because it is equal in reliability to the older method, and the diagnosis may be made in at least 77.3 per cent. of cases in ten days compared to six weeks by the subcutaneous or intraperitoneal methods, which should also be used as controls.

12. A positive laboratory result by either method determines the diagnosis of tuberculosis of the genito-urinary tract; of renal tuberculosis in the female, the exact focus in the male to be determined by additional clinical and laboratory means.

13. A single negative laboratory result, regardless of thoroughness of examination, does not determine an absolute negative diagnosis of renal tuberculosis, as the manifestation of this disease is essentially intermittent. Negative results obtained in three successive weekly examinations should, however, bear considerable weight in the diagnosis.

No. 15

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TWO INSTANCES OF CHRONIC FAMILY JAUNDICE.[1]

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In June, 1910, Tileston and Griffin,[2] of New Haven, published a report of four families in which this disease appeared in several members of each family. They presented a thorough review of the bibliography of the subject, the major part of which came from the French, though in both the German and English medical literature the condition was by no means ignored. I will take the liberty of giving a short *resume* of their work before presenting my own cases:

Basing their opinions upon their own cases as well as upon those of foreign authors, they conclude that the following points are characteristic of the disease: That it is a chronic, non-obstructive jaundice, with enlargement of the spleen, occurring usually in hereditary form, or in several members of one family, and dating from birth, or first being noticed during adolescence. The icterus is not intense, there are no signs of obstruction of the bile ducts, and symptoms of cholemia, such as itching, xanthemas, and multiple hemorrhages, are lacking. Enlargement of the spleen is almost a constant feature, while enlargement of the liver is unusual.

"Bilious attacks" are extremely common, especially in youth. Headache, diarrhea, and slight fever are occasionally noted. After a day or two the attack passes off, to recur several times in a year. Gall-stone colic is frequently associated, as is, less often, perisplenitis. No hemorrhages are encountered other than epistaxis, which is not uncommon. The jaundice is pronounced in the conjunctiva and the skin of the body is yellow, while the face is of a peculiar characteristic buff color, varying in intensity from time to time, fatigue and worry tending to increase it.

[1] Read before the Pathological Society of Philadelphia, January 23, 1913.
[2] Amer Jour. Med. Sci., June, 1910.

The urine is highly colored, urobilin is present in the majority of cases, as is also urobilinogen, while bile is almost always absent. Urochrome is constantly increased, which accounts for high coloring.

The stools are well-colored, and urobilin and bile are always present, giving marked reactions to corrosive sublimate and Schlesinger's test. Moller has shown the total urobilin excretion in urine and feces to be considerably increased. Constipation is often present.

The blood-serum always shows the presence of bile-pigment, but not of urobilin. The important feature which gives a clue to the nature of the disease is the decreased resistance of the red blood cells to hemolyzing agents, that is, the increased fragility, which was first discovered by Chauffard. The method for testing for it is as follows: The red blood cells are separated from the plasma, washed, and then placed in a series of test-tubes in which are varying strengths of hypotonic salt solutions, varying from 0.7 per cent. down to 0.36 per cent. NaCl. In normal blood, hemolysis starts at 0.44 per cent., while in this disease it starts almost always at some point higher and not infrequently at 0.6 per cent. or even 0.7 per cent. This marked decrease in resistance is all the more important, because in chronic obstructive jaundice the resistance is normal or increased. Hemolysins have never been found in the blood-serum. A moderate anemia, with red counts between 3,000,000 and 4,000,000, is common. Abnormally high counts have been found. Color-index is usually normal. Average size of red cells is reduced. Reticulation of red cells as shown by the "vital" method of staining is apparently a constant feature. Nucleated red cells and anisocytosis are not rare. Leukocytes are normal.

Pathology. At autopsy liver appears normal, no obstruction of bile ducts, and no cirrhotic changes. Gallstones have been found several times. Spleen enlarged and shows evidences of old perisplenitis. Increase is in pulp, the trabeculæ being, as a rule, not much enlarged. Microscopically there is a marked engorgement, with blood, this being most evident in the pulp. The bone-marrow is found to be in a state of intense reaction.

Etiology. The red cells being more fragile, are readily destroyed, which leads to the anemia and in turn to an increase in free hemoglobin, out of which bile-pigment is made, and hence pleiochromia and icterus. Such a hemolytic icterus has been produced experimentally by Lesné and Ravaut by the injection of hemolytic agents, which is followed by jaundice, increase in size of spleen, and appearance of urobilin, bile, or hemoglobin in urine. Increased destruction of red cells probably takes place in the spleen, and hence its increase in size.

In conclusion, the following cases are presented:

Family No. I.

CASE I.—B. A. R., male, aged nineteen years; student. Came to the hospital complaining of yellow discoloration of skin. For three days the patient had noticed a gradually increasing jaundice; there had been no disturbance of digestion nor indiscretion of diet. Bowels were moderately constipated. There was no itching of the skin nor disturbance of vision or other symptoms due to jaundice, except a slow pulse, 58, which was soft and regular; appetite was fair; no fever.

Previous Medical History. Health was always good. Had had three or four attacks of jaundice a year as long as he could remember, but none so marked as this one. Had rheumatism three years before, otherwise negative.

Family History. Grandfather and father had had frequent attacks of jaundice and pain in the upper right abdomen. Sister had somewhat similar attacks, though milder.

Physical Examination. Slender young man of moderate nutrition. Oral mucous membrane pale; tonsils normal. The skin was of a deep yellow tinge; pigment was uniformly distributed over the body; sclera were deeply stained. Lungs and heart were normal. Liver was not palpable; flatness extended from the fifth interspace to the costal margin. The spleen, though not palpable, was considerably enlarged to percussion.

Blood-pressure: Systolic, 110; diastolic, 60. Urine was negative.

Blood Examinations: Hemoglobin, 65 per cent.; red cells, 300,000,000; leukocytes, 7600.

Feces: Clay-colored at beginning of attack for one day, at other times were brown. Occult blood was negative.

Fragility Test: Increased from 0.44 per cent. normal to 0.6 per cent. saline.

Patient's condition remained stationary for five days, after which the jaundice began to clear up. Temperature reached 100° for one day, and then remained normal to subnormal. Pulse rate, 50 to 60. Ten days later he returned for a blood analysis. There was no increase in fragility of erythrocytes at that time. Blood-serum was still greenish yellow, and showed strong bile bands in the spectrum. General condition was much improved, but still showed some jaundice. Seven months later the blood-count was normal. Coagulation time was eight minutes. Fragility increased to 0.48 per cent. NaCl. Bile in the serum. Three months later he was readmitted to the hospital. A tinge of jaundice had remained over the whole of the body. Just previous to admission he was suddenly seized with cramp-like pain in the pit of the stomach, which doubled him up, but it passed away in five minutes. Examination at that time revealed an enlarged and readily palpable spleen and hepatic dulness extending 4 cm. below the ribs in the parasternal line. Stools were dark in color.

Blood Examination: Hemoglobin, 79 per cent; red cells, 3,820,000; leukocytes, 6900. There was some polychromatophilia, also slight anisocytosis and poikilocytosis; red cells were fragile and many were crenated.

Fragility: Hemolysis started at 0.48 per cent. NaCl and was complete at 0.42 per cent.

Serum: Spectroscope showed bile bands. Hemolytic to blood cells of six cases showing normal resistance, but did not affect cells from seven other cases.

Case II.—N. J. R., father of Case I. Health when presented was good. Had always had a tendency to bilious attacks and headaches. As a boy these would come on after the slighest change in his daily routine, diet, or after any excitement. Attacks began with headache, followed by nausea and vomiting, constipation, and drowsiness for several days. Bowels were constipated, requiring cathartics each day. If they missed a day an attack of headache, etc., would result. After onset of attack, thorough evacuation or vomiting would often bring it to an end. For some years these attacks were followed by mild jaundice. Occasionally, and especially seven or eight years before, he had distinct jaundice. General health not especially affected. Had gained weight in last few years.

Past Medical History. Otherwise negative.

Family History: Father had a tendency to sallowness. Brothers and sisters had no similar trouble. Son had a distinct type of familial trouble. Daughter had slight signs of similar trouble.

Physical Examination. Had an anemic appearance. Eyes were a bit puffy. Slightly sallow complexion. There was no definite jaundice. Liver and spleen were not palpable. Urine and stools were normal. Blood examined for fragility showed normal resistance. Serum was hemolytic to two cases and negative to one.

I did not have an opportunity to examine personally the next two cases:

Case III.—Grandfather of Case I.

Blood Examination: Red blood cells were fragile to 0.54 per cent. Serum was negative.

Case IV.—Sister of Case I.

Blood Examination: Erythrocytes showed normal resistance. Serum showed no bile bands. Hemolytic to two cases.

Family No. II.

Case V.—A. L., male, aged twenty-five years; single. No recent illness. Had diphtheria and measles in childhood. For years had been subject to "bilious attacks," three or four times a year. Was almost always slightly jaundiced, the sclera especially being jaundiced during the "bilious attacks." Had had several attacks of severe pain in gall-bladder region, which he believed to

be due to gall-stones. Attacks were always worse in summer or when he was tired out or worried. Pulse was infrequent, often reaching a rate of 54.

Family History. Grandmother was always jaundiced. Father was jaundiced and had "bilious attacks" of the same type as the patient, which were much more frequent in his earlier years. Next to his oldest sister had attacks each month, unassociated with her menses; she was always moderately jaundiced. Stools were frequently clay-colored; urine was dark. General health was never very good. The next two children were free from jaundice or "bilious attacks." The next three were always slightly jaundiced, and were subject to attacks similar to those of the patient. One had had two severe attacks of gall-stone colic.

Physical Examination. Examination of the patient showed that he had not had an attack for several months except from a slight icteroid hue of skin.

Blood: Hemoglobin, 100 per cent.; red cells, 5,200,000; leukocytes, 4700.

Serum was greenish yellow, and presented strong bile bands in spectroscope. Fragility of erythrocytes started at 0.48 per cent. NaCl. Urine was negative. Stools were dark brown.

A summary of the cases in these families was as follows: The jaundice was in every instance chronic, non-obstructive, and familial in type. In the first family, four members of which had had attacks of varying severity, it may be noted that it extended over three generations, and that the attacks were more frequent and more severe in youth. The spleen was enlarged in each attack, at which times there was a moderate degree of anemia.

Throughout the course of Case I, which we have been able to follow more closely than any of the others, it will be observed that the fragility of the red blood cells ran *pari passu* with the "bilious attacks" and the increase in intensity of jaundice.

Case II did not show any increase in fragility, nor did Case IV, both of which had been free of attacks for some time. Case III showed a lessened resistance of red cells up to 0.54 per cent. NaCl.

The second family presented interesting features, in that three generations were involved and no less than five children in one immediate family were subject to the condition at that time. Owing to the fact that most of the family were living in Canada and New England, I have been unable so far to carry out further studies on the individual cases.

In one point my findings do not confirm those of Tileston and Griffin, as I have found in the serum of three members of this group isohemolysins to be present in ten out of a series of nineteen experiments.

In conclusion, I wish to extend my thanks to Drs. Stengel and Arthur Landry for the opportunity of studying these cases.

No. 16.

A Clinical Study of Vagotonia

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A CLINICAL STUDY OF VAGOTONIA*

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PHILADELPHIA

In 1899 there appeared in the *New York Medical Journal* three admirable articles by Meltzer,[1] in which he deals with the function of inhibition. He speaks of the entire life of the animal as being a delicately adjusted equilibrium between excitation and inhibition and cites instances in which the slightest deviation of the resultant in the nervous mechanism of an organ may lead to the most serious consequences.

A later article[2] by the same author deals with the relation of inhibition to some forms of disease, and though it has paved the way for the more recent work brought forth by Eppinger and Hess[3] in Vienna, only the latter work will be discussed here.

It is my purpose to present a brief summary of their work together with a clinical analysis of a number of cases which I have studied during the last year. At the present time, though I am by no means prepared to support all of their hypotheses, the results are of interest and worthy of further study.

Owing to the difficulty of accurate diagnosis of neuroses, these authors have made a clinical study of such conditions, basing their work chiefly on varying conditions of irritability of the vagus and sympathetic nervous system. They divide the nervous system into the animal and vegetative, the former being represented by all the fibers running to voluntary muscles and sense organs, the latter by fibers supplying smooth muscle organs, as intestines, vessels, ducts of glands, etc.

Recognizing the difficulty in separating either anatomically or physiologically the fibers running to these organs, they attempted and succeeded in making a pharmacological separation, demonstrating the specific action possessed by epinephrin in stimulating the sympathetic system and the selective action of the pilocarpin group, i. e., pilocarpin, atropin, physostigmin and muscarin, for the vagus and vagus extended, or so-called autonomic system. The action of these drugs on the organs

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* From the William Pepper Laboratory of Clinical Medicine. Under the J. Alison Scott Research Fund.

1. Meltzer: New York Med. Jour., May 13, 20, 27, 1899, lxix, 661, 669 and 739.
2. Meltzer: Med. Rec., June 7, 1902, lxi, 881.
3. Eppinger and Hess: Sammlung klinischer Abhandlungen über Pathologie und Therapie der Stoffwechsel und Ernährungsstörungen, 1910.

involved is best demonstrated by Table 1. The accompanying diagram illustrates the autonomic and sympathetic systems anatomically.

Epinephrin being constantly secreted by the adrenals and general chromaffin system, must have a constant stimulating effect on the sympathetic system, and Eppinger and Hess[3] hold that it may be possible that a physiological analogue to epinephrin may exist for the autonomic nervous system. The central nervous system may be the general controller of the antagonistic systems and a disturbance of control, too much or too little irritability, too much or too little nerve tonus of one antagonist may bring about a pathological condition. On this ground they have attempted to clear up the so-called neuroses, studying clinically the irritability of the autonomic system, believing that a fluctuation in tonus or irritability might give an explanation for clinical symptoms.

TABLE 1.—Action of Drugs

Organs	Pilocarpin	Atropin	Epinephrin
Iris sphincter	Stimulates	Paralyzes	.....
Dilator	.....	.....	Stimulates
Ciliary muscle	Stimulates	Paralyzes	.....
Salivary glands	Stimulates	Paralyzes	Stimulates (?)
Sweat glands	Stimulates	Inhibits	Inhibits
Heart muscle	Inhibits	Stimulates	Stimulates
Vasomotor to head	.....	Contracts (?)	Contracts
Esophagus	Excites	Relaxes	Relaxes
Cardia	Excites	Paralyzes	Paralyzes
Stomach	.....	.....	.....
Tonus	Increases	Diminishes	.....
Peristalsis	Increases	Paralyzes	Paralyzes
Secretion	Increases	Diminshes	Diminishes (?)
Pancreatic secretion	Excites	Inhibits	Inhibits
Bronchi	Excites	Inhibits	Inhibits
Gall Bladder	Contracts	Relaxes	Relaxes
Intestinal Musculature	Excites	Paralyzes	Paralyzes

High tonus in one system is accompanied by increased irritability in the other, and the antagonism must be also a pharmacodynamic one; individuals susceptible to epinephrin being only slightly susceptible to pilocarpin and vice versa. The name *"vagotoniker"* they apply to those constitutions which show functional increased tonus and increased susceptibility to pilocarpin, as well as an insusceptibility to sympathetic stimulation. The entire system or but a single branch may be involved. It finds its expression in latent increase of function and gives in this way to specific irritation a better point of attack than where no increase of tonus exists. Such specific irritation may arise from noxa in the form of bacterial toxins, as during or after acute infections, drugs or the products of deranged metabolism, mechanical irritation and so forth.

SYMPTOMS OF IRRITABLE VAGUS IN RELATION TO INDIVIDUAL ORGANS

Eye.—Ciliary muscle cramp, which is increased by pilocarpin and decreased by atropin; accommodation paralysis as observed after severe infection and finally strabismus may all be explained by vagal irritability.

Salivary Glands.—Salivation due to autonomic irritability may occur in nervous people, in *vagotonikers* and tabetic crises.

Skin.—Sweating is typical, and in crises of many infections, when associated with a slow pulse, suggests increase in tonus of heart vagus. Cold hands and feet suggest stimulation of dilators of peripheral vessels by the autonomic poison.

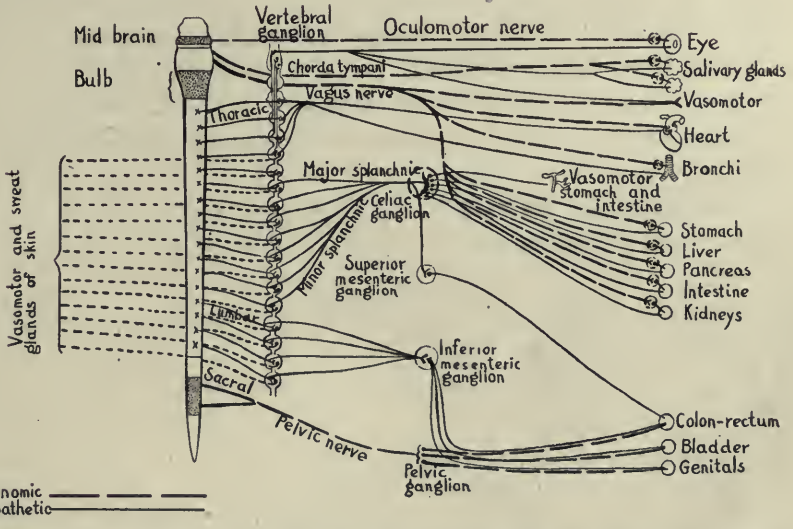

Diagram of the anatomical relations of the autonomic and sympathetic systems (Meyer and Gottlieb, Experimentelle Pharmakologie, 1911, p. 128.)

Heart.—Bradycardia is common in "vagotonikers," especially in the young, and, owing to vagus irritation of toxic or mechanical origin, may be observed in convalescence after acute infections, icterus, brain pressure and many heart poisons.

Nervous block, as described by Hering,[4] is improved by atropin, while some forms of angina pectoris may be attributed to vagal irritability causing narrowing of coronary vessels.

After digitalis the early appearance of bradycardia with gastro-intestinal disturbances is proof of special irritability in autonomic area. The combination of atropin with digitalis in such cases is held to be of great therapeutic value.

4. Quoted by Eppinger and Hess; see Note 3.

Lung and Respiration.—Bronchial asthma, which can be produced by peripheral irritation of vagus in animals, these authors assert is a classical example. Being due to a cramp of bronchial musculature, the air in the lung alveoli is not pressed out, the autonomic stimulation leads to muscle cramp with increase of secretion and further hindrance to air going out. Epinephrin checks secretion and atropin tends to relieve the spasm. Strumpell speaks of narrowing of the glottis in this condition, the recurrent laryngeal nerve being related to the autonomic system. Respiratory arhythmia is common.

Stomach.—The vagus influences the form, peristalsis and secretion, and where there exists a physiologically high tonus there may be a great increase in gastric juice and hyperacidity without complaints. Pylorospasm with increased acidity, spasmodic hourglass constriction, cardiac and esophageal spasm, and finally vomiting accompanied by ptyalism in the early stages of pregnancy may all be due to an irritable vagus.

Intestine.—Peristalsis is increased. There may be either diarrhea or constipation.

Blood-Picture.—Eosinophilia occurs especially where the skin, lung or intestinal element is present, and is increased after pilocarpin, often markedly.

Urine.—Rich in phosphates and oxalic acid, especially where there is high gastric acidity; may be slight dysuria.

The so-called vagotonic disposition is described by Eppinger and Hess as follows:

> Often familial in type, this condition is seen most often in young people of nervous tendency who are subject to cold hands and feet, which are frequently bluish and mottled. They have a tendency to swallow when talking, to flush readily and to sweat readily; skin is moist, there is slight internal strabismus, increased power of accommodation, loss of sensation of touch in throat and larynx. Pulse is naturally slow. Marked respiratory fluctuations are present, due to irregularity in contraction of diaphragm. Reflexes increased, dermographism marked. The first complaint may be of gastric or intestinal disturbance.

COMBINATION OF VAGOTONIA WITH OTHER DISEASES

The presence of an irritable vagus, they say, may materially influence the symptoms in the following conditions:

Gastric ulcer or carcinoma, cholangitis, gall-stones, cholecystitis, stone in the kidney tract, tabetic crisis, hyperthyroidism, the menopause, puberty and menstruation, and skin conditions such as urticaria.

The advantage of atropin to combat anaphylaxis and the probable upset nervous mechanism in certain cases of tuberculosis due to destruction of epinephrin in chromaffin system are at least suggestive of a disturbance of this type.

This question will not be dealt with beyond calling attention to the fact that epinephrin in the blood has an influence on the internal secretions of the pancreas, which in turn controls production of sugar in the liver. When there is too much epinephrin in the blood the internal secretion from the pancreas is inhibited and the liver can produce sugar excessively.

In cases of vagotonia there is a marked increase in tolerance for grape sugar up to 200 or 300 gm., while in cases of sympathetic neurosis there is a corresponding decrease in tolerance.

The test for vagotonia is as follows (Fleischmann):[5] Before the injection of pilocarpin the average pulse and respiratory rates are taken, the blood-pressure estimated and smears made for a differential blood-count. Pilocarpin, ¼ grain, is then given hypodermically and during the hour the general reaction is observed, as sweating, salivation, lacrimation, increase in nasal secretion, fibrillation, flushing, chills and cold extremities. The pulse and respiratory rates are taken every two or three minutes, the blood-pressure estimated at longer intervals and at the end of one hour more smears are made for a differential blood-count.

A strong general reaction accompanied by a marked increase in eosinophils in the blood, an increase in tolerance for grape-sugar up to 200 or 300 grams, and a cardiorespiratory arhythmia may be interpreted as a positive result.

The following cases, having shown symptoms suggestive of an irritable autonomic system, were tested; Table 2 showing the results:

REPORT OF CASES

Case 1.—Diagnosis: cardiac extrasystole. Arhythmia.

Capt. R. S., aged 45, presented himself five years ago complaining of attacks of palpitation of the heart or "flutterings" with missed heart-beats. Pulse in interval is slow and feet always cold. Patient is constipated when he stops using tobacco, which he has used to excess. During the attacks of fluttering which are brought on by slight exertion or mental excitement, he voids a large amount of urine.

Aside from the preceding symptoms he has no complaint. Physical examination and history are negative.

Case 2.—Diagnosis: gastric neurosis.

K. G. F., female, aged 17, complains of regurgitation of food. Three years ago, just preceding onset of menstruation, patient developed regurgitation of food a few minutes after each meal, lasting for about five minutes. This continued for two years, when one year ago the attacks began to persist for about an hour at a time. Patient has a tendency to colds, nasal congestion, and is troubled with much nasal secretion; occasionally vomits freely. She has gaseous eructations toward end of regurgitations.

Examination shows tonsils large, hands and feet always cold and clammy. Roentgen ray shows curvature of spine to left from tenth dorsal to third lumbar

5. The technic of this test is the one used by Dr. P. Fleischmann in the Second Medical Clinic of Krankenhaus Charité, Berlin, to whom I am indebted for it.

TABLE 2.—Test for Vagotonia in Ten Cases*

Before Pilocarpin					After Pilocarpin gr. ¼†									
Case	Pulse	Resp.	Blood Pres.	Eos. Pct.	Pulse	Resp.	Blood Pres.	Eos. Pct.	Saliva c.c.	Sweat	Dext. Lev.	Flush	Fibril	Notes
			142				155		++					
1	22	4	58	0.5	27-21	3-4	70	2.5	120	Profuse	....	++	+	Chill
			115				120		++					
2	19	6	75	3.0	26-24	1-6	73	5.0	260	Profuse	200 D	++	+	Nausea
			135				195				75 L			
3	16	4	75	53.0	23-18	2-5	80	65.5	++	Profuse	200 D	++	+	.....
			122				133		++					
4	23	5	70	1.5	31-24	2-4	40	5.0	124	Profuse	....	++	+	Nausea
			112				130							
5	22	5	90	3.0	25-20	4.7	...	7.0	++	Profuse	75 L	++	+	.....
			154				150							
6	25	6	120	0.0	29-24	4-7	85	14.5	++	Profuse	75 L	++	+	.....
			112				130		++					
7	18	6	40	0.5	28-20	2-6	...	8.5	100	Profuse	....	++	+	.....
									++					
8	16	5	...	....	21-17	3-5	...	3.0	215	Profuse	200 D	++	+	.....
			115				115							
9	18	5	85	0.0	17-16	4-5	78	2.0	+	Moderate	....	+	—	.....
			110				110							
10	20	5	60	0.0	26-20	4-5	78	1.0	++	Moderate	....	+	—	.....

* Cases 9 and 10 showing negative reactions are tabulated as controls.
† In all cases cold extremities followed the pilocarpin; no sugar.

vertebrae. After bismuth an increasing incoordination of peristalsis ending in faintness and vomiting. Fluoroscope showed on three examinations a fluttering at pylorus. Response to treatment good.

Case 3.—Diagnosis: angioneurotic edema.

Mrs. B., aged 52, a brass-worker, since 1904 has been subject to localized edema every three or four weeks without fail. It is boggy, firm and does not pit on pressure; it gives a sense of pressure but no pain. In 1907 menstruation ceased. The attacks, which always occurred between the menstrual periods, have continued to date with almost the same regularity. The edema is localized to arms and face, and sometimes spreads to upper part of chest and lasts from four to eight days. Over thirty attacks have occurred in the University Hospital, sixteen being accompanied by convulsions. Urticarial eruptions frequently accompany the swellings.

Fifty-two blood-counts in 1908 showed an average eosinophilia of 60 per cent., while in 1909 there was an average of 59 per cent. They show an increase during an attack with reduction during the intervals.

Case 4.—Diagnosis: angioneurotic edema.

J. L., schoolgirl, aged 14, last spring, two or three months before menstruation was established, first noticed localized swellings of upper eyelids and at times over brow. The swellings, which are boggy, do not pit on pressure and give no pain, have varied in size, being aggravated by overexertion or mental excitement, and decreased within twelve hours after patient has been in bed. She has had one urticarial wheal on foot. In the past few months she has noticed that her feet and hands are usually cold and feet often wet with sweat; there has been an increasing nasal secretion and marked constipation.

Mother had similar swellings which were especially bad during the menopause, at childbirth and at times when under severe mental anxiety; she has an enlarged thyroid and is of pronounced nervous temperament.

The patient on examination shows slight enlargement of right lobe and isthmus of thyroid, extremities cold, pulse somewhat rapid, waves irregular at times, knee-jerks increased, slight muffling of systolic sound at apex; otherwise no abnormalities detected.

Case 5.—Diagnosis: fractured coccyx. Enlarged thyroid.

J. K., female, occupation, housework, complains of nervousness and enlarged thyroid. Patient has always had the swelling over the thyroid region unaccompanied by any symptoms until nine months ago, when she had a severe fall, landing on buttocks. Diagnosis of fractured coccyx was then made. Since the fall there have developed general nervousness and mental depression. For three weeks patient has daily periods of unconsciousness lasting from fifteen minutes to one half hour, preceded by chills. Cold and sweating of extremities warned her of attack each time. Patient never utters a cry and no convulsive movements have been noted in any seizure. Attacks are apparently vasomotor in origin. Three months ago patient had frequent attacks of vomiting. Patient is constipated.

Mother has similar swelling in neck.

Examination shows ocular symptoms negative, both tonsils enlarged, adenoids and symmetrical swelling of thyroid which moves with larynx in swallowing; reflexes very prompt; alimentary glycosuria is negative.

Transferred to surgical service, section of coccyx removed and patient discharged free from all symptoms except the thyroid enlargement.

Case 6.—Diagnosis: Basedow's disease.

Mrs. S. O., aged 44, housewife, complains of swelling of neck, indigestion and palpitation of the heart. She presents the cardinal symptoms of hyperthyroidism, the onset of which followed an attack of rheumatism five years ago. She also complains of nausea, vomiting, eructations of gas and diarrhea.

Slight exophthalmos, lagging of upper lid and widening of palpebral angle are all present. Thyroid is enlarged and firm. Pulsation and palpable venous

thrill are present. Heart: soft systolic murmur at apex and both basal valve areas. Kidneys palpable. No alimentary glycosuria.

Section of thyroid at operation showed epithelial proliferation excessive.

CASE 7.—Diagnosis: bronchial asthma.

W. D., aged 28, drug clerk, eight years ago, following an attack of influenza, developed wheezing respiration, dyspnea and cough. He has had frequent recurrences since then, lasting from twenty-four hours to three months and typical of bronchial asthma. During attacks he is intensely nervous, voids urine frequently and has hot and cold flushes, and epigastric pain. He is greatly helped by epinephrin. Overeating and excitement precipitate attacks. Patient is constipated. He has had nasal catarrh with hypersection and sneezing for ten years; has had polyps and necrotic turbinate bone removed.

Patient given atropin and epinephrin to control attacks.

CASE 8.—Diagnosis: gastric neuroses.

R. C., aged 18, single, farmer, briefly presented the following condition: Gaseous eructations, pulse-rate 60, cold extremities, gastric hyperacidity, constipation.

Examination shows dermographism, large tonsils, cold and mottled extremities, especially hands.

Of the few cases so far examined in which symptoms suggested the possibility of an increased tonus of the sympathetic system, but one gave a positive reaction.

This test, as carried out by Fleischmann, is as follows: The day before the test 100 gm. of dextrose are given on an empty stomach, and the first five hourly specimens of urine are collected, mixed and polarized. The following day another 100 gm. of dextrose are given, and one-half hour later 10 minims of epinephrin (1:1,000) are injected hypodermatically. Just previous to this injection, blood-smears are made for a differential leukocyte count and the average pulse and respiratory rate and blood-pressure estimated. After epinephrin the blood-pressure, pulse and respiratory rates are taken every two minutes, and the patient is observed for tremor and palpitation. At the end of one hour more smears are made for another differential leukocyte count. The urine is collected and polarized as after the first 100 gm. of dextrose. A positive reaction is obtained when there is a marked increase in lymphocytes at the end of one hour, a rise of at least 15 mg. mercury in blood-pressure, a decrease in sugar tolerance and the development of a tremor and sometimes cardiac palpitation.

The patient above mentioned came to the hospital complaining of bilateral paralysis of wrists, a tremulous voice and localized sweating over abdomen. He showed pigmentation of legs and peripheral olive-green staining of cornea, increased reflexes, some tremor and a reduction in sugar tolerance.

The results of his reaction are as follows: Blood-pressure increased from systolic 120 to systolic 140, lymphocytes from 28 per cent. to 43 per cent.; 9.1 gm. of sugar were recovered, as contrasted with a trace before injection of epinephrin. A markedly increased tremor of hands was noted, but no palpitation.

The presentation of the results of the cases, which includes less than half of the series studied, is made with the view of approaching a little nearer to an accurate diagnosis of those diseases so frequently filed away in the records of even the best-regulated hospitals, as neurasthenia, gastric neurosis, etc.

It must be admitted that hypotheses are abundant, and as has been said, I have not been able to support all of them by the analyses of these few cases; certain of them are, however, confirmed, and this newer method of approaching a clear-cut diagnosis is not only of interest, but is worthy, I believe, of still further investigation and development.

Since starting this work my attention has been attracted to the study of a series of cases presented by Barker[6] in 1912, to an article by Neuhof[7] in the same year in which he discusses reflex vagus phenomena, but does not deal with the question of drug reactions, and to Abrams'[8] text-book on spondylotherapy.

In conclusion I wish to extend my thanks to Dr. Stengel for the opportunity of studying the cases, nearly all of which were on his service in the University Hospital, and to Dr. H. B. Wilmer for case 4.

6. Barker and Sladen: Tr. Assn. Amer. Phys., 1912, xxvii, 471.
7. Neuhof: Am. Jour. Med. Sc., May, 1912, cxliii, 724.
8. Abrams: Spondylotherapy, 1912.

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HYGIENE OF THE OPERATING-ROOM.[1]

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The days of empiricism have been succeeded by scientific regulation and control, and asepsis has succeeded antisepsis. Asepsis must be guarded, guided and completed by a thorough consideration of all hygienic conditions. The operating-room is certainly in as great a need of hygienic regulation as the room of a sufferer from contagious disease. While in a measure hygienic principles are observed in surgical clinics by the euclusion of dirty fingers from sterile objects, this will no more control operative results than simple disinfection of typhoid stools would always eradicate an epidemic.

In a rather thorough search of the literature I find only a few articles dealing with the arrangement and construction of amphitheaters or surgical departments, and a great number about the bacteriology. While this is perhaps the most important of the subdivisions of our subject, it is by no means the only one worth considering. It has undoubtedly revolutionized the operating-room, and we can now make such an apartment, for all practical purposes, non-infective. When we shall have brought the ventilation, heating, surface covering, and the like to the same state of perfection, we shall be ready for any great new advance science has in store.

In the presentation of this subject I shall consider briefly the surgical clinic as a unit for the hospital, the operating-room itself, the personnel, and the methods to be followed in a bacteriological examination of the room, the personnel, and the conduct of an operation.

In this country more than abroad the surgical clinic is for the use of all the surgeons of the staff. While not always practicable it is for obvious reasons better to have separate operating-rooms for each division or service. In either case it should represent a compact separate or separable part of the hospital.

I shall outline an arrangement which seems to facilitate carrying out the hygienic regulations to appear below. The clinic should consist of two parallel rows of rooms ending in the operating-room with which they separately communicate, but the parallel rooms should not inter-communicate. At the side of the operating-room should be the surgeon's dressing-room and the recovery-room. The parallel sets shall consist respectively of the nurse's dressing-room, preparation-room, and sterilizing-room in one row, the reception-room and anesthetizing-room in the other. To the side of the surgeon's dressing-room and receiving rooms may be the hallway for exit from that end of the series. The other end may be entered on the patient's side or the preparation side.

Spectators may enter from a separate entrance on the far side of the rooms, to seats at the extreme far side or corner. These spectators should be so placed that they do not endanger the operation. Glass plates will form a suitable barrier, and can be erected in front of the seat. In a hospital at Naples a gallery runs around the walls, guarded by glass plates extending to the ceiling.

The general construction of operating suites offers little upon which to dwell. The joints of course must be tight, the windows and door frames flush with the wall, and all parts set with some non-porous material, or covered with non-absorbent paint, the latter being preferable as it permits of easy renewal with added advantage of fresh appearance. The rounded corners are quite the accepted thing nowadays. Plumbing should be as simple as possible and workable by the knee. The outer doors and windows should be fitted with grooved metallic window stripping. Double windows permit of temperature regulation

[1]Read before the Clinical Congress of Surgeons, Philadelphia, 1912.

better than single. This window arrangement is nearly steam and formalin proof, but should these methods of disinfection be contemplated it would be well to add waxed canvas buffers to the window and door frames.

In the arrangement of rooms as described above, door-knobs are unnecessary except at corridor entrances. If there are double-swinging doors, no handles are necessary, or at most simple, smooth, right-angular grips. It would seem better, however, to have the communicating doors of the two series of rooms of the sliding variety worked by pneumatic power and operated by a foot crank on the floor or on the wall near the door.

The floor and walls are usually treated with mosaic and marble with paint above the wainscoting. This matter will be referred to again. The material used is of small importance so that it be impermeable and easily cleaned.

The draining of the floor should not be in the middle nor yet toward the preparation-rooms. It should rather be, in the above plan, toward the far end of the suite, under the seats or gallery, and beside it should be an outlet of the steam system, permitting disinfection of drained material.

Lighting from above and to one side is desirable, but the former is usually sufficient. Where possible, the skylight should run down to the north side. The lighting should never cause any focusing of rays upon the table. Reflected light may be arranged at will. The surface covering of the operating-room is nearly always white. I do not know why this is other than to supply a surface which does not absorb light, but I am not sure this color is desirable, as the constant strain upon the eyes of the operator cannot be beneficial. A neutral color, say of pale gray or brown, would absorb very little light.

This brings us to a consideration of what seems to me next in importance to asepsis —that is, heat and ventilation. As I understand the requirements of operating-rooms in this regard, they should have a constant slow supply of fresh air at about 75° F. and about 75 to 80 per cent relative humidity. There should at no time be any draft, but the temperature and humidity should be uniform throughout the operating unit. The influence of this will be felt by patient, surgeon, and nurse. In the first place, if the patient remain under identical atmospheric conditions from the time he enters the anesthetizing room until he shall have recovered completely from the ether, no sudden readjustment on the part of his circulation will be necessary. For this reason I have included the recovery-room at the side of the operating-room. Moreover, this uniformity would be appreciated by those who are now going from the cool dressing-room, to the warm operating-room, to the hot sterilizing-room.

The arrangement of this scheme is one of engineering. In the first place a definite course of air-flow must be established. It should flow away from the operating-room of course. In order not to interfere with this in the course of the row of rooms the steam must be forcibly conducted away from the sterilizing-room, and it can be utilized by carrying it to heat the entering air. The air should enter the operating-room near the top, preferably by electric fans; it should be passed into a chamber heated by steam from the general system, perhaps augmented as above, and filtered through wetted cotton-wool. Its admission to the room is to be guarded by some device which will spread it and avoid drafts. In order to establish the air-flow, exhaust fans can be installed at the end of a room series. Engineers can discover from the cubic contents of rooms the proper size of the inlets and fans. Notter in his hygiene indicates that at 75° F. with a rate of 2 to 3 feet per second and 80 per cent of saturation draft would not be felt. It seems probable that this degree of humidity would not be uncomfortable to surgeons and would not permit of rapid heat radiation. A higher degree of humidity would, however, be advantageous, for it would cause a slight precipitation on walls and

floors, obviating dust and delaying drying of blood and pus. Exhaust tubes near the floor are also desirable. If the above system cannot be used large inlets may be put in any convenient place if the air be warmed. If cold, Notter says air must be admitted 9 or 10 feet from the floor and directed to the ceiling, where the outlets should also be placed, but not so as to draw fresh air directly to them. If the air has been warmed outlets may be anywhere. The subject of protection of the operating suite by differing pressures may be found by consulting Ochsner's Hospital Construction, wherein he describes how the air may be drawn away from clinic departments by lowering pressures in other parts of the building. The position of the operating suite is better at the top of the building because of purer air. The practice of putting a ventilator over the operating area is only defensible when it is well protected.

The Hygiene of Conducting the Rooms. —In approaching the subject of bacteriology of the operating-room we naturally ask ourselves first how the operating-room should be made clean; secondly, how the operating-room is made dirty. I beg leave to take these up in reverse order, for the reason that material I have to present concerns a control of cleanliness. The operating-room may be made dirty chiefly by dirty feet, dirty hands, and the bacteria coming from the nasopharynx of people therein. No one untrained in surgical asepsis should enter the operating-rooms during operations or preparation for them. The surgeon should acquaint himself with the qualifications of each attendant. There should be a routine examination of the nurses and orderlies for the presence of angina, infected wounds, and the like. I think it advisable to consider the room infected at all times and to observe the methods for cleaning to appear later. Clinical and experimental evidence points to faulty technique and careless surgery as the most prolific sources of infection. A case of the former is shown in which the surgeon did a plastic operation upon the face. Washings from the inside of his gloves remained sterile. A student who merely held instruments and sponges gave a culture of *M. albus* and *M. aureus* in the washings from the inside of his gloves.

For the bacteriological control of the operating-room it is advisable to examine rigidly the solutions, tap-water, brushes, dressings, gloves, powder, and sterilizing apparatus. To this may be added culturing the hands of surgeons, assistants, and nurses. The walls, floors, and air may be tested after pus cases or when infection has occurred without good cause. The gloves if new and whole are impermeable, as shown in work done by E. A. Schumann and myself some years ago. We have lately investigated the inside of rubber gloves as they come from operations. The inner surface has been innocent of pathogenic bacteria except in the case cited above. About half of the gloves showed *B. mycoides* or *B. subtilis*. These organisms were also found in the powder used for drawing on the gloves. The autoclave was found to be able to kill *B. coli* and *B. subtilis* spores when these organisms were exposed in glass tubes sealed after withdrawing as much air as possible. All cultures in the operating-room control should be made aerobically and anaerobically.

The tap-water in this clinic contained 830 bacteria per cubic centimeter; no colon bacilli, but a few of the fermenting aerogenes group. These organisms while not highly pathogenic belong to a group sometimes responsible for human infections. You will notice no colon bacilli were found. Nevertheless the presence of a fermenting organism and 830 bacteria per cubic centimeter in tap-water indicates that it should not be used for hand-scrubbing before operation. Therefore sterile water is indicated for the mechanical cleansing of the hands.

If a surgeon finds his hands infected after a pus case he should refrain from operating for some days or permit his skin to be artificially sweated out in a dry-air chamber. The emptying of the sweat glands and loosening of the sebaceous se-

cretions materially reduce the number of organisms on the skin and render cleansing easier.

The teaching surgeon must wear a face mask. My blood agar plates show from Dr. Martin's mask after operating and teaching about one hour streptococci, pneumococci, diphtheria bacilli, *M. catarrhalis, Sp. linguale,* and sarcina, while the mask of his assistant, who talked very little, worn about two hours, gave streptococci, *M. catarrhalis* and *Sp. linguale.*

The question of hand and operation site disinfection I will not here discuss, further than to say that in cases of exposure cultures should be made both aerobically and anaerobically.

The subject of bacteria in the air has been frequently discussed. There is no doubt that pathogenic bacteria may be found in the air. That the air of an operating-room should be free of them goes without saying. The number of bacteria of all kinds in the air of a room is in direct relation to the dryness of the atmosphere and surfaces and the activity of the persons therein. In this clinic during a ward class there were four bacteria per liter, while in the private operating-room during a quiet operation there were two per liter. The former number I might add is about what one finds on a quiet, sunny day in the country.

I wish here to mention a method I employed in the air examinations. The filter was of the Frankland type, modified by the addition of a small quantity of gelatin to a filter of alternate layers of mineral wool and powdered glass. The gelatin makes the filter very tight, air passing through quite slowly, about 20 liters an hour. From the air in this clinic I obtained *B. mycoides,* a white coccus indistinguishable biologically from *M. pyogenes albus,* yet non-pathogenic, but liquefying gelatin too rapidly to be called the *M. epidermis albus.* In the air from the quiet operating-room only *B. mycoides,* a mold, and a few colored air colonies were cultivated.

The subject of bacteria in quiet and moving air was well discussed by Hunter Robb (*Am. Jour. Obstet.,* 1909). A most beautiful demonstration of the effect of simple cleaning is to be found in his paper. The walls of his operating-room were thoroughly cleansed with soap and water. Four days later he obtained 20 colonies in 710 minutes, and three weeks later practically none grew on plates exposed for the same length of time. The room had been unused. Under operating conditions before cleaning he had found as many as 1700. He finds that washing the floor with an antiseptic solution much reduces the number of bacteria in the air. He does not find that the electric fan running in the room has much effect upon the numbers falling upon his plates, and concludes that the fans should be without danger. He says that in a quiet room, Sunday, with the fan going, practically no bacteria fell upon the plates. His worn shows definitely the effect of settling. This admirable paper should be read by all interested in the subject. His method of measuring the air bacteria may be open to some objections, but the effect of moving air upon bacterial content is beautifully expressed.

The organisms that have been found in the air in clinics and throughout hospitals in order of their pathogenicity are: streptococcus, staphylococcus, colon bacillus, *B. pyocyaneus, Bact. diphtheriæ, B. mucosus aerogenes, B. aerogenes capsulatus,* and a few others of less importance. This of course does not take into consideration air organisms. These organisms are brought in chiefly by the feet and in the throat. This indicates that the mere wearing of a white gown by a spectator is useless and that the face mask is imperative for the onlooker as well as for the surgeon. The propulsion of the bacteria from the speaking mouth was shown several years ago by Flügge, who obtained on plates exposed several minutes before a speaking person cultures of *B. prodigiosus,* which organism had been introduced into the mouth artificially. The introduction of dirt on the feet seems to be a very important factor, when we consider

that Robb found a diminution in air bacteria when the floors were cleaned with an antiseptic solution. The wearing of street shoes into operating-rooms should therefore be prohibited. Shoes, boots, or gum slippers kept in a disinfectant solution should be supplied.

Disinfection.—A word first concerning infected objects in the operating-room. They are usually of such a nature that bacteria will continue to grow. If surfaces of pus gauze or pus drops dry, bacteria may escape. Such materials should never be put upon the surgical tables, nor should pathological specimens be opened in the operating-room. A bucket of disinfectant solution should be kept for the receipt of all infected objects discarded from the operating area, and an attendant should remove with a mop, wetted with a disinfectant, all infective material dropped upon the floor.

My reason for suggesting the observation of spore-bearing air organisms and the anaerobic culturing of all objects likely to affect the operation site is that while they may not be themselves pathogenic they may settle upon a dried serous surface, adhere, and by a non-specific reaction on the part of the body irritate the part sufficiently to make it more permeable for pathogenic organisms either from within or without. For this reason non-pathogenic organisms should be excluded if possible.

By inference from Robb's work it would seem advisable to wash the walls and floor after each operation. The cleansing of all surfaces should be done with an effective soap or powder like naphthalene or gold dust and followed by hot water. A hot-water flush hose is desirable. For sterilizing the walls and floor a solution of 1:1000 bichloride of mercury or 1:20 carbolic acid with 5 per cent glycerin is the best. The glycerin is used to hold the carbolic in solution, soften any organic matter on which the germicide may fall, and to protect the carbolic when the water shall have evaporated from the surface to be disinfected.

In especially important instances disinfection by formalin spray or the generation of formaldehyde gas by the following formula should be done: Potassium permanganate 17 oz., formaldehyde solution 20 oz., per 1000 cubic feet. Objects in the operating-room do not readily hold formaldehyde odor, but should it remain the spraying of a little ammonia water about will rapidly remove it.

For hardware of any kind that will endure direct water sterilization, 100° C. for twenty minutes should be employed. Dressings and the like require steam at 100° C. + with 10 pounds pressure for fifteen minutes.

Conclusions.—In drawing practical conclusions from the foregoing in the order first mentioned, I would say that all surfaces of the operating-room should be made clean each day by thorough cleansing with water and soap or flushing by a pipe attached to the boiler. After pus cases it should receive treatment of carbolic or bichloride, or under serious conditions by formaldehyde gas. It should be protected by the exclusion of any hospital attaché having angina, infected wounds, and the like; by prohibiting the wearing of street shoes; by the wearing by all persons therein of a gown and mask covering all but the eyes; and by the bacteriological examination of all materials used in the room, of the air and surfaces when infections have occurred without good cause.

No. 18

THE GONOCOCCUS COMPLEMENT-FIXATION TEST AND ANALYSIS OF RESULTS FROM ITS USE*

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In recognition of the recent admirable work by Schwartz and McNeil on the complement-fixation test in gonococcic infections, the fact must not be overlooked that Müller and Oppenheim, in 1906, were the first to apply this reaction to a gonorrheal affection and consequently are entitled to the distinction of being termed the originators. The present popularity of this test has been the outgrowth of the suggestion made by Schwartz and McNeil — namely, that of the employment of a polyvalent antigen. As a result of their labors, these workers contend, and seem to have proved conclusively, through animal experimentation: (1) "that the different strains of the gonococcus differ markedly one from another — so much so that the antibodies produced in the body by the toxin of one strain will in many instances not bind the complement in the presence of an antigen prepared from another strain. Therefore, if only one strain is used in the preparation of the antigen, a great many negative results would be obtained in positive cases; (2) an antigen prepared from many strains fixes the complement whenever one of its component strains does so, and consequently the necessity of testing a serum against a number of antigens separately is avoided. It is not to be denied that there probably are other strains of gonococci differing widely from any present in the polyvalent antigen, so that at times a negative result will be obtained in a positive case."

While we recognize the fact that a negative reaction may mean nothing, in fact, may be erroneously contradictory, the significance, on the other hand, of a positive reaction has been so great — more specific, in fact, than when the lipotropic antigen, commonly employed in the performance of the Wassermann reaction, is utilized — that we have applied the test for the past few months in a large series of diverse cases with the most gratifying results. Without entering on any discussion of the theories governing fixation or deviation of complement and hemolysis, it is our purpose, at this time, merely to outline our own technic, desig-

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nating certain points of superiority which it may possibly possess over that employed by Schwartz and McNeil, and to submit the analysis and results of cases in which serums were subjected to the gonococcus complement-fixation test.

DISCUSSION OF TECHNIC

The technic utilized and advocated by Schwartz and McNeil in the performance of the complement-fixation test for gonococcic infections embodies what may be termed the minimal quantity method. That is, the smallest quantities of the component substances entering into the reaction are employed that are compatible with practicability. Other than as a matter of economy and questionable conservation of time, this method possesses no recommendation. In fact, from a comparative study of the results in a series of cases in which Schwartz and McNeil's method is contrasted with the technic analogous to the Wassermann reaction as employed by us, it has appeared that the advantage has always rested with the latter or larger quantity method, in that the hemolytic readings seemed to be easier of interpretation.[1] Moreover, we have been impressed by the fact that the quantity of antigen employed in the former case, as will be seen presently, is too great — being indeed as much as, and more than, that utilized by us with five to ten times the gross quantity of material.

Schwartz's latest method and the technic which we practiced for a time in comparison with our own gonococcus complement-fixation test is as follows, so far as the quantities of the ingredients participating in the reaction are concerned: (1) patients' serum, 0.02 c.c.; (2) salt solution, sufficient to equalize the volume in each tube; (3) antigen (routinely 0.3 c.c. in one tube and 0.15 c.c. in another tube, of a commercial preparation diluted 1 to 10). These quantities are determined by preliminary standardization with a fresh known negative and a known positive serum; the positive showing the true antigenic dose, and the negative the highest quantity of antigen which will allow complete hemolysis; in the actual test this large quantity of antigen is placed in one tube and one-half the quantity in a second tube; (4) complement 0.1 c.c. of a dilution 1 to 10; (5) amboceptor, 0.1 c.c. representing twice the lowest quantity that will completely hemolyse 0.1 c.c. of the cell suspension with 0.1 c.c. of complement in one hour; (6) sheep's red blood-cells (5 per cent. suspension), 0.1 c.c. Incubation is made for one-half hour at 37 C.

1. If it be necessary or desirable to practice economy in the performance of the test, we would suggest, in preference to the very small quantities employed by Schwartz and McNeil, which are more or less tedious to manipulate, just half of the component materials as employed in our technic. We have resorted to this modification on several occasions with results just as satisfactory as those obtained by the regular method.

in a water-bath or for one hour in dry heat, before and after the addition of the hemolytic system.

The technic on which we have learned to place the greatest reliance is essentially that utilized by us in the performance of the Wassermann reaction, merely substituting the gonococcus specific antigen for the syphilitic lipotropic antigen, employing always the carefully standardized single complement unit and the routine standardization of antigen and amboceptor. Briefly, the steps in the daily procedure are as follows:

AUTHORS' TECHNIC

As previously stated, a detailed description of the fundamental serological principles involved in the complement-fixation or deviation reaction, will be omitted from this discussion. We shall submit, therefore, merely a table representing the routine procedure in the performance of the gonococcus complement-fixation test (Table 1).

We believe that the features of importance in the proper execution of this test are: the required inactivations, the exact standardization of the hemolytic amboceptor and suspension of sheep's red blood-corpuscles, and paramountly, the complement and antigen each time the reaction is undertaken.

ANTIGENS

The preparation of the various ingredients entering into the reaction will receive no consideration at this time—with one exception, namely, the antigen. This, however, is of such importance with respect to the gonococcus complement-fixation test that it must receive some discussion, since on the integrity and specificity of this antigen depends the whole value of the test in gonorrheal infections. The necessity of a polyvalent antigen is indisputable, presumably owing to the diversity of the strains of the gonococcus. The only question of importance is, how may this antigen be prepared to the best advantage? Schwartz and McNeil in their latest communication say that the "various strains of gonococci are grown on salt-free veal-agar, neutral in reaction to phenolphthalein; 24-hour old cultures are washed off the agar-slants with distilled water and the resulting suspension is heated for two hours in a water-bath at 56 C. It is then centrifuged and passed through a Berkefeld filter. No salt is added to this antigen until it is desired to use it, when it is made up to 0.9 per cent. strength by adding one part of 9 per cent. saline solution to nine parts of antigen. Following Schwartz and McNeil's instructions to the letter, we have prepared monovalent, trivalent and hexavalent antigens[2] and have employed them comparatively in a large series of cases

2. In the near future we shall report the results of our studies of various gonococcal antigens, variously extracted; also the results comparatively of non-specific antigens prepared from certain Gram-negative and Gram-positive diplococci, as the *Micrococcus catarrhalis*, the meningococcus, the pneumococcus, the *Streptococcus pyogenes*, etc.

with the result that the hexavalent preparation gave the highest percentage of positive results and in every way appeared to be the most reliable of the three antigens. Even with the hexavalent antigen,[3] prepared as described above, we have been forced, in comparative studies, to the conclusion that it has not been so useful or reliable as when prepared in the following manner: Forty-eight-hour old cultures of the same six strains of gonococci, grown on blood-agar, were washed off in sterile distilled water; shaken for one hour; and autolyzed for twenty-four hours in a

TABLE 1—

	No. Test Tube	Antigen (Dilution 1:10) c.c.	NaCl Solution (0.85%) c.c.	Patient's Serum (Inactivated) c.c.	Known Positive Serum (Inactivated) c.c.	Known Negative Serum (Inactivated) c.c.	Complement (Dilution 1:10) c.c.		Hemolytic Amboceptor (Antisheep) (Titre = 1:2,000) (Dilution 1:1,000) c.c.	Red Blood Corpuscles (Sheep's 5% Washed Suspension) c.c.
Tests for complement standardization.....	1	0.2 ?	1.3	...	...	...	0.2	Incubation at 37 C. for one hour.	1.0	1.0
	2	0.2 ?	1.2	...	...	...	0.3		1.0	1.0
	3	0.2 ?	1.1	...	...	...	0.4		1.0	1.0
	4	0.2 ?	1.0	...	...	...	0.5		1.0	1.0
Tests for antigen standardization and controls	5	0.05	1.5	...	0.1	...	0.4 ?		1.0	1.0
	6	0.1	1.4	...	0.1	...	0.4 ?		1.0	1.0
	7	0.2	1.3	...	0.1	...	0.4 ?		1.0	1.0
	8	0.3	1.2	...	0.1	...	0.4 ?		1.0	1.0
	9	0.2	1.3	...	...	0.1	0.4 ?		1.0	1.0
	10	0.4	1.1	...	...	0.1	0.4 ?		1.0	1.0
	11	0.6	0.9	...	...	0.1	0.4 ?		1.0	1.0
Tests and controls for the suspected serum	12	0.2	1.3	0.1	...	...	0.4		1.0	1.0
	13	0.2	0.9	0.1	...	...	0.8			
	14	0.2	1.3	0.1	...	...	0.4		1.0	1.0
	15	...	1.5	0.1	...	...	0.4		1.0	1.0
									1.0	1.0

thermostat at the temperature of 37 C. and heated in a water-bath at 60 C. for one-half hour. Before use, this antigen is diluted one to ten by the addition of 0.85 per cent. salt solution. In spite of our efforts[2] thus far, we have been unable to produce an antigen showing results quite as clean-cut, although they have been just as constant, as that marketed by

3. We are indebted to Dr. A. P. Hitchens of H. K. Mulford Co. for the six strains of gonococci employed in this work. It should be noted in this connection that it is assumed that the cultures are true gonococci, since their staining and morphological characteristics are identical with that micro-organism, although they grow readily on blood-serum or even on plain agar-agar. The dispute has arisen as to whether a Gram-negative diplococcus, growing in this wise, should or should not be designated as the *Micrococcus catarrhalis.*

Parke, Davis & Co. The probable explanation is that their antigen is made up from twelve strains of the gonococcus, while ours contains but six.[3]

ANALYSIS OF CASES TESTED

The result of our work has been little more than a corroboration of the reports that have emanated from Schwartz and McNeil[4] and those who have confirmed their results. We believe, however, that, by utilizing the technic herein described, we have added to the accuracy of the test

TEST REACTIONS

	Results (Immediately or Morning After Refrigeration)	Objects of the Reactions.
Incubation at 37 C. for one and one-half hours.	Incomplete hemolysis.....	To determine quantity of complement to be used in test proper.
	Incomplete hemolysis.....	To determine quantity of complement to be used in test proper.
	Complete hemolysis.....	To determine quantity of complement to be used in test proper.
	Complete hemolysis.....	To determine quantity of complement to be used in test proper.
	Incomplete hemolysis.....	To determine quantity of antigen to be used in test proper.
	Partial hemolysis.....	To determine quantity of antigen to be used in test proper.
	No hemolysis.....	To determine quantity of antigen to be used in test proper.
	No hemolysis.....	To determine quantity of antigen to be used in test proper.
	Complete hemolysis.....	To prove that the antigenic dose is not in itself anticomplementary.
	Complete hemolysis.....	To prove that twice the antigenic dose is not in itself anticomplementary.
	Incomplete hemolysis.....	To prove that thrice the antigenic dose is not in itself completely anti-complementary.
	No hemolysis..... (Positive reaction hemolysis =1 unit)	To determine quantitatively the degree of complement fixation.
	No hemolysis..... (Positive reaction hemolysis =2 units)	To determine quantitatively the degree of complement fixation.
	Complete hemolysis..... (Complete negative reaction)	Shows that there was no immune body present in the patient's serum with the aid of the antigen to fix the complement.
	Complete hemolysis.....	Proves that the immune body itself will not fix complement.

as applied by them and have thereby improved the findings to the credit of the test and its value in clinical diagnosis. To the increased positive results we attribute the accurate standardization of antigen on each occasion and the employment of a standardized single complement unit. For the sake of convenience, our results with respect to the serums tested, numbering in all 204, have been tabulated (Table 2).

4. Schwartz and McNeil: "The Complement-Fixation Test in the Diagnosis of Gonococcic Infections," Am. Jour. Med. Sc., May, 1911; "The Complement-Fixation Test in the Differential Diagnosis of Acute and Chronic Gonococcic Arthritis," ibid., September, 1912; "Further Experiences with the Complement-Fixation Test in the Diagnosis of Gonococcus Infections of the Genito-Urinary Tract in the Male and Female," ibid., December, 1912.

TABLE 2.—RESULTS WITH RESPECT TO SERUMS TESTED
KNOWN NON-GONORRHEAL AFFECTIONS

No.	Remarks	Result of Gonococcus Complement-Fixation Reaction. Antigens				
		Monovalent (Unfiltered)	Trivalent (Filtered)	Parke, Davis & Co. (Schwartz Technic.)	Hexavalent Unfiltered	Parke, Davis & Co.
1	Typhoid fever	.....	.....	.....	Negative	Negative
2	Typhoid fever	.....	.....	.....	Negative	Negative
3	Typhoid fever	.....	.....	.....	Negative	Negative
4	Tuberculous otitis media and pulmonitis	.....	.....	.....	Negative	Negative
5	Tuberculous otitis media and pulmonitis	.....	.....	.....	.....	Negative
6	Pulmonary tuberculosis	.....	.....	.....	Negative	Negative
7	Diabetes mellitus	.....	.....	.....	Negative	Negative
8	Diabetes mellitus	.....	.....	.....	Negative	Negative
9	Diabetes mellitus	.....	.....	.....	Negative	Negative
10	Acute streptococcic endocarditis	.....	.....	.....	Negative	Negative
11	Chorea; secondary endocarditis	.....	.....	.....	Negative	Negative
12	Malaria	.....	.....	.....	Negative	Negative
13	Syphilis	.....	.....	.....	.....	Negative
14	Congenital syphilis	.....	.....	.....	.....	Negative
15	Syphilis	.....	.....	.....	.....	Negative
16	Luetic rhinitis	.....	.....	.....	.....	Negative
17	Syphilis	.....	.....	.....	.....	Negative
18	Syphilis	.....	.....	.....	Negative	Negative
19	Normal, no symptoms of gonorrhea	.....	.....	.....	.....	Negative
20	Normal, no symptoms of gonorrhea	.....	.....	.....	.....	Negative
21	Normal, no symptoms of gonorrhea	Negative	Negative	Negative	.....	Negative
22	Tuberculous osteitis and pulmonitis	.....	.....	.....	.....	Negative
23	Chronic hypertrophic arthritis	.....	.....	.....	Negative	Negative
24	Subacute arthritis (sacro-iliac and elbow)	.....	.....	.....	Negative	Negative
25	Arthritis (both ankles); six weeks	.....	.....	.....	.....	Negative
26	Tuberculous arthritis (knee)	.....	.....	.....	Negative	Negative
27	Chronic synovitis (midtarsal)	.....	.....	.....	.....	Negative
28	Optic atrophy	.....	.....	.....	.....	Negative
29	Neurasthenia	.....	.....	.....	.....	Negative
30	Neurasthenia	.....	.....	.....	.....	Negative
31	Chancroid	.....	.....	.....	.....	Negative
32	Vesical calculus; cystitis	.....	.....	.....	.....	Negative
33	Hemiplegia	.....	.....	.....	.....	Negative
34	Myelitis; incontinence of urine	Negative	.....	.....	.....	Negative
35	Case of rape; no signs of infection	Negative	.....	Negative	.....	Negative

36	Epididymitis, possibly tuberculous, but undoubtedly gonorrheal	.....	.....	.....	Medium +	Medium +
37	Urine cloudy and full of shreds	Medium +	.....	.....	.....	Strongly +
	PATIENTS CLINICALLY CURED					
38	Several attacks of gonorrhea; no signs at present	.....	.....	.....	.....	Weakly +
39	Convalescent after treatment for 2½ years for chronic urethritis and prostatitis	.....	.....	.....	.....	Negative
40	Syphilis; had three doses of neosalvarsan; convalescent from chronic urethritis	.....	.....	.....	.....	Negative
41	Convalescent from chronic prostatitis	.....	.....	.....	.....	Negative
42	Convalescent from chronic urethritis, three attacks; first 3 years ago	.....	.....	.....	.....	Negative
43	Convalescent from first attack of gonorrhea 8 weeks previously	.....	.....	.....	.....	Negative
44	Convalescent from attacks of gonorrhea 13 and 2 years previously	.....	.....	.....	Negative	Negative
45	Gonorrhea several years ago; no symptoms now	.....	.....	.....	Negative	Negative
46	Clinically convalescent from gonorrhea acquired 2 years ago	.....	.....	.....	.....	Negative
47	Convalescent from urethritis and prostatitis; received 8 c.c. of antigonococcic serum 15 months ago for gonorrheal pleurisy	.....	.....	.....	Negative	Negative
48	Polyarthritis; has gonorrheal history	.....	.....	.....	.....	Negative
49	Gonorrhea three times; asserts a cure; complains of impotence	Medium +	.....	.....	.....	Strongly +
50	Gonorrhea 3 years ago; epididymitis 9 months ago; prostatorrhea; wants to marry	.....	.....	.....	.....	Negative
51	Gonorrhea three or four times; now clinically cured	.....	.....	.....	.....	Negative
52	Gonorrheal history; apparently cured	Negative	Negative	Negative	.....	Negative
53	Gonorrhea 5 months ago; cured	.....	.....	.....	.....	Negative
54	Contracted gonorrhea 1½ years ago; convalescent from chronic prostatitis	.....	.....	.....	.....	Negative
	CASES ACCIDENTALLY DISCOVERED. CLINICALLY CURED					
55	Confessed to gonorrhea 2½ years ago; under antisyphilitic treatment	Weakly +	.....	.....	.....	Weakly +
56	Luetic rhinopharyngitis; no inquiry as to gonorrhea	.....	.....	.....	.....	Weakly +
	ACUTE ANTERIOR URETHRITIS					
57	Gonorrhea of 12 hours duration (first attack)	.....	.....	.....	.....	Negative
58	Gonorrhea of 10 days' duration (first attack)	.....	.....	.....	.....	Negative
59	Acute gonorrhea (first attack)	.....	.....	.....	.....	Negative
60	Gonorrhea of 11 days' duration (first attack)	.....	.....	.....	.....	Negative
61	Gonorrhea of 7 days' duration (first attack)	.....	.....	.....	.....	Negative
62	Gonorrhea of 4 weeks' duration (first attack)	.....	.....	.....	Negative	Negative

TABLE 2.—Results with Respect to Serums Tested.—(Continued)

No.	Remarks	Result of Gonococcus Complement-Fixation Reaction. Antigens: Monovalent (Unfiltered)	Trivalent (Filtered)	Parke, Davis & Co. (Schwartz Technic.)	Hexavalent Unfiltered	Parke, Davis & Co.
	ACUTE AND SUBACUTE ANTERO-POSTERIOR URETHRITIS					
63	Gonorrhea of 6 weeks' duration (first attack)	.....	.....	.....	.....	Medium +
64	Gonorrhea of 5 weeks' duration (first attack)	.....	.....	.....	.....	Negative
65	Gonorrhea of 4 weeks' duration (first attack 10 years ago)	.....	.....	.....	.....	Negative
66	Gonorrhea of 2 weeks' duration (first attack)	.....	.....	.....	.....	Negative
67	Gonorrhea of 10 days' duration (first attack 1 year ago	.....	.....	.....	.....	Negative
68	Gonorrhea of 7 weeks' duration (first attack)	.....	.....	.....	.....	Negative
69	Gonorrhea of 4 weeks' duration (first attack)	.....	.....	.....	Negative	Negative
70	Gonorrhea of 6 weeks' duration (first attack)	.....	.....	.....	Negative	Negative
71	Gonorrhea of 7 days' duration (three previous attacks)	.....	.....	.....	Negative	Negative
72	Gonorrhea of 10 days' duration (six previous attacks)	.....	.....	.....	Negative	Negative
73	Gonorrhea of 5 week's duration (first attack 2 years ago)	.....	.....	.....	.....	Negative
	CHRONIC URETHRITIS PLUS ACUTE EXACERBATION					
74	Gonorrhea 1 year previously; received 8 c.c. of antigonococcic serum; duration of present attack is 4 weeks	.....	.....	.....	.....	Weakly +
75	Gonorrhea 2 years previously; duration of present attack is 4 weeks	.....	.....	.....	.....	Medium +
76	Gonorrhea 1 year previously; duration of present attack is 3 weeks	.....	.....	.....	.....	Strongly +
77	Gonorrhea 1 year ago; acute exacerbation at present	.....	.....	.....	.....	Weakly +
78	Gonorrhea 8 times previously; duration of present attack is 17 days	Strongly +	Weakly +	Strongly +	.....	Strongly +
79	Gonorrhea 5 years ago; duration of present attack 10 days	.....	.....	.....	Weakly +	Weakly +

80	Gonorrhea 4 years ago; present attack of 8 months' duration	.....	.....	.....	.....	Medium +
81	Gonorrhea several times; present attack of 2 months' duration	.....	.....	.....	.....	Negative
82	First attack; duration of 6 months	.....	.....	.....	.....	Negative
83	Contracted gonorrhea 3 years ago	.....	.....	.....	.....	Weakly +
84	First attack; duration 3½ months	.....	.....	.....	.....	Negative
85	First attack; duration 6 months	.....	.....	.....	.....	Negative
86	First attack; duration 2½ months	.....	.....	.....	.....	Strongly +
87	Gonorrhea twice previously; present attack 3 months duration	.....	.....	.....	.....	Weakly +
88	First attack; duration 8 months	.....	.....	.....	.....	Negative
89	Gonorrhea several times; urine contains shreds	.....	.....	.....	.....	Negative
90	First attack; duration 5 months	.....	.....	.....	.....	Negative
91	Chronic gonorrhea; duration unknown	.....	.....	.....	.....	Negative
92	Gonorrhea 7 times in 12 years; duration of present attack unknown	.....	.....	.....	.....	Strongly +
93	Gonorrhea 4 years ago; duration of present attack unknown	.....	.....	.....	.....	Medium +
94	Gonorrhea 2 times previously; duration of present attack 2 years	Medium +	.....	Medium +	.....	Medium +
95	Gonorrhea 1½ years ago; present attack 5 months' duration	.....	.....	.....	.....	Negative
96	Gonorrhea 16 years ago; present attack of 9 months' duration	.....	.....	.....	.....	Negative
97	First attack; duration 1 year	.....	.....	.....	.....	Negative
98	First attack; duration 4 months	.....	.....	.....	.....	Negative
99	Urethritis for 12 years	.....	.....	.....	.....	Negative
100	Chronic urethritis; duration unknown	.....	.....	.....	.....	Strongly +
101	Gonorrhea one year ago; pain in heel	.....	.....	.....	.....	Negative
102	Chronic recurrent urethritis	Negative	Negative	Negative	.....	Negative
103	Gonorrhea 2 years ago; duration of present attack unknown	.....	.....	.....	.....	Negative
104	Gonorrhea 2 years ago; slight morning discharge	.....	.....	.....	.....	Negative
105	Gonorrhea 3 times previously; present attack 3 months duration	.....	.....	.....	.....	Weakly +
106	Gonorrhea 5 years ago; present attack 4½ months' duration	.....	.....	.....	.....	Strongly +
107	Gonorrhea previously; duration of present attack 6 months	.....	.....	.....	Weakly +	Weakly +
108	Gonorrhea 3 years ago; bilateral buboes	.....	.....	.....	.....	Negative
109	First attack; duration 15 months; had four inoculations of Gonorrhea Bacterin 1 year ago	.....	.....	.....	Negative	Negative
110	Chronic urethritis; duration unknown	.....	.....	.....	Negative	Negative
111	Gonorrhea for years; several recurrences	.....	.....	.....	Negative	Negative
112	First attack; duration 2 months	.....	.....	.....	Negative	Negative

TABLE 2.—Results with Respect to Serums Tested.—(Continued)

No.	Remarks	Result of Gonococcus Complement-Fixation Reaction. Antigens				
		Monovalent (Unfiltered)	Trivalent (Filtered)	Parke, Davis & Co. (Schwartz Technic.)	Hexavalent Unfiltered	Parke, Davis & Co.
113	Recurrent; duration at least 1 year	.....	.....	.....	.....	Weakly +
114	Second attack gonorrhea 1 year ago; prostatitis and seminal vesiculitis treated for 1 year; practically cured at present	.....	.....	.....	.....	Negative
115	Duration 6 months	.....	.....	.....	.....	Negative
116	Gonorrhea 4 years ago; recurrent prostatitis, seminal vesiculitis and stricture ever since	.....	.....	.....	.....	Weakly +
117	Gonorrhea 1 year ago and 5 months ago	.....	.....	.....	Medium +	Medium +
118	First attack of gonorrhea 1 year ago; never cured	Negative	.....	.....	.....	Strongly +
119	Gonorrhea 8 years ago; not treated; now has shreds in urine	.....	.....	.....	.....	Negative
120	Gonorrhea 6 years ago; discharge at present	.....	.....	.....	.....	Strongly +
121	First attack of gonorrhea 1 year ago; chronic posterior urethritis and prostatitis	.....	.....	.....	.....	Medium +
122	First attack of gonorrhea 3 months ago	.....	.....	.....	.....	Negative
123	Duration uncertain	.....	.....	.....	.....	Weakly +
124	Has had gonorrhea nine times; last attack 6 months ago	Negative	Negative	Negative	.....	Negative
125	First attack of gonorrhea 9 years ago; last attack 2 years ago	.....	.....	.....	.....	Negative
126	Duration unknown	.....	.....	.....	.....	Negative
127	Gonorrhea 3 years ago and continuously ever since	.....	.....	.....	.....	Medium +
128	Gonorrhea past 4 years; second attack	.....	.....	.....	.....	Negative
129	First attack of gonorrhea 5 years ago; never cured	.....	.....	.....	.....	Doubtful
130	Duration unknown	Medium +	.....	.....	.....	Strongly +
131	Gonorrhea twice before; present attack of 4 years' duration	Medium +	.....	Medium +	.....	Strongly +
132	Gonorrhea 9 months ago; discharged as cured from another hospital; still has shreds in urine	.....	.....	.....	.....	Weakly +
133	Duration one year	.....	.....	.....	.....	Negative
134	Gonorrhea five times	.....	.....	.....	.....	Medium +
135	Duration unknown	.....	.....	.....	.....	Negative
136	Gonorrhea four times; last time 8 months previously	.....	.....	.....	.....	Weakly +
137	Gonorrhea 4 years ago; last attack 2 months ago	.....	.....	.....	.....	Negative

138	Ten or twelve attacks of gonorrhea; last attack 6 months ago	.....	.....	.....	.....	Negative
139	Gonorrhea 2 years previously; shreds in urine..	.....	.....	.....	.....	Weakly +
140	Gonorrhea one year ago.....	Weakly +	Weakly +	.....	.....	Strongly +
141	First attack; duration 1 year.....	.....	.....	.....	.....	Negative
142	Chronic hypertrophic changes in knee joint; prostatitis	Negative	.....	.....	.....	Negative
143	Gonorrhea for four years.....	.....	.....	.....	.....	Strongly +
144	Gonorrhea contracted 5 years ago; now has mild prostatitis and stricture	.....	.....	.....	.....	Negative
145	Gonorrhea 4 years ago; now has prostatitis and seminal vesiculitis	.....	.....	.....	Negative	Negative
146	First attack of gonorrhea 6 years ago; present recurrence one year ago (mild).....	.....	.....	.....	.....	Negative
147	Duration unknown; has taken gonorrheal phylacogen without benefit.....	.....	.....	.....	.....	Medium +
148	Several attacks of gonorrhea over period of 10 years; had forty-four inoculations of gonococcic bacterin in 6 months 2 years ago.....	.....	.....	.....	.....	Weakly +
149	Same case 1 week later; under treatment.....	.....	.....	.....	.....	Negative
150	Gonorrhea 2 years ago.....	.....	.....	.....	.....	Negative
151	Gonorrhea first time 5 years ago; twice since; duration of prostatis 1½ years; three recurrences; last one 9 months ago.....	.....	.....	.....	.....	Negative
152	Recurrence of above case 2 months later.....	.....	.....	.....	.....	Negative
153	Gonorrhea 26 months ago; very slight; involvement of prostate; urine clear.....	.....	.....	.....	Negative	Negative
154	Duration unknown; case 155, 10 days after treatment	Negative	.....	Negative	.....	Negative
155	Gonorrhea 5 years ago.....	.....	.....	.....	.....	Weakly +
156	First attack; duration 6 months.....	.....	.....	.....	.....	Weakly +
157	Above case three weeks later.....	.....	.....	.....	.....	Weakly +
158	Gonorrhea 3 years previously.....	.....	.....	.....	.....	Weakly +
159	Gonorrhea 15 years ago; last 2½ years ago; premature ejaculation	Weakly +	Weakly +	.....	.....	Strongly +
160	Gonorrhea 7 and 2 years; present attack of 4 mos.' duration; had 5 injections of phylacogen	.....	.....	.....	.....	Medium +
	STRICTURE					
161	Gonorrhea 3 years and 4 months ago.....	.....	.....	.....	.....	Medium +
162	Gonorrhea 14 and 12 years ago.....	Negative	Negative	Negative	.....	Negative
163	Gonorrhea for 1½ years.....	.....	.....	.....	.....	Negative
164	Two attacks of gonorrhea; last one 18 months ago	Negative	.....	Strongly +	.....	Weakly +
165	Gonorrhea for 4 years with recurrences.....	.....	.....	.....	Strongly +	Medium +
166	Duration unknown	.....	.....	.....	.....	Strongly +

TABLE 2.—Results with Respect to Serums Tested.—(Continued)

No	Remarks	Result of Gonococcus Complement-Fixation Reaction. Antigens: Monovalent (Unfiltered)	Trivalent (Filtered)	Parke, Davis & Co. (Schwartz Technic.)	Hexavalent Unfiltered	Parke, Davis & Co.
		EPIDIDYMITIS				
167	Duration three weeks	.....	.....	.....	.....	Negative
168	Same as 167, two weeks later	.....	.....	.....	.....	Weakly +
169	Epididymitis 2½ months ago; still unresolved; prostatitis	.....	.....	.....	.....	Medium +
170	First attack of gonorrhea; duration unknown	.....	.....	.....	.....	Medium +
171	Gonorrhea and epididymitis 2 years ago; second attack at present	Weakly +	Weakly +	.....	.....	Strongly +
172	Gonorrheal history; slight swelling of epididymis	Negative	Negative	.....	.....	Medium +
173	Previous attack of gonorrhea; present attack of six weeks' duation	.....	.....	.....	.....	Medium +
174	Gonorrhea three or four times; last time six weeks ago	.....	.....	.....	.....	Weakly +
175	First attack of gonorrhea; duration six months	Negative	Weakly +	Strongly +	.....	Strongly +
176	Gonorrhea twice previously; present attack of six weeks' duration	Negative	Strongly +	.....	.....	Strongly +
177	Acute attack of nine days' duration	.....	.....	.....	.....	Weakly +
178	Acute attack of seven days' duration; denies gonorrhea	.....	.....	.....	.....	Medium +
179	Gonorrhea three times previously; epididymitis 15 years ago, plus present attack	.....	.....	.....	.....	Strongly +
180	Duration two months	Strongly +	Medium +	.....	.....	Strongly +
181	Duration unknown	.....	.....	.....	.....	Medium +
182	Bilateral epididymitis, probably tuberculous; has pulmonary tuberculosis; gonorrhea 10, 5 and 2 years ago	.....	.....	.....	.....	Negative

183	Chronic hypertrophic arthritis; duration 1 year; abdominal section 3 years ago; no G. U. symptoms at present	.....	.....	.....	.....	Medium +
184	Arthritis knee for 10 years; urethritis for 6 months, since when knee has been worse	.....	.....	.....	.....	Weakly +
185	Multiple subacute arthritis; no history of gonorrhea	Weakly +	.....	Weakly +	.....	Weakly +
186	Arthritis both ankles; gonorrhea for 3 years; synovitis 3 weeks after gonorrhea	.....	.....	.....	.....	Weakly +
187	Duration 10 weeks	.....	.....	.....	Weakly +	Weakly +
188	Pain in left shoulder; no inquiry as to gonorrheal history	.....	.....	.....	.....	Weakly +
		GYNECOLOGICAL CASES				
189	Pain in right ovarian region; leukorrhea; dysuria; frequency	.....	.....	.....	.....	Negative
190	Eclampsia	.....	.....	.....	Negative	Negative
191	Puerperal sepsis	.....	.....	.....	Negative	Negative
192	Pelvic abscess; puerperal sepsis	.....	.....	.....	Weakly +	Weakly +
193	Abscess of uterine cornua; vaginal smear shows Gram-diplococci	.....	.....	.....	Negative	Negative
194	Abscess in cornua of uterus; delivered of dead fetus	.....	.....	.....	Negative	Negative
195	Leukorrhea; dysuria; frequency	.....	.....	.....	Negative	Negative
196	Vaginal smear shows Gram-diplococci	.....	.....	.....	Negative	Negative
197	Vaginal smear shows Gram-diplococci	.....	.....	.....	Negative	Negative
198	Tenderness in hypogastrium; vaginal smear shows Gram-diplococci	.....	.....	.....	Negative	Negative
199	Tenderness in hypogastrium; vaginal smear shows Gram-diplococci	.....	.....	.....	Negative	Negative
200	Salpingitis	.....	.....	.....	.....	Negative
201	Pyosalpingitis	.....	.....	.....	Negative	Negative
202	Gonorrheal urethritis; salpingo-oöphorectomies	.....	.....	.....	.....	Medium +
203	Bartholinitis and cyst	Negative	.....	.....	.....	Negative
204	Gonorrheal endometritis	.....	.....	.....	.....	Strongly +

Reviewing our experience with the gonococcus complement-fixation test in general, it may be stated that a negative reaction is not decisive against the presence of a gonorrheal infection, and this is particularly true during the first six weeks of a primary acute urethritis either anterior or posterior in the absence of any complication, previous to which time we have never obtained a positive reaction; on the other hand, the supervention, even during the acute stage of the disease, of complications such as epididymitis, arthritis, prostatitis, etc., is prone to result in the production of a positive reaction. On the contrary in our experience, a positive reaction has been pathognomonic of a focus of gonococcal infection and has assisted many times in elucidating obscure or doubtful lesions. In fact, it appears that the gonococcus-fixation test enjoys greater specificity than does the Wassermann reaction, since thus far we have found no alien infection or condition capable of producing a positive reaction. This much certainly cannot be claimed for the Wassermann reaction. Moreover, there is no drug, as there is in syphilis, which is capable of causing the reaction to be negative during the existence of the disease. The probable explanation for the greater specificity of the gonococcus complement-fixation test rests in the fact that with gonococcic infections we employ a specific antigen—the gonococcus—while in the case of syphilis a non-specific or lipotropic antigen is employed.

The analysis of our cases further illustrates another interesting feature, namely, the persistence in some cases for a short time of a positive reaction, after an apparent clinical cure. This has occurred so often that we no longer discharge a patient cured or give him a clean bill of health so long as he gives a positive reaction, provided he has not been the recipient of immunotherapy. Usually a persistent positive reaction will become negative in two or three weeks following clinical cure with or without a continuation of treatment. The only explanation is that it requires an indefinite time for the antibodies, formed during the course of infection, to disappear from the blood. Torrey,[5] in animal experimentation, has found that the antibodies in immunized rabbits begin to disappear after ten days, and that the elimination is practically complete by the fiftieth day in all cases, disappearing much earlier in many instances. Thus a patient, evidencing a positive reaction two months after presumed clinical cure, should be regarded as still harboring a latent gonorrheal focus. Such experience, adopted either as routine procedure in the management of treatment, or discovered accidentally when gonorrheal infection or its symptoms were denied, or demonstrated by submitting suspected or positive syphilitic serum to the gonococcus-fixation test, has been encountered in a large number of cases.

Because of the generally acknowledged difficulties, at times, of differentiating the pelvic lesions in women, notably certain of the inflammatory

5. Torrey: Jour. Med. Research, 1910, No. 1.

from the cystic and neoplastic conditions, and also the differential diagnosis among gonorrheal, tuberculous and pyogenic infections themselves, the gonorrheal-fixation test seems destined to play a rôle. As in the male, in whom a positive reaction seems never to occur, at least so long as the infection is confined to the anterior urethra, so in the female we have been unable to obtain a positive reaction unless the disease has ascended to the level of the uterus.

An interesting, if not important, feature connected with this work is the comparative importance and value of the serological and bacteriological examination of cases of suspected gonorrheal infection. It is, to-day, a fact that the judiciary courts of our land require that the presence of the gonococcus be demonstrated, culturally, in order to establish its indisputable and legal identity. Based on this qualification, there are many cases of gonococcic infection impossible to determine, and we do not hesitate to state that, in our judgment, many such cultures are in reality the *Micrococcus catarrhalis* and not the gonococcus. This applies particularly to such isolation of the diplococcus of Neisser from chronic inflammatory processes. Moreover, it must be generally recognized that the demonstration of a Gram-negative diplococcus in smear is often insufficient and faulty evidence on which to base a diagnosis of gonococci. Therefore, it is most fortunate that in the chronic stage of the disease with complications, the complement-fixation test seems to be signally meritorious, while in the acute, subacute and frequently in the chronic forms of the diseases when the gonococcus may be demonstrated bacteriologically, the serological test promises little or nothing.

CONCLUSIONS

Detailed and careful analysis of the gonococcus complement-fixation test, performed with the serums of the cases tabulated in our series, would seem to justify the following assertions:

1. A positive reaction is invariably reliable and always denotes the presence of a focus of gonococcic infection.

2. A negative reaction frequently fails to determine the presence of disease especially in the acute and subacute stage when the disease is limited to the urethra, and never when it is confined to the anterior urethra or vagina alone.

3. In no alien non-gonorrheal infections of systemic disease has a positive reaction been obtained; the test, therefore, appears to be absolutely specific.

4. A positive reaction has been found to be present in 21.05 per cent. of patients clinically cured. Such patients, therefore, should not be discharged from treatment or observation until a negative reaction has been obtained.

5. Not infrequently, either when suspicious lesions are presented or accidentally, positive reactions will be discovered in patients denying gonorrhea.

6. In only 9.09 per cent. of cases of acute and subacute anteroposterior urethritis has the complement-fixation test resulted positively. The earliest appearance of a positive reaction in a primary attack of posterior urethritis, without complication, occured in the sixth week.

7. In a number of cases of chronic recurrent urethritis with acute exacerbations, the test was invariably positive; many of these patients undoubtedly had prostatitis.

8. The reaction resulted positively in one-third of all cases of chronic posterior urethritis; undoubtedly many of these cases had a mild or low-grade prostatitis.

9. In 52.08 per cent. of cases of chronic prostatitis a positive reaction was obtainable.

10. Two-thirds of all stricture cases demonstrated a positive test.

11. In epididymitis a positive complement-fixation test was observed in 87.5 per cent. of cases. If, from our series, one case probably tuberculous, may be eliminated, and a time duration of five weeks can be imposed, the positive result in this form of disease has been 100 per cent.

12. In arthritis, undoubtedly gonorrheal in character, positive reactions were obtained in 100 per cent. of cases.

13. In the diagnosis and differential diagnosis of pelvic disease in women, the gonococcus-fixation test is destined, unquestionably, to play an important rôle. We have been unable to obtain any positive results in uncomplicated urethritis, vulvovaginitis and Bartholinitis, and it would appear that the infection must ascend at least to the level of the uterus in order to produce a positive blood response.

14. Inoculations of gonococcus bacterin, antigonococcic serum, etc., may in themselves by the production of immune bodies be causes of positive reactions. How long these immunizing effects may endure is unknown, but we have observed patients, treated by immunotherapy, who one year later demonstrated negative complement-fixation reactions.

15. Although the bacteriological demonstration of the gonococcus culturally is the only absolute method for its identification in chronic inflammatory processes, the method as a routine procedure is impractical and susceptible of many failures and fallacious results, so that the complement-fixation test is not only less laborious, but is productive of a higher percentage of positive findings.

16. Finally, we hope and trust that the complement-fixation tests in gonococcic infections, as the Wassermann reaction in syphilis, are demonstrating their reliability and value to the extent that they will be recognized as indispensable, so soon as the courts shall rule that each applicant for marriage licensure must produce a health certificate properly attested.

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THE IVES REPLICA DIFFRACTION GRATING IN SPECTROSCOPIC ANALYSIS.

BY GORDON J. SAXON[1]

(From the Pepper Laboratory of Clinical Medicine, University of Pennsylvania.)

(Received for publication, January 3, 1914.)

In the examination of fluids giving absorption spectra it has been found convenient to use the Ives replica diffraction grating. This little device is used extensively by physicists in determining wave lengths.

In the examination of dilute solutions by transmitted light it enables one readily to take advantage of the fact that prolongation of the column under examination is equivalent optically to concentration of the fluid, as is the case in colorimetric determinations. Fluids containing oxyhemoglobin in such high dilution that it is impossible to get an absorption spectrum through a column 0.3 meter long, may display very distinct bands when a column a meter long is used.

I have demonstrated the absorption bands of oxyhemoglobin in a solution containing one drop of blood in 2 liters of water, simply by examining it through great depths of the fluid.

I have found it convenient to have three troughs of varying lengths, one-third meter, two-thirds meter and one meter respectively, the cross sections of which give a horizontal dimension of 5 mm. and a perpendicular dimension of 20 mm.[2] They are made by flattening glass tubing and cutting or grinding off the top Parallel plane plates of glass are cemented on the ends. The best cement to use, I have discovered, is dislysin, obtained from bile since it withstands the action of acids, alkalies, ether, alcohol or chloroform. It is used hot and the surfaces to be cemented should first be submerged in boiling water.

[1] Woodward Fellow in Physiological Chemistry.

[2] The troughs used were made by Dolbey and Company, Philadelphia.

Figure 1 shows the arrangement of grating G, slit for transmission of light S, trough MN, collecting lens L, and source of illumination F. The best illumination is obtained from a Welsbach mantle; electricity may well be used however, especially if inflammable solutions are to be examined. The focal length of the lens is 15 to 20 cm. The source of light is placed at a distance from the lens equal to its focal length, thus producing parallel rays which pass through the glass plate N and through the solution. The rays emerge at M and strike a screen having an oblong slit about 0.5 mm. wide at S. The screen may be constructed from a thin piece of sheet copper or tin mounted on a stand by means of a clamp. This would permit up and down adjustment. The distance from the trough to the slit should be as small as possible but the difference from the slit to the diffraction grating G is arbi-

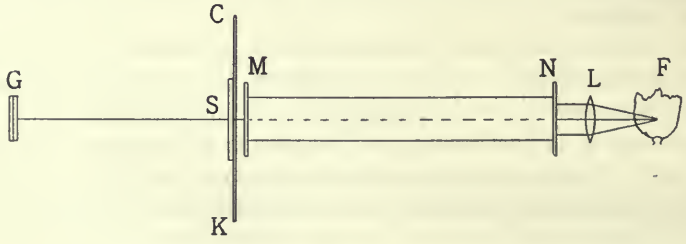

Fig. 1.

trary. It is, however, advisable to make the distance S–G at least 1 meter if measurements are desired.

The grating is transparent. If the eye is placed behind it the direct image of the slit S, can be seen. On either side of this direct image will appear spectra of light, the whole spectrum being visible at the same time. If rays of light from other sources should be seen in looking at the spectra, they may be cut off by use of a cardboard screen CK. This should extend 30–40 cm. either side of the slit. It may stand on the table in a perpendicular position and be made to support itself against the stand holding the slit screen.

The absorption spectrum does not consist of sharp lines missing from the continuous spectrum of the flame, but whole regions of the spectrum are absorbed. It is obvious that not only a color of

one wave length is absorbed in this region but many adjacent colors. If therefore it is desired to state between which wave lengths the absorption is to be found it may be easily determined. The width of the bands will vary with the concentration of the solution and length of the column through which the light has passed. The edges of the bands are not very sharply defined but they are much more easily identified than with an instrument permitting a view of only a small part of the spectrum.

Comparison of the absorption spectrum can easily be made with the direct or unabsorbed spectrum of the source. If the upper rays from the lens pass above the solution and the slit be made long enough to allow them to pass through, the observer can see

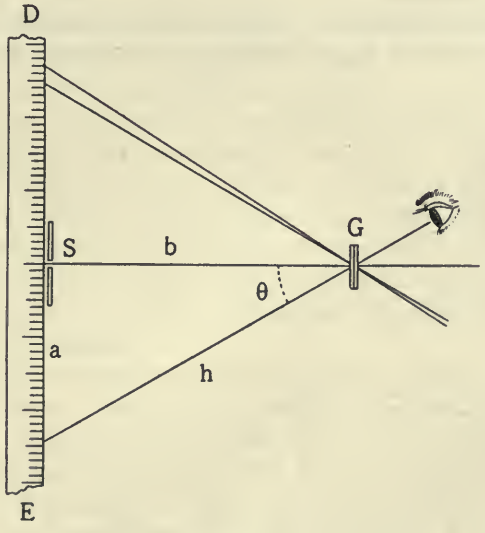

Fig. 2.

both these spectra at the same time. This renders the absorption spectrum more striking by comparison.

If it is desired to measure the wave lengths of the absorption bands a meter stick may be placed along *S*, as in figure 2. In looking at the spectrum with the eye the position of the band can be noted on the meter scale and the distance, *a*, determined. It is well to obtain this position on both sides of *S*, and take the aver-

age value of a thus obtained. The wave length of the particular band located at a is calculated by the formula:

$$l = \frac{d \cdot a}{h} \text{ or } l = \frac{d \cdot a}{\sqrt{a^2 + b^2}}$$

In which l = wave length, a = distance, along the meter stick, from the slit S to where the band is seen. d = distance between lines on the diffraction grating.

In purchasing a diffraction grating the number of lines ruled per centimeter or per inch, should be obtained from the maker. With age this distance may change, the grating should therefore be tested occasionally by means of light of known wave-length, *e.g.*, sodium flame.

The grating costs $12. This, together with the cost of the troughs gives one a highly efficient spectroscope at a very low cost.

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A METHOD FOR THE DETERMINATION OF THE TOTAL FATS OF UNDRIED FECES AND OTHER MOIST MASSES.

BY GORDON J. SAXON.[1]

(From the Pepper Laboratory of Clinical Medicine, University of Pennsylvania.)

(Received for publication, January 3, 1914.)

The procedure about to be detailed is essentially a combination of the excellent method of Folin and Wentworth[2] for the determination of the total fats of powdered dried feces, and the method of Meigs[3] for the determination of the fat of milk.

It is as follows: In the case of feces the twenty-four-hour specimen is collected and very thoroughly mixed until a homogeneous paste results. If the stool is liquid, infusorial earth is slowly added while thoroughly mixing, until a workable paste results. The mass is weighed before and after a sample for extraction is chosen. If the mixing has been done in an ordinary mortar it is advisable to remove the mass quantitatively, and place it on waxed paper for weighing.

The sample for extraction is placed in a 100-cc. glass stoppered, graduated cylinder. Care must be taken not to smear the neck of the cylinder. This may be avoided by removing from various portions of the mass small bits with the aid of short capillary tubes sealed at both ends. The tubes and the portions of specimen which they carry are dropped into the cylinder. The specimen may be as much as 5–6 grams.

Add 20 cc. of distilled water and 1–2.5 cc. of concentrated hydrochloric acid (depending on amount of sample) and again sufficient

[1] Woodward Fellow in Physiological Chemistry.

[2] Folin and Wentworth: this *Journal*, vii, p. 421, 1910.

[3] Arthur V. Meigs: *Phila. Med. Times*, July 1, 1882; Arthur V. Meigs and Howard L. Marsh: *The Medical Record*, Dec. 30, 1911; Croll: *Biochem. Bull.* for June, 1913.

water to make a total bulk of 30 cc. Add exactly 20 cc. of ether and shake vigorously for five minutes. Allow to stand a few seconds, remove stopper and add exactly 20 cc. of 95 per cent alcohol and again shake for five minutes.

Stand the cylinder aside. The ether containing practically all of the fat will come to the top as a colored transparent layer. The ether layer is blown off into a tall 150–200 cc. beaker. This is accomplished in the same manner that water is blown from a wash bottle. The submerged end of the delivery tube is bent upward as in the pipette described by Meigs and Marsh[4] in order that there be no upward currents which would set in motion the subjacent alcohol-water-feces layer. In this manner practically all the ether can be removed. The thin layer which remains is diluted with 5 cc. of ether, slightly agitated and blown off. This is done in all five times, care being taken each time to wash down the sides of the cylinder. The stopper also should be washed.

Twenty cc. of ether are again added, and the cylinder shaken for five minutes and set aside. When the ether has nearly stratified blow it off and wash as before. During the second washing process the stratification will complete itself. Evaporation is carried on until no trace of alcohol which has been carried over by the ether remains. From this point on the method is that of Folin and Wentworth. To the residue add 30 cc. of low-boiling petroleum ether (should distil over below 60°C.) and allow to stand over night. Petroleum ether for this work should be frequently tested for a residue on evaporation. If a residue is left the ether should be redistilled.

Filter the fatty petroleum ether, catch filtrate and washings in a tall, weighed, 100-cc. beaker, evaporate off the solvent, dry the beaker at 90°C., desiccate and weigh. Subtract the weight of the beaker from the last weighing and the result is the weight of neutral fat, free fatty acids and the fatty acids of the soaps contained in the specimen extracted.

The fatty acid titre is obtained by dissolving the contents of the beaker after the weighing just mentioned in 50 cc. of benzol, heating almost to the boiling point, adding two drops of a 0.5 per cent alcoholic solution of phenolphthalein and titrating with a decinor-

[4] This *Journal*, xvi, p. 152, 1913.

mal solution of sodium alcoholate. Each cubic centimeter of the standard solution used in the titration represents 28.4 mgm. of stearic acid.

The difference between the gravimetric and the volumetric determinations is the weight of the neutral fat. In the preparation of the sodium alcoholate solution absolute alcohol and freshly cut bright metallic sodium are used; otherwise it is the same as the standardization and preparation of any other standard solution.

Following are determinations showing the accuracy of the method: Three specimens of stearic acid were melted, mixed with infusorial earth and extracted by the wet method with the following results:

0.0830 gram stearic acid used..... 0.0828 gram recovered.
0.0970 gram stearic acid used..... 0.0964 gram recovered.
0.1632 gram stearic acid used..... 0.1622 gram recovered.

In order that a comparison might be made between this method and that of Folin and Wentworth, a dried, powdered stool was used to which infusorial earth had been added previous to drying in sufficient quantity to render workable the specimen, which was large and semi-liquid when it came to the laboratory.

TABLE I.

WEIGHT OF SPECIMEN	WEIGHT OF TOTAL FATS	FATTY ACID TITRE IN GRAMS	PERCENTAGE OF TOTAL FATS
0.5	0.0306	0.017	6.12
0.5	0.0301	0.017	6.02
0.5	0.0306	0.017	6.12
0.5	0.0311	0.017	6.22

Average of four determinations, 6.12 per cent.

This table shows the results of four determinations by the wet method.

The next table shows results by the Folin-Wentworth method. Under the heading "Re-extract" is placed the amount of fat extracted by the wet method from the contents of the thimble after thirty hours' extraction by the Folin-Wentworth method. It will be seen that the combined fatty residues of the extract and the re-extract are equal to the result obtained from a like amount of specimen by the wet method.

It might be said here that both methods give identical results on specimens to which infusorial earth has not been added. It would seem that the earth causes a small portion of the fats to enter into a colloidal combination which ether will not break up. In the wet method this combination is destroyed in an ether-water-alcohol menstrum.

TABLE II.

NO.	WEIGHT OF SPECIMEN	WEIGHT OF TOTAL FATS	FATTY ACID TITRE IN GRAM
1	0.5	0.0275	0.0113
2	0.5	0.0274	0.0099
3	0.5	0.0276	0.0114

TABLE III.

NO.	WEIGHT OF TOTAL FATS IN RE-EXTRACT	FATTY ACID TITRE IN GRAMS OF RE-EXTRACT
1	0.0040	0.0022
2	0.0033	0.0025
3	0.0040	0.0022

TABLE IV.

NO.	FOLIN-WENTWORTH EXTRACT PLUS RE-EXTRACT BY WET METHOD	PERCENTAGE OF TOTAL FATS
1	0.0275 + 0.0040 = 0.0315	6.30
2	0.0274 + 0.0033 = 0.0307	6.14
3	0.0276 + 0.0040 = 0.0316	6.32

Average of three determinations, 6.25 per cent.

Compare the average of four determinations in Table I with that of three determinations in Table IV.

If it is thought advisable by the worker to extract larger samples it is necessary only to increase the size of the extraction cylinder and increase the quantities of water, alcohol and ether proportionately.

I have extracted the entire twenty-four-hour excreta of an eight months' old infant in a 250-cc. cylinder. In this case the quantities of reagents used were two and one-half times the amount used in a 100-cc. cylinder.

No. 21

Extracted from the American Journal of the Medical Sciences,
March, 1914, No. 3, vol. cxlvii, p. 366.

IRON INFILTRATION IN THE FIXED AND WANDERING CELLS OF THE CENTRAL NERVOUS SYSTEM.

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In a contribution to (*a*) iron infiltration in ganglion cells and (*b*) forced movement due to cellular degeneration of the cerebellum following rattlesnake poisoning,[1] I pointed out the existence of iron infiltration in the cortical ganglion cells both as an end result and as an intermediate process of the absorption of hemoglobin. The ganglion cells gave the biochemical tests for hemosiderin. The microchemical reactions in the large pyramidal cells in the cortex were striking and characteristic. In the Weigert-hematoxylin method the entire cell with its processes took a deep black stain. In thin sections this pigmentation seemed to consist of fine black granules; these black granules could be followed into the dendrites and produced an appearance of fragmentation. With the slow eosin stain the ganglion cells gave the same brilliant red reaction as did the red-blood corpuscles. With the potassium ferrocyanide and alcohol differentiation, the ganglion cell as a whole gave a diffuse blue reaction without, however, the ganular arrangement of the hematoxylin stained cell. This microchemical reaction in the ganglion cells was not obtained throughout the entire cortex, but only in the neighborhood of the diseased vessels.

Weber[2] has described iron infiltration in the cortical ganglion cell with similar changes to those here noted, but no other contribution, so far as I have been able to determine, has been made to this subject. This condition must indeed be a rare one, as I have noted it, as a complete reaction, only in the case above noted, reported by me. It is not a constant reaction in hemorrhage into the cortical tissues. I have looked for it in a large number of brains showing cortical hemorrhage, with degeneration of the red cells in various stages, but have not been able to find it. In a series of experiments on young puppies an artificial thrombosis of the venous sinuses was produced in an attempt to simulate the venous

[1] Contributions from the William Pepper Laboratory of Clinical Medicine, 1900, vol. i.
[2] Monats. f. Psychiat. u. Neurol., 1898, No. 3.

capillary hemorrhage observed in some cases of the cerebral palsies of childhood. Capillary hemorrhages in the tissues were produced, but no end reaction of hemosiderin was noted. What might be considered an intermediate reaction was the rule. Cells took a deep black stain with hematoxylin and a deep red stain with eosin, but no blue reaction with potassium ferrocyanide was obtained. The results of this series of experiments were inconclusive in demonstrating a structural change in the ganglion cell as a result of long-standing venous stasis or thrombosis occurring at the time of delivery. The experiments have not yet been completed, but so far as they have been carried, an artificial infiltration of hemosiderin in the ganglion cells has not been produced. In line with this work a careful investigation of ganglion cells was made in the brain from a patient who suffered from an extensive hemorrhagic encephalitis in the cortex. The iron reaction was not demonstrated in the cortical ganglion cells in this case, but was obtained in a striking cell reaction in the plasma-like cells of the pia arachnoid and in the elastic coats of the smaller cortical arteries. The case was as follows:

The patient, R. L., a white male, was admitted to the hospital, June 1, 1910 in a semi-unconscious condition, in which he remained for four days. On admission his neck was rigid, his pupils contracted and his reflexes exaggerated, and since the stuporous condition had been present, any attempt to move his neck or bend his back caused him to cry out as if from pain. Pressure upon the muscles also caused him pain. He had incontinence since his admission. Owing to the noise he made during any attempt to examine his heart and lungs an accurate knowledge of their condition could not be ascertained. There was, however, apparently no valvular lesions of the heart. In the left lung many dry rales could be heard in front. The patient had an old, depressed fracture near the Rolandic area on the left side. There was a large discolored area over the right temporal region; and just below the left deltoid muscle, on the outer side of the arm, was a large bruise. He had been operated upon shortly before coming into the hospital for a tuberculous fistula in ano, which was still unhealed. His wife later stated that he had been operated upon for fracture of the skull in September, 1908, which explained the depression.

On June 25, an examination of the eyes showed the eye-grounds to be normal, though the optic nerves were very small. A lumbar puncture revealed nothing abnormal macroscopically or microscopically, but a Noguchi (Butyric acid) test was positive. The urine had a specific gravity of 1026, and contained a trace of albumin; the microscope revealed granular casts, triple phosphates, and amorphus urates. The temperature was normal except for a slight rise during the fortnight preceding his death. The patient died July 25.

The autopsy on July 26 permitted the following diagnosis: Dilatation of the left ventricle; fibroid myocarditis; congestion and edema of the lungs; an old fracture of the skull; a chronic internal hemorrhagic pachymeningitis. The general postmortem examination showed nothing more than the above diagnoses suggest. The man was fairly well-nourished; the lungs were free and crepitated throughout, but contained frothy bloody fluid, while the pleural cavities were empty. The heart was flabby, with the muscle pale in color, but no lesion was found in the valves, and the coronary arteries were smooth. The papillary muscles were slightly fibroid at the tips, and the aorta showed a few yellowish patches. The liver contained an excess of blood, and the spleen was large and soft. The pancreas, adrenals, ureters, and bladder were normal. The kidneys showed no noteworthy change. There was no gross lesion of the intestines, but these with the stomach, which presented a dark reddish mucosa, were distended. In the abdominal aorta were a few calcareous patches.

The skull showed a fracture partly linear in type, extending from the right posterior fossa just behind the foramen diagonally across the median line to the left fossa, and running superiorly and anteriorly close to the juncture of the parietal and temporal bones and terminating anteriorly at the base of the skull, just posterior to the orbital plate of the frontal bone on the left side. There were two small circular deficiencies in the frontal bone of the left side near the median line.

The brain showed slight excess of fluid. The inner surface of the dura, over practically the entire hemisphere, but especially posteriorly and superiorly, was thickened and darkened in color. At points the thickening appeared to be due to fibrin. At other places it appeared to be newly formed tissue. Posteriorly the superior surface of the brain showed a distinct depression underneath the thickest portion of the dura. The cord presented no gross lesions.

The gross examination of the brain, after hardening, showed at the base an advanced grade of arteriosclerosis of all the vessels composing the circle of Willis. There was a slight trace of chronic leptomenigitis over the base of the frontal lobes, and there were evidently adhesions between this and the dura of the base, which were torn away when the brain was removed. There was a yellowish pigmentation of the pia arachnoid over the base of the frontal lobe on the left side and the tip of the left temporosphenoidal lobe. Pons and medulla were normal. There was an extensive hemorrhagic internal pachymeningitis involving the dura over the convexity of the left hemisphere. On this side of the brain the pia arachnoid was adherent to the dura over the anterior half of the frontal lobe. The pia arachnoid as far back as the midparietal area had a yellowish red appearance, as if infiltrated with an old transformed blood pigment. Here and there throughout the frontal

lobe, and more particularly where the pia arachnoid was adherent to the dura, the cortex was friable and softened as if from a recent thrombotic lesion. Sections of the cortex showed that the reddish-yellow pigmentation extended into the gray corticle matter, while the underlying white matter had the appearance of yellow softening. The dura over the right hemisphere was the seat of a localized pachymeningitis (6 cm. long by 3 cm. broad) situated over the inferior parietal area. The internal surface of the dura was perfectly normal, both in color and texture. The external surface of the dura was roughened, indurated, and of an orange-brown to black color. Section of this area showed this condition for the most part to be extradural. The pia arachnoid on the right side was normal in texture and color, as was also the cortex. Transverse section of the brain showed nothing abnormal.

Microscopic Examination of the Brain Cortex. The left cerebral cortex showed here and there throughout the frontal area capillary hemorrhages, both old and relatively recent. The cortex in sections taken from the frontal lobe showed necrotic areas extending from the surface into the subcortical tissue in the form of pyramids. At the apices of these pyramidal areas of degeneration there was proliferation or neuroglia, while the body of the pyramids was composed of partially degenerated cortical substance and partially organized cortical scar tissue. New-formed capillaries were scattered throughout this area, and here and there capillary hemorrhages were present. A large number of a vesicular type of cell, containing an orange-brown pigment, was scattered through the areas of degeneration.

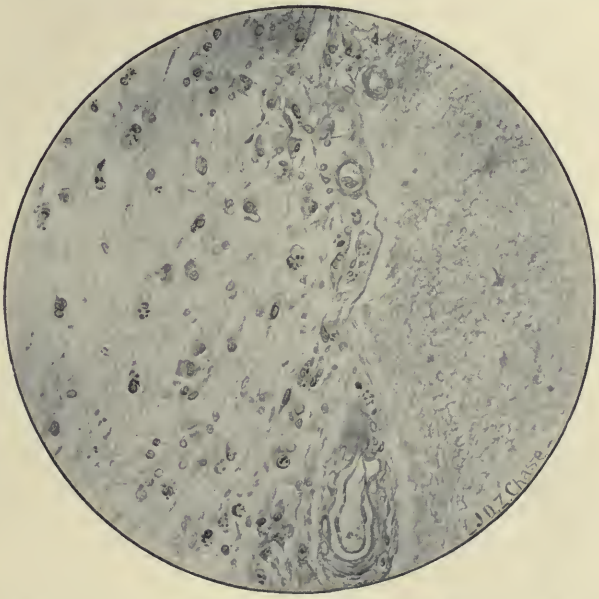
The dura mater over the left side of the brain was composed of laminæ of fibrous tissue, containing a great number of cells, mostly with connective-tissue nuclei, and containing pigment varying in color from light yellow to black. On the inner surface there was a layer of pseudomembrane, composed of trabeculæ of connective tissue, new-formed capillaries, and a great number of red-blood corpuscles. The connective-tissue cells contained yellow and black pigment.

Throughout the motor cortex on the left side there was a proliferation of neuroglia cells in the tangential and subtangential layers. The pyramidal cells showed a mild grade of chromatolysis, and appeared to be fewer in number than in the normal cortex. The left parietal and occipital areas were normal in structure. The cortical tissues of the right side revealed no abnormal change.

The pia arachnoid of the frontal and motor area of the left side showed a proliferation of the connective-tissue trabeculæ with an infiltration of red-blood cells, in various stages of degeneration into the interstices. Scattered through the arachnoidal spaces were found a great number of cells of a large vesicular type, filled with pigment varying in color from a light yellow to an orange-

brown. The relative number of these cells in the sections depended upon the degrees of hemorrhagic infiltration in the meninges and neighboring tissues.

All the tissues showing this condition were stained for hemosiderin. The Berlin-blue reaction was not obtained in any of the cortical cells. A well-defined, clear-cut, blue reaction was obtained, however, in many of the vesicular type of cells in the pia arachnoid.



Section through cortex and pia arachnoid. The small cells with hard granules gave the typical hemosiderin reaction. The small vessels also presented the Berlin-blue reaction.

The elastic tissue in the small arteries of the pia arachnoid and superficial areas of the cortex took the same clear-cut, blue color as did the plasma cells above mentioned. None of the other tissues gave this reaction. Sections of the bloodvessels gave a microscopic picture, very much as that seen in the sections stained with a selective elastic stain. While the cortical ganglion cells did not give the stain reaction for hemosiderin in the sections stained with iron hematoxylin, both the neurogliar nuclei and the partially degenerated cortical ganglion cells in the neighborhood of the pyramidal areas of necrosis, gave a jet-black reaction, which I have already pointed out in a previous contribution to this subject may be considered as a partial or transitional stage between hemoglobin and hemosiderin.

Sections from the spinal cord showed a degeneration in the cross-pyramidal tract of the right side.

The area of external pachymenigitis on the right side was found,

under the microscope, to be composed of (*a*) a normal dura somewhat thickened, and (*b*) external to this a thick layer composed of fibrous tissue, but relatively looser in consistence than that of the normal dura, containing a large number of connective-tissue nuclei and here and there large areas in which red-staining nuclei the size of red-blood corpuscles, but irregular shape, were found arranged in columns and nests. Connective-tissue nuclei and large irregular cells scattered here and there contained masses of dark pigment having the appearance of melanin.

The biochemical microscopic reactions here noted threw some light on the minor changes in the nerve cells, leading to a disturbance of function, without apparent structural change. The partial reactions to iron infiltration shown in the ganglion cells, together with the complete reaction noted in the arachnoidal free cell, and in the coats of the capillary vessels were evidently only temporary in nature. These changes may entirely disappear, and studies of these tissues in after life would leave us without any evidence of these previous pathological lesions. In a complete stage of iron infiltration in the cortical ganglion cells, complete chromatolysis with disintegration of the cell occurs. In the minor grades of iron infiltration, relatively little structural change is noted in the cell. Granted such a transitory process, extensive disturbance of the function may result without leaving any changes evident in the histological picture. In cases of mental retardation, high-grade imbecility, etc., the brain structure not infrequently presents a normal histological picture. There is no reason, in this group of cases, why the functional activity of the brain cells should be subnormal. In conditions of prolonged passive congestion at birth, and more particularly in those cases where the microscopic examination of the newborn children, dying at or shortly after birth, reveals an osmotic extravasation of red-blood cells in the tissues, a medium grade of iron infiltration may well have taken place, leaving the brain structure normal, but with deficient functional power.

The meaning of the hemosiderin reaction in the elastic coats of the capillary vessels is not altogether clear, None of the other coats of the vessels gave this reaction. It is evidently a selective reaction, due to some changes in the elastic fibres. It was not present in the larger vessels within the area of blood extravasation and degenerating blood cells.

The extensive vascular changes noted in this case are of considerable interest from the standpoint of legal medicine. As a result of traumatism of the head, with a linear fracture, the following series of changes are presented: Extensive cerebral necrosis with hemorrhagic extravasation into the cortex on the side of the lesion, internal hemorrhagic pachymeningitis on the opposite side, together with an area of external hemorrhagic pachymen-

ingitis on the posterior part of the dura on the side opposite to the bone lesion. There is a history of the excessive use of alcohol. How far are these changes due to the original trauma? It has been held by experts in court that concussion with a prolonged period of unconsciousness, without evidence of focal lesion or lesions, produces disturbances of the physical or nervous health of the individual, from which he practically never recovers. The lack of resistance to stress and strain in such cases, a matter of clinical observation, not only in medicolegal cases but also in cases without a cause for legal action is probably due to relatively minor changes in the cerebrovascular system as compared to those here noted.

In the history of this case, it is shown that the patient apparently recovered from the cerebral injury after the operation. A succession of vascular accidents beginning with the cerebral injury and possibly influenced by the excessive use of alcohol took place, leading finally to his death. It is reasonable to suppose that these changes would not have taken place in the absence of any such injury. Certainly, the exact nature of the changes was determined by the extensive laceration of the brain tissue incidental to the original brain injury. Extravasating hemorrhage into the pia arachnoid is a relatively rare pathological phenomenon. I have seen it in one other case affecting approximately the same distribution in the brain in a case of locomotor ataxia.

The necrosis of the cortical and subcortical tissue is difficult to explain. It is possible that the inflammatory adhesions of the pia arachnoid to the dura produced partial or complete obstruction of the cortical capillary or both the capillary and the cortical venous circulation. The pyramidal shaped areas would be more in favor of a subcortical capillary destruction.

No. 22

The Relative Value of the Various Methods for the Determination of Functional Kidney Sufficiency.

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It has long been conceded that the chief function of the kidney is the excretion, by processes of selective absorption, filtration and osmosis, of the effete products conveyed to it by the blood, but the precise manner in which this is accomplished by the intrinsic renal mechanism is a much mooted question and one that bids fair to attract and distract the attention of physiologic chemists of future generations. Nevertheless, the great importance of definitely determining the sufficiency or insufficiency of one or both kidneys is to the surgeon a problem of paramount consideration, and has furnished the incentive to urologists, universally, to announce untold functional kidney tests. The very variable value of these methods has demanded repeated attempts to perfect a thoroughly reliable technic. The result has been that during the past two decades the advancement in renal surgery has been second to none.

In defining the present status of kidney diagnosis, a twofold consideration becomes apparent: First, the instrumental, including the development of segregators, cystoscopes, ureteral catheters, etc., for the collection of the respective urines; and, second, the various functional tests, comprising the physical, chemical and physiological. The first is as important as it is interesting, but the compass of this article forbids its presentation.

It must be admitted that the most scientific as well as the most correct method of determining the function of the kidney is by quantitative metabolic study. Unfortunately, this can be carried out only under exceptional conditions, and even then it is not infallible, as has been shown by von Noorden.[1] Consequently it has been necessary to resort to other methods, more practical, to demonstrate the health or disease, that is, the functional activity of the kidney.

Ludwig demonstrated by animal experimentation that the work performed by each kidney varied at any given time. Israel, Kapsammer[2] and Albarran,[3] subsequently confirmed this deduction on the human body, the last stating that for short periods the difference amounted to thirty per cent., but that it decreased to ten per cent. for longer intervals and that in the course of ten or twelve hours the work of the two kidneys was identical. Casper and Richter[4] have at all times vigorously assailed this doctrine and maintain that healthful kidneys simultaneously excrete practically identical urines. This dictum is substantially supported by Bardier, Fedorow, Frenkel and Friedrich Strauss. This is the keynote of modern renal diagnosis and sounds the fundamental law governing its utility and practicability.

The requisition demanded of any functional renal test is twofold; first, the determination of the combined functional ability of both kidneys, and secondly, the functional sufficiency of each kidney separately. In the practice of the surgeon

[1] Von Noorden: Metabolism and Practical Medicine, Vol. 2, p. 502.
[2] Kapsammer: *Wien. klin. Wochenschr.*, 1903, p. 1417.
[3] Albarran: Ann. d. Mal. d. Org. Genito-urin., 1904, p. 81.
[4] Casper and Richter: Funktionelle Nierendiagnostik, 1901. Handbuch der Cystoskop., 1905.

this means the diagnosis and the extent of disease of a given kidney, and the prognosis in view of nephrotomy or nephrectomy relative to the sufficiency or insufficiency of the sister organ. The ideal qualifications for such a test should be (1) applicability with as simple a technic as possible; (2) ease of application and accuracy of interpretation; (3) avoidance of general anesthesia if possible; (4) determination, within narrow limits, of the amount of work performed by normal kidneys; (5) constancy of variation in the presence of uniform abnormal conditions; (6) indication of functional alteration, when such exists, even in the absence of histo-pathological changes; (7) demonstration of routine function, as well as the compensatory ability of the kidney under strain.

Deductions will be least erroneous if dependent upon two or more functional tests. The urologist is quite as much entitled to be conversant with the history and physical examination of his patient, before rendering an opinion on the result of his functional test, as is the pathologist to know the history and have the gross specimen for study, prior to submission of his diagnosis, following the microscopical examination of tissue.

Quantity of Urine. It must have been recognized by Aesculapius that the daily output of urine bore a definite relationship to the health or disease of the kidneys. Consequently, it is logical to infer that in the twenty-four-hour collection of urine, we have the oldest as well as the most important measure of the functional integrity of the kidneys.

Odor Tests. Among the earliest observations on the variations in renal elimination was the diminution or absence in nephritics of that peculiar odor commonly present following the eating of asparagus. Again the absence of the odor of violets in the urine of gouty subjects after the injection of turpentine was noted. These have been merely interesting phenomena and are not only too crude, but have never been applied in practical functional tests.

Physical, Chemical and Microscopical Tests. The color, concentration, specific gravity, albumin and casts are no longer regarded as conclusive evidence of renal insufficiency. It is now well known that both albumin and casts may be absent in nephritis and, conversely, that the presence of these accidental urinary constituents does not insure primary disease of the kidney.

Normal Urinary Constituents. For a time it was believed that the problem of renal sufficiency or insufficiency could be solved by the quantitative estimation of urea, chlorids, phosphates, total nitrogen, creatinin, etc. Such a method is manifestly fallacious, inasmuch as the output of these substances is markedly influenced by the amount carried to the kidneys for elimination. To be of any value these tests must be dependent upon definite known intake of fats, carbohydrates, proteids, etc. This involves extensive and impractical metabolic studies and even then, as has been stated, the results are not always dependable.

Valueless as may be the determination of the amount of these salts in the total urine, it is indisputable that their comparative determination, from synchronously catheterized ureters, is decidedly valuable. Particularly is this true if there be much difference between the two sides. It should be remembered, however, that the most important of these substances is urea, and its determination is merely a link in the chain of functional renal diagnosis. Moreover, the Doremus ureometer is the instrument commonly employed for its determination and the results are very inaccurate.

Drugs. The toxicity produced by certain drugs in renal disease probably directed

attention to the fact that they might be employed advantageously in functional kidney diagnosis. Among the various drugs in which this retardation of elimination occurred may be named alkaline carbonates, Dover's powder, iodin, mercury, potassium, quinin, sodium and salicylate preparations. (Chauvet,[5] Charcot and Cornil,[6] Duckworth,[7] Roberts,[8] and Todd.[9])

Fuchsin. The factors operating to jeopardize the efficiency of any drug for purposes of testing the activity of the kidneys must be patent to all. Consequently, drugs failed of their purpose and have long since been forgotten. (The recently advocated phenolsulphonephthalein may prove an exception to this statement.) Various anilin dyes grew into favor and have effectually supplanted drugs. The first of these was fuchsin, introduced in 1877 by Bouchard.[10] The idea was clever but the selection of the dye proved to be a failure and was little used, the results being very unsatisfactory.

Methylene Blue. Twenty years later, Achard and Castaigne[10] introduced methylene blue as a test for the functional ability of the kidney. This marked the advent of so-called chromocystoscopy, which in its present perfected technic is the greatest boon of urological diagnosis. By the employment of a dye it became possible to render opinion relative to the activity of the kidneys without resort to ureteral catheterization, an act which, theoretically at least, it must be admitted, may be conducive to the production of an ascending infection of the ureter and kidney, remote as the possibility may be. In the employment of methylene blue, as in all other anilin dyes used for the determination of the kidney function, the following factors are considered in drawing conclusions: (1) The initial onset of the excretion of the coloring matter, (2) the time of maximum intensity of the elimination, and (3) the duration of the excretion. The Germans soon raised serious objections to the use of methylene blue. It was found that it was occasionally eliminated as colorless chromogen and therefore possessed inconstancy of color reaction. Pugnat and Revilliod[11] have shown that occasionally the dye undergoes unknown chemical changes in the body and even in health can not be demonstrated at all in the urine.

The dye does not make its appearance as a color substance for one half hour following injection, the elimination for the fifteen minutes previously occurring as a chromogen, requiring the addition of acetic acid and heat to elicit the color. Furthermore, the duration of elimination, even in health, may continue for six days and during this period, as pointed out by Chauffard[12] and Chauffard and Cavasse,[13] the excretion is not continuous and uniform, but irregular and intermittent. Therefore, because of its inconstancy, variability and prolonged duration of elimination methylene blue has fallen into disrepute.

Rosanilin. Rosanilin or rosanilin trisulphate of soda was first introduced by Lepine[14] in 1898. It possesses the following advantages over methylene blue. It is eliminated with greater intensity, the duration of excretion describing a monocyclic curve in contradistinction to the polycyclic curve of methylene blue; also the duration of its elimination is not so prolonged. Furthermore, it is almost entirely eliminated by the kidneys. Rosanilin is one of the very best dye substances that can be utilized for testing the renal activity. Nev-

[5]Chauvet: Thèse, Paris, 1877.
[6]Charcot and Cornil: On Bright's Disease (Millard), 1878.
[7]Duckworth: St. Bartholomew's Hospital Reports, 3, p. 216.
[8]Roberts: A Practical Treatise on Urinary and Renal Diseases, Including Urinary Deposits, 1865.
[9]Todd: Clinical Lectures on Certain Diseases of the Urinary Organs, and on Dropsies, 1857.
[10]Achard and Castaigne: Bull. et mém. soc. méd. d. hôp. de Paris, April, 1897; p. 637. *Gaz. Hebd. de Méd.*, Paris, 1897, No. 37, p. 433.
[11]Pugnat and Revilloid: *Arch. Gén. de Méd.*, 1902, Vol. 8, p. 19.
[12]Chauffard: *La Presse Méd.*, 1898, Vol. 1, p. 13.
[13]Chauffard and Cavasse: *La Presse Méd.*, 1898, Vol. 1, p. 129.
[14]Lepine: *Lyon Méd.*, 1898.

ertheless, it has never attained any popularity.

Cryoscopy. By virtue of the process of osmosis, occurring through the capillary walls, Bowmann's membrane and the epithelium of the tubules, the waste products of metabolism conveyed by the blood are eliminated. Raoult and Van't Hoff have shown that osmotic pressure is proportional to the molecular concentration of the solution. Dreser[15] demonstrated that the molecular concentration of a fluid could easily be determined by the determination of the lowering of the freezing point and, subsequently, Koranyi[16] in 1898 was the first to make practical the method of determining by cryoscopy the work performed by the kidney. The correctness and the value of Koranyi's work has been ably confirmed by many investigators (Albarran, Bernard and Bousquet,[17] Bouchard,[18] Claude and Balthazar,[19] Lindemann,[20] Moritz,[21] Richter and Roth,[22] Senator[23]) and the practical value, clinically, of the determination of the lowering of the freezing point as a test of renal sufficiency is to-day staunchly supported and practiced by no others than Casper and Richter,[4] Kümmell[24] and Rumpel.[25] The first do not place reliance solely on cryoscopy, but draw their conclusions from the associated physical, chemical, urea, and phloridzin findings of synchronously catheterized specimens. The last two vigorously uphold the merits of cryoscopy, emphasizing the importance of comparative determinations of the lowering of the freezing points of both blood and urine as originally recommended by Bernard.[26] Under normal conditions the urine exhibits a range from —0.9 degrees to —2.3 degrees, and healthy kidneys in a given subject may give a variation of 0.4 degrees. Moreover, conditions of polyuria and anemia seriously embarrass the results of lowering of the freezing point of the urine. The normal blood, on the other hand, shows a fairly constant freezing point of —0.55 degrees to —0.57 degrees. The bone of contention lies in the question whether or not operation shall be done in case of a freezing point under —0.60. Albarran, Kapsammer and Rovsing head the affirmative; Kümmell the negative, with a statistical report commanding the greatest consideration.

My belief, relative to the value of cryoscopy *per se*, as a test to determine the renal function, is that as an indication or contraindication to surgical intervention, it is of importance only in relation to the urines of each kidney separately as compared with the molecular concentration of the blood and that cryoscopy of the total urine alone or of the blood alone is valueless. Such a procedure entails bilateral synchronous ureteral catheterization, an act which can not be accomplished in all cases. Therefore, although in disease of the kidneys the molecular concentration of the blood is increased and the freezing point lowered, while the molecular concentration of the urine is diminished and the freezing point raised, and comparative cryoscopy of the blood and urine the most accurate way of determining this molecular concentration, so many factors influence the character of the urinary output, as, water, diet, etc.,

[15]Dreser: *Arch. f. exper. Pathol. u. Pharmacol.*, 1892, Vol. 29, p. 303.

[16]Koranyi: *Zeitschr. f. klin. Med.*, 1897, Vol. 33, p. 1. Ibid., 1898, Vol. 34, p. 1.

[17]Albarran, Bernard and Bousquet: Sur la cryoscopie appliquée à l'exploration de la fonction rénale, 41ème session de l'association franc. d'urologie, Paris, 1900, p. 495.

[18]Bouchard: Molécule urinaire. *Jour. de Physiol. et de Path. Générale*, 1899, Vol. 1, No. 3.

[19]Claude et Balthazar: La cryoscopie des urines dans les affections du coeur et des reins. *La Presse Méd.*, 1900, No. 37.

[20]Lindemann: Die Konzentration des Harnes und Blutes bei Nierenkrankheiten, mit einem Beitrage zur Lehre von der Urämie. *Deutsches Archiv. f. klin. Med.*, 1899, Vol. 65.

[21]Moritz: Über den klinischen Wert der Gefrierpunkts-bestimmungen. *St. Petersburger med. Wochenschr.*, 1900, No. 22.

[22]Richter and Roth: Experimentelle Beiträge zur Frage der Niereninsufficienz. *Berliner klin. Wochschr.*, 1899, No. 30 and 31.

[23]Senator: Weitere Beiträge zur Lehre vom osmotischen Druck i. thierischen Flussigkeiten. *Deutsche med. Wochenschr.*, 1900, No. 3.

[24]Kümmell: *Münchner med. Wochenschr.*, 1900, No. 44; Verhandl. d. Deutschen Gesellsch. f. Chir., 30 Kongress, Berlin, 1901; Ibid. 31, Kongress, Berlin, 1902.

[25]Rumpel: *Beitrag z. klin. Chir.*, 1901, Vol. 29; *München. med. Wochenschr.*, 1903, No. 1, 2 and 3.

[26]Bernard: *La Presse Méd.*, Feb. 17, 1900, p. 159.

and the technic of cryoscopy is so laborious that, for the urologist, it is already devoid of popularity.

Phloridzin. In 1885 von Mering demonstrated that peculiar property possessed by phloridzin of producing an artificial glycosuria following its subcutaneous injection, by virtue of its specific action on the renal parenchyma. Casper and Richter[4] in 1899 were the first to introduce the use of this substance in functional kidney diagnosis. For a decade phloridzin was heralded by many as deserving of first choice in the realm of functional renal tests. Achard and Delamere[27] showed that it was superior to methylene blue in acute and subacute nephritis, since, in these affections, the excretion of the latter might be not only normal but increased. Some have drawn their conclusions from the percentage of sugar eliminated; others from the total quantity excreted, and Kapsammer, particularly, has directed attention to the time of appearance of sugar in the urine.

The original popularity of phloridzin is rapidly disappearing. Grave fallacies exist. It would appear that the test is too sensitive and allows of exaggerated ideas of the extent of disease encountered. On the other hand, wide variations have been observed in normal kidneys and even an absence of glycosuria after injection has been noted in cases demonstrating no pathological condition. Therefore, owing to serious inconsistencies, the fact that the solution must be prepared fresh each time and the necessity of laborious, prolonged and sometimes impossible technic, phloridzin has been found to be less dependable than many other functional tests.

Electrical Conductivity. The determination of the electrical conductivity of the urine was announced by Schäfer[28] and Koeppe.[29] It depends upon the salt content of the fluid, that is, the number of ions, and is measured in ohms of resistance. Consequently, it takes into consideration, principally, the mineral content of the urine. The application of this method employs the Kohlrausch apparatus after the principle of the Wheatstone bridge. As in cryoscopy, the test to be of real value must be a comparative one of both the blood and urine. Turner[30] states, "A blood of high resistance indicates that the proportion of salts in the blood is small or that the proportion of corpuscles present is large. Hence a high resistance of the blood but a low resistance of the urine is indicative of health." Consequently, a hemorenal salt index has been suggested. This has been determined to be 3. An index above 3 means increased health; below 3, disease, and contraindicative of surgical intervention.

The method possesses certain advantages over cryoscopy, in that much smaller quantities of blood and urine are required, and that it can be carried out much more quickly. Electrical conductivity as a functional kidney test is destined never to become popular, because of the complexity and cost of the apparatus, the training and skill required for its performance and the fallacious results obtainable unless the water intake and diet are accurately determined.

Indigocarmin. Indigocarmin was first employed by Heidenhain[31] in studying the physiology of the kidneys. He also proved that the elimination through the kidneys occurred constantly as a blue coloring matter and that the anilin color substance is excreted from the epithelium of the tubules, while from the glomeruli a colorless secretion takes place. Völcker and Joseph[32] in

[27] Achard and Delamere: Bull. et mém. de la Soc. Méd. des hôp., Anr. 7, 1899, p. 379.

[28] Schäfer: Reaktion, Leitfähigkeit und Gefrierpunktserniedrigung des normalen menschlichen Harnes. Inaug.-Dissert. Giessen, 1900.

[29] Koeppe: Zur Kryoskopie des Harnes, *Berliner klin. Wochenschr.*, 1901, No. 28.

[30] Turner: *Edinburgh Med. Jour.*, Apr., 1907; Practical Med. Chem., 1904, p. 190.

[31] Heidenhain: (1) Handbuch de Physiologie; (2) Arch. f. mikrosk. Anatomie, Vol. 10, p. 1; (3) Pflügers Archiv., Vol. 9, p. 1.

[32] Völcker and Joseph: *München med. Wochenschr.*, 1903, No. 48.

1903, however, were the first to introduce carmin ceruleum as a valuable measure in functional kidney diagnosis. Since that time this anilin substance has enjoyed an ever increasing and amazing popularity. (Bier,[33] Blum and Prigl,[34] Blumreich,[35] Döderlein and Krönig,[36] Fueth,[37] Hofmeier,[38] Joseph,[39] Karo,[40] Kapsammer,[41] König,[42] Kümmell,[43] Keydel,[44] Kakels,[45] Rosemann,[46] Riese,[47] Rauscher,[48] Sellheim,[49] Suter,[50] Thelen,[51] Thomas,[52] Vogel,[53] Zangemeister.[54])

Indigocarmin exhibits the following points of superiority over any other anilin substance used for the determination of the renal fuction: (*a*) Earliness of onset of elimination, (*b*) constancy of color reaction, (*c*) intensity of color excretion, (*d*) shorter duration of elimination, (*e*) ease of application of test, (*f*) ability to draw immediate conclusions, and, not least of all, (*g*) absence of dependence upon ureteral catheterization.

[33]Bier: *Deutsche med. Wochenschr.*, 1904, No. 19.
[34]Blum and Prigl: Quoted by Knorr, *Zeitschr. f. gynäkol. Urol.*, 1908, Vol. 1, No. 1, p. 64.
[35]Blumreich: *München med. Wochenschr.*, 1904, No. 22.
[36]Döderlein and Krönig: Quoted by Knorr, *Zeitschr. f. gynäkolog. Urolog.*, 1908, Vol. 1, No. 1, p. 64.
[37]Fueth: *Zentralbl. f. Gynäkol.*, 1904, No. 17.
[38]Hofmeier: *Münschen med. Wochenschr.*, 1904, p. 455.
[39]Joseph: Diagnose der chirug. Nierenkrankungen unter Verwertung der Chromocystoskopie, 1906.
[40]Karo: (1) *München med. Wochenschr.*, 1904, No. 3; (2) *Monatsschr. f. Urologie*, Vol. 9, No. 1.
[41]Kapsammer: (1) *Weiner klin. Rundschau.*, 1904, No. 6; (2) *Weiner med. Wochenschr.*, 1904, No. 26 and 51; (3) *München med. Wochenschr.*, 1905, No. 17.
[42]König: Quoted by Knorr., *Zeitschr. f. gynäkolog. Urolog.*, 1908, Vol. 1, No. 1, p. 64.
[43]Kümmell: Verhandl. d. deutsch. Gesellsch. f. Chirurgie, 1904.
[44]Keydel: *Zentralbl. f. Harn-und Sexual organe*, Vol. 16, No. 5.
[45]Kakels. *Zentralbl. f. Chirurgie*, 1905, p. 1273.
[46]Rosemann: *Deutsch. med. Wochenschr.*, 1904, No. 38.
[47]Riese: *Deutsch. med. Wochenschr.*, 1905, No. 3.
[48]Rauscher: *München med. Wochenschr.*, 1904, No. 37.
[49]Sellheim: *Beitr. z. Geburtsh. u. Gynäkol.*, Vol. 9, p. 413.
[50]Suter: (1) Korrespondenzbl. f. Schweizer Artze, 1904, No. 18; (2) *Zeitschr. f. Urolog.*, 1908, Vol. 2, No. 5, p. 423.
[51]Thelen: *Zeitschr. f. Urologie*, 1908, Vol. 2, No. 2, p. 140.
[52]Thomas: (1) Chromocystoscopy in Functional Renal Diagnosis Based upon the Employment of Indigocarmin. *Surg., Gynecology and Obstet.*, April, 1909, pp. 368-375; (2) Chromoureteroscopy in Functional Kidney Diagnosis, *Zeitschrift für Urologie*, 1911, Vol. 2, or *Surgery, Gynecology and Obstetrics*, March, 1911.
[53]Vogel: *Deutsch. med. Wochenschr.*, 1905, No. 7.
[54]Zangemeister: (1) *Deutsch. med. Wochenschr.*, June, 1903; (2) *Zeitschr. f. Geburtsh. u. Gynäkol.*, Vol. 55.

It is rarely necessary to observe more than the initial onset of the excretion of the dye. Although other substances, as, rosanilin and phenolsulphonephthalein are more completely eliminated by the kidneys, that fact seems to play no part in the value of indigocarmin as a functional test even when resort is had to quantitative determinations. Oppenheimer[55] has attempted to use the Duboscq colorimeter to determine the percentage of dye output, but found that the color did not lend itself satisfactorily to colorimetric readings on account of peculiar color reactions produced by the urinary constituents. I have succeeded in reducing this difficulty by using urine instead of water as a diluting medium both for the control and specimen. Moreover, even in employing distilled water, I have found the chlorimetric readings just as trustworthy in the case of indigocarmin as with other substances, notably, phenolsulphonephthalein, which has been enthusiastically launched recently as a valuable renal test by Rowntree and Geraghty.[56] Although it is acknowledged that 25 per cent. of the dye is excreted by the kidneys during the first 24 to 48 hours after injection, I have found that 10.17 per cent. is eliminated in the first 3 hours, and that only 4.7 per cent. of the total output for the first 4 hours is eliminated during the fourth hour, whereas, in the case of phenolsulphonephthalein, 8.1 per cent. is eliminated under similar conditions, proving, therefore, that the elimination of indigocarmin is more rapid and presumably of shorter duration than phenolsulphonephthalein. I have also proved that the onset of elimination of indigocarmin is earlier than phenolsulphonephthalein. In short, from my experience, although at times additional data are to be obtained from a study of the

[55]Oppenheimer: Verhandlungen der deutschen Gesell. f. Urol., 1909, 2nd Kongress, p. 289.
[56]Rowntree and Geraghty: An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, *Jour. of Pharmacol. and Exper. Therapeut.*, July, 1910, Vol. 1, No. 6.

intensity and duration of the elimination, it is my belief, that a correct observance of the onset of the excretion of the dye is all-sufficient for the practical purposes of the surgeon.

Albarran's Experimental Polyuria Test. In 1904, Albarran established two facts: First, that a diseased kidney functionates more uniformly than its sister organ and that the uniformity of function is directly proportional to the extent of the disease, and, secondly, that in the case of unilateral disease, if the kidneys are subjected to additional forced work, the increase of function occurs on the healthy side. These facts form the basis of the experimental polyuria test. The fundamental procedure in the technic is bilateral synchronous ureteral catheterization. Urine is collected from the two sides for one half an hour. The patient is then required to drink 400 to 600 c.c. of water, and the urine again collected at half-hour intervals for three hours. Conclusions are then based upon the amounts collected before and after the forced intake of water, with particular reference to the work performed by each kidney. In determining the efficiency of the renal function, Albarran does not depend upon the polyuria test alone, but in conjunction with cryoscopy, phloridzin, total chlorids and urea.

The test is not only impractical, but may be fallacious for the following reasons: (1) Except in the female, it is frequently impossible to employ ureteral catheters that will completely occlude the ureters, thereby preventing an escape of urine around one or both catheters into the bladder; (2) two or three hours is an unjustifiably long time to allow the ureteral catheters to remain *in situ;* (3) the inability of some patients to drink the quantity of water required; (4) occasionally it will be impossible to produce polyuria by the increased water intake; and (5) in slight lesions of the kidneys, there will be no perceptible difference between the functions of the two sides.

Phenolsulphonephthalein. The latest functional renal test is that by the use of phenolsulphonephthalein, first prepared by Remsen[57] and later described by Sohon.[58] During the present year, Rowntree and Geraghty[59] have made an exhaustive study of the experimental and clinical value of this substance as an agent in the determination of the sufficiency of the kidneys. They are enthusiastic over its merits, stating that it possesses advantages over all other functional tests and is better adapted for the purposes of such a test than any other drug thus far utilized.

The technic of the test again necessitates the synchronous bilateral catheterization of the ureters. The patient is required to drink 600 to 800 c.c. of water prior to the administration of the drug. Urine is collected from both sides for the physical and chemical tests. One cubic centimeter of solution, containing six milligrams of phenolsulphonephthalein is then injected subcutaneously, and the urines collected in test tubes, each containing one drop of twenty-five per cent. sodium hydroxid. The drug in acid urine produces an orange yellow, but in alkaline solution is transformed into a brilliant amethystine red. In addition to noting the onset of elimination, Rowntree and Geraghty lay particular stress on the quantitative percentage elimination of the drug for the first two hours after injection. This they claim is most important and reliable because of the relatively high, speedy and complete elimination of phenolsulphonephthalein. For quantitative determinations, the Duboscq colorimeter is most serviceable and very accurate.

I have made comparative studies of the value of phenolsulphonephthalein and indigocarmin on the human body. Although my evidence is not extensive enough to be

[57] Remsen: *Amer. Chem. Jour.*, Vol. 6, 280.
[58] Sohon: *Amer. Chem. Jour.*, Vol. 20, 257.

	Onset.	Percentage of Elimination.				
		1st Hour.	2d Hour.	3d Hour.	4th Hour	Total
Indigocarmin	R-10 minutes L-7 "	3.35	5 47	1.35	0.51	10.68
Phenolsulphonephthalein	R-18 minutes L-18 "	11.00	6.95	3.22	1.89	23.06

conclusive the findings in the submitted normal case will suffice to illustrate my results thus far. (See table on next page.)

It will be observed, contrary to the statement of Rowntree and Geraghty, concerning the early appearance, rapidity and completeness of the elimination of this phthalein by the kidney, that the initial onset of the excretion of the drug is delayed approximately twice as long as indigocarmin and that during the fourth hour after injection the percentage of excretion of phenolsulphonephthalein was over three times that of indigocarmin, indicating that the duration of elimination of the former was longer than the latter. Moreover, the employment of the phenolsulphonephthalein is attended with the following disadvantages: It depends upon bilateral ureteral catheterization, which is an impossibility in some cases. By virtue of the use of an alkaline color indicator the technic is slightly more complicated than in some other tests. The quantitative determination unduly prolongs the examination. I can not agree that the colorimetric properties of phenolsulphonephthalein lend themselves for Duboscq readings to a better advantage than indigocarmin, and in the latter case the onset of elimination is of greater practical value than quantitative determinations. Its sole advantage over indigocarmin lies in the lesser amount employed for injection, being in the ratio of 1:20, consequently less productive of local irritation. This, however, is inconsiderable and negligible when properly used as opposed to the other merits of indigocarmin.

SUMMARY AND CONCLUSIONS.

1. The most accurate and dependable test for the determination of the renal function is that by quantitative metabolic study. Unfortunately, save in rare instances, such a study is not only impractical, but impossible, and for the purposes of the surgeon, wholly superfluous.

2. It is admittedly beyond dispute that the association of two or more tests is productive of more reliable data than the results of a single determination.

3. Any test or tests dependent upon synchronous bilateral catheterization of the ureters, for collection of urine from the respective sides, has a limited field of applicability, inasmuch as, in those advanced cases where it is commonly most desirable to determine the sufficiency or insufficiency of one or both kidneys, it will be impossible to catheterize both or either ureter. Consequently, the vast majority of the functional kidney tests which have been advanced and recommended are doomed to unpopularity and inevitable oblivion.

4. It will be found, that in seventy-five per cent. of cases, with a symptomatology referable to vesical and renal conditions, the simple cystoscope will localize the lesion in the bladder. Supplement this, when indicated, with one of the anilin-dye tests (chromoureteroscopy), notably indigocarmin, and the field of kidney diagnosis can be boldly trespassed.

5. The cystoscope of greatest utility is the type of the Buerger-Brown or Schlagintweit instrument, because, by virtue of the irrigation-evacuation principle, the mechanism is such as to render simple cystoscopy, chromoureteroscopy or ureteral catheterization possible, whereas, by the use of other instruments, such acts may be impossible in troublesome cases of pyuria and hematuria.

6. A review and analysis of the various tests herein discussed demonstrate that, for the surgeon, at least, the most valuable functional kidney test is that by the employment of indigocarmin, not that it may

be infallible—none fulfills that exigency—but because it is paramountly the most practical.[59]

DISCUSSION.

Dr. A. I. Murphy, Pittsburg: In Casper's polyuria test we occasionally see that the output of urine from the diseased kidney exceeds the output of urine of the healthy kidney. My experience with phloridzin is limited to cases of prostatic hypertrophy with unmistakable signs of renal disease; and in these cases the phloridzin produced glycosuria in three or four hours after injection and in some cases it never occurred; this I think is a disadvantage, showing that in these cases of hypertrophy of the prostate phloridzin is not a valuable functional test. The determination of the urea output is very valuable and perhaps has been the most reliable test when used in unilateral kidney disease.

I wish to speak now concerning the newest method of determining renal function, that is by the means of phenolsulphonephthalein test which was first developed by Rowntree and Geraghty of Baltimore during the last year.

Phenolsulphonephthalein is a crystalline powder, bright red in color, somewhat soluble in water, more soluble in salt solution and more so in alcohol. It is one of the members of the phenophthalein family. It is eliminated almost entirely by the kidney. In experimental work upon animals and human beings no toxic conditions were ever found even after the administration of one-gram doses. The dose used in the functional test is 0.6 mg. This is injected with an accurate syringe subcutaneously in the upper arm. One half an hour previous to the test the patient should drink 200 to 400 c.c. of water in order to insure a free excretion of urine. The time of appearance in the urine varies from five to eleven minutes and its presence in the urine is detected by adding a drop of a weak sodium hydroxid solution which changes the acidity of the urine to alkaline thus bringing out the color of the drug, which appears in the early stages of the test as a very faint pink, growing a deeper red as time progresses. In unilateral kidney disease one hour for the collection of the urine suffices to determine the function of each kidney. In cases where only a urethral catheter is used as in prostatic hypertrophy it is better to collect the urine for two hours, each hour's secretion being collected in a separate vessel. This is necessary so that we can estimate total function of one kidney with the function of the other in order to find which kidney is diseased. The amount of the drug eliminated varies from forty to sixty per cent. the first hour, and twenty to twenty-five per cent. in the second hour. Elimination is practically over in two hours.

Owing to its beautiful and stable color the phenolsulphonephthalein is very suitable for colorimetric work. The colorimeter I use for this estimation is the Duboscq and the technic is quite simple. The urine for the estimation is diluted up to 1000 c.c. with distilled water and is made positively alkaline by a drop of sodium hydroxid and this is compared with a standard solution of phenolsulphonephthalein containing 3 mg. to the 1000 c.c. which is just one half the amount of the phenolsulphonephthalein injected. The two cups of the colorimeter are filled with these solutions and a comparison made by the colorimeter. The indicators on the colorimeter point to the Vernier scale. After the solutions have been equalized in color by adjusting the colorimeter, the indicator on the side of the urine test is read and the estimation of the amount of phenolsulphonephthalein excreted is then an easy problem in arithmetic.

This test has given excellent information of the functional power of both kidneys. However the findings must not be considered alone, but must be compared with a thorough clinical examination of the patient. I think that not enough work on nephritis cases has been done with this test to estimate its value there. But undoubtedly it will open a great field for investigators of nephritis and also in eclampsia. In prostatic hypertrophy cases, it is an excellent index to the renal function, the importance of this aid being invaluable to the surgeon when in doubt as to the best time of operation for his patient.

[59] For the detailed technic of the employment of indigocarmin consult the author's article on Chromoureteroscopy in Functional Kidney Diagnosis, *Zeitschrift für Urologie*, 1910, Vol. 2, or *Surgery, Gynecology and Obstetrics*, March, 1911.

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CONTRIBUTIONS

FROM THE

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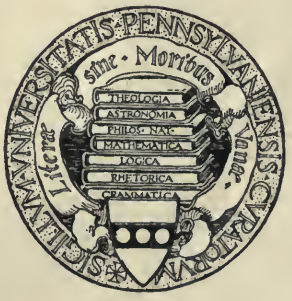
(REPRINTS)



No. 9

PHILADELPHIA
1918

CONTENTS

FURTHER OBSERVATIONS ON THE EMPLOYMENT OF SPECIFIC AND NON-SPECIFIC ANTIGENS IN THE PERFORMANCE OF THE GONOCOCCIC COMPLEMENT-FIXATION TEST *

B. A. THOMAS, M.D., R. H. IVY, M.D.,
AND
J. C. BIRDSALL, M.D.
PHILADELPHIA

In a former paper[1] read before the Philadelphia County Medical Society May 28, 1913, it was conclusively shown by Thomas and Ivy in an analysis of over 200 cases in which monavalent, trivalent, hexavalent and a commercial antigen of twelve strains of gonococci were employed, that the gonococcus complement-fixation test possesses great specificity so far as positive results are concerned. The results have proved that "the different strains of the gonococcus differ markedly one from another — so much so that the antibodies produced in the body by the toxin of one strain will in many instances not bind the complement in the presence of an antigen prepared from another strain. Therefore, if only one strain is used in the preparation of the antigen, a great many negative results would be obtained in positive cases; an antigen prepared from many strains fixes the complement whenever one of its component strains does so, and consequently the necessity of testing a serum against a number of antigens separately is avoided. It is not to be denied that there probably are other strains of gonococci differing widely from any present in the polyvalent antigen, so that at times a negative result will be obtained in a positive case."

Convinced from our previous study of the specificity of the gonococcus complement-fixation test in gonorrheal infections, namely, that although a negative reaction may be obtained in gonorrheal subjects and consequently is devoid of reliance, a positive reaction is most dependable and was not obtained in a large series of infectious and other diseases.

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* Read before the Philadelphia Pathological Society, May 28, 1914.

* From the Department of Genito-Urinary Surgery and Laboratories, Polyclinic Hospital, and the William Pepper Laboratory of Clinical Medicine.

1. Thomas, B. A., and Ivy, Robert H.: The Gonococcus Complement-Fixation Test and Analysis of Results from its Use, The Archives Int. Med., 1914, xiii, 143.

In this article, in view of the finding of many Gram-positive and Gram-negative bacteria in the urine after massage of the prostate gland and seminal vesicles in the involvement of these organs in neisserian infection, a study has been made with respect to determining the specificity of the gonococcus antigen in the complement-fixation test by employing non-specific antigens made up from the various bacteria isolated from time to time.

Antigens were prepared from the following micro-organisms and utilized routinely in a series of serums from gonorrheal subjects:

Nine strains of gonococci.[2]
Fifteen strains of meningococci.[2]
Six strains of streptococci.
Six strains of the *Micrococcus albus.*
Six strains of the pneumococcus.
Six strains of *Micrococcus aureus.*
Three strains of the *Micrococcus catarrhalis.*[2]
Six strains of *Corynebacterium pseudodiphtheriticum.*
Six strains of *Bacillus coli.*

TECHNIC OF THE PREPARATION OF THE ANTIGENS

As in the former work the best results were obtained with antigens prepared in the following manner:

Forty-eight-hour old cultures were washed off in sterile distilled water, shaken for one hour, and autolyzed for twenty-four hours in a thermostat at the temperature of 37 C. and heated in a water-bath at 60 C. for one-half hour. Before use this antigen is diluted 1 : 10 by the addition of 0.85 per cent. salt solution. The quantities of each antigen used is determined by preliminary standardization. The technic on which we have learned to place the greatest reliance is essentially the same as that employed by us in the performance of the Wassermann reaction — substituting the specific or non-specific antigen in each case for the syphilitic antigen, using always the carefully standardized single unit of complement and the routine standardization of antigen and amboceptor. This technic is fully described in the former paper on this subject.

Two hundred and sixteen serums in all were tested by the employment of various non-specific antigens. These added to the results of the previous work number 420 serums in which the complement-fixa-

2. We are indebted to Dr. Parks of the Research Laboratory, New York Department of Health, for the fifteen strains of meningococci and the three strains of *Micrococcus catarrhalis,* and to Dr. A. P. Hitchens of H. K. Mulford Company for the nine strains of gonococci employed in this work.

tion test has been employed, using specific gonococcus and non-specific antigens.[3]

Of the 216 cases in which both the specific and non-specific antigens were employed, we have grouped the cases according to their clinical diagnoses.

1. Patients clinically cured, 9 cases.
2. Acute anterior urethritis, 10 cases.
3. Acute and subacute anteroposterior urethritis, 40 cases.
4. Chronic posterior urethritis, 84 cases.
5. Stricture, 7 cases.
6. Epididymitis, 30 cases.
7. Arthritis, 30 cases.
8. Gynecological affections, 3 cases.
9. Vulvovaginitis, 1 case.
10. Sexual impotence, 2 cases.

Results of Complement-Fixation Reactions with Specific Gonococcic and Non-Specific Antigens in Two Hundred and Sixteen Cases

Antigens	No. Cases	Results No Positive	No. Negative
Specific; Gonococcic:			
Nonovalent	216	67	149
Parke, Davis & Co.	216	67	149
Non-Specific:			
Micrococcus catarrhalis.....	180	5	175
Pneumococcus	216	4	212
Micrococcus aureus	216	3	213
Streptococcus	216	1	215
Corynebacterium pseudodiphtheriticum	160	1	159
Meningococcus	216	1	215
Micrococcus albus	216	....	216
Bacillus coli	160	....	160

Of this series of cases 135 serums gave negative results with the employment of specific and non-specific antigens.

Sixty-seven serums gave positive results with specific gonococcus antigens.

Fifteen serums gave positive results with non-specific antigens.

Of the complement-fixation tests, using non-specific antigens, the *Micrococcus catarrhalis* antigen gave positive results in 5 cases, the pneumococcus in 4, the *Micrococcus aureus* in 3, the streptococcus in 1, the *Corynebacterium pseudodiphtheriticum* in 1 and the meningococcus in 1 case.

3. Standardization of all the specific and non-specific antigens at the conclusion of this study as compared with their antigenic properties in the beginning demonstrated no deterioration.

Four of the foregoing non-specific antigens gave positive results when all other non-specific and specific antigens resulted negatively. They were:

1. Pneumococcus, 4 cases.
2. *M. aureus,* 3 cases.
3. *M. catarrhalis,* 1 case.
4. *Corynebacterium pseudodiphtheriticum,* 1 case.

Six of the foregoing fifteen non-specific fixation reactions occurred conjointly with the specific fixation reaction. They were:

The *Micrococcus catarrhalis,* 4 cases.

The streptococcus, 1 case.

The meningococcus, 1 case.

Our explanation for these occurrences in the complement-fixation reaction is that frequently a mixed infection complicates the gonorrheal urethritis, prostatitis, seminal vesiculitis, etc., also that not infrequently the gonococcus has ceased to be viable and that the active cause for the inflammation is a superimposed bacterium.

CONCLUSIONS

1. Although mixed infections are commonly found, antibodies of the non-specific organisms rarely bind complement in the presence of the non-specific antigens, and when such is the case, it can be attributed to the implantation of a superimposed mixed infection.

2. The specificity of the gonococcus complement-fixation test when positive in cases of neisserian infection seems to be clearly established; a negative reaction, on the contrary, means absolutely nothing from the clinical point of view.

3. Those organisms in cases of mixed infection capable of binding complement in our studies have been the *Micrococcus catarrhalis,* the pneumococcus, the *Micrococcus aureus,* the streptococcus, the *Corynebacterium pseudodiphtheriticum* and the meningococcus.

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OBSERVATIONS ON THE GONOCOCCUS COMPLEMENT-FIXATION TEST EMPLOYING SPECIFIC AND NON-SPECIFIC ANTIGENS [1]

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SUPPLEMENTARY to work undertaken about a year since and announced for subsequent publication in an article[2] read before the Philadelphia County Medical Society, May 28, 1913, we desire at present to summarize briefly the results obtained in an analysis of several hundred sera subjected to the complement-fixation test employing specific gonococcal and non-specific antigens.

In the performance of the complement-fixation reactions for suspected gonococcic and syphilitic infection there is little or no fundamental variance in the technique other than in the employment of different antigens. Whether it be the Wassermann or the gonococcus complement-fixation test, one essential stands paramount, namely, the absolute standardization of all substances entering into the reaction, and this applies more particularly to the gonococcic antigen, because of its greater instability owing to watery extraction, than to the alcoholic syphilitic extract.

An important feature of the complement-fixation reaction in gonococcic infections is the necessity of employing a polyvalent antigen, owing to the apparent diversity in the various strains of the gonococcus. Thus we have utilized in our studies mono-, tri-, hexa-, and nonavalent antigens, prepared by washing off in sterile distilled water forty-eight-hour-old cultures of gonococci grown on blood agar; these suspensions were shaken for one hour, autolyzed for twenty-four hours in a thermostat at temperature of 37° C., and heated in a water bath at 60° C. for one-half hour. Before use, these antigens are diluted 1:10 by the addition of 0.85 per cent salt solution.

Although a negative reaction may be erroneously contradictory, a positive result is most reliable, in fact, more specific than the Wassermann, since thus far we have discovered no alien infection capable of producing a positive result, nor have we found any drug (as mercury in the treatment of syphilis) influential in negativing a positive reaction. These facts naturally and early suggested the advisability of comparative studies, using non-specific with the specific antigens in the performance of these serological reactions. Accordingly, polyvalent antigens were prepared from various non-gonorrhœal gram-negative and positive bacteria; namely, the micrococcus catarrhalis, the diplococcus meningitidis, the streptococcus pyogenes, the pneumococcus lanceolatus, the micrococcus albus and aureus, the colon bacillus, and the cornybacterium pseudodiptheriæ.

The technique which we have employed with the greatest satisfaction is essentially that used in the performance of the Wassermann reaction, substituting merely a specific gonococcic or non-specific antigen for the syphilitic lipotropic antigen, always using the accurately standardized single-complement unit, the required inactivations and the routine standardization of antigen, hæmolytic amboceptor, and suspension of sheep's red blood-corpuscles. A detailed description of the fundamental serological principles involved in complement fixation or deviation will be omitted. A study of the accompanying table will, it is believed, serve to give a comprehensive understanding of the technique and actual steps of the routing performance of the reactions.

Reviewing the results of our work to date with especial reference to a study of specific versus non-specific antigens to be reported in detail in a later communication, we have deduced the following facts:

1. Very rarely have polyvalent antigens prepared from meningococci, pneumococci,

[2] "The Gonococcus Complement-Fixation Test and Analysis of Results from its Use."

[1] Read before the Philadelphia Genito-Urinary Society, January, 1914. Received for publication, January 31, 1914.

TABLE OF TEST REACTIONS

	No. Test Tube	Antigen (Dilution 1:10)	NaCl Solution (0.85%)	Patient's Serum (Inactivated)	Known Positive Serum (Inactivated)	Known Negative Serum (Inactivated)	Complement (Dilution 1:10)	Incubation at 37° C. in water-bath for one-half hour.	Hæmolytic Amboceptor (Antisheep) (Titre = 1:2000) (Dilution 1:1000)	Red Blood-Corpuscles (Sheep's 5% washed suspension)	Incubation at 37° C. in water-bath for three-quarters of an hour.	Results (immediately or morning after refrigeration)	Objects of the Reactions
		cc.	cc.	cc.	cc.	cc.	cc.		cc.	cc.			
Tests for Antigen Standardization and Controls	1	0.5	1.5		0.1		0.4		1.0	1.0		Incomplete hæmolysis	To determine quantity of *antigen* to be used in test proper.
	2	0.1	1.4		0.1		0.4		1.0	1.0		Partial hæmolysis	
	3	0.2	1.3		0.1		0.4		1.0	1.0		No hæmolysis	
	4	0.3	1.2		0.1		0.4		1.0	1.0		No hæmolysis	
	5	0.2	1.3			0.1	0.4		1.0	1.0		Complete hæmolysis	To prove that the antigenic dose is not in itself anticomplementary.
	6	0.4	1.1			0.1	0.4		1.0	1.0		Complete hæmolysis	To prove that twice the antigenic dose is not in itself anticomplementary.
	7	0.6	0.9			0.1	0.4		1.0	1.0		Incomplete hæmolysis	To prove that thrice the antigenic dose is not in itself completely anticomplementary.
Tests for Complement Standardization	8	0.2	1.3				0.2		1.0	1.0		Incomplete hæmolysis	To determine quantity of *complement* to be used in test proper.
	9	0.2	1.2				0.3		1.0	1.0		Incomplete hæmolysis	
	10	0.2	1.1				0.4		1.0	1.0		Complete hæmolysis	
	11	0.2	1.0				0.5		1.0	1.0		Complete hæmolysis	
Tests and Controls for the Suspected Serum	12	0.2	1.3	0.1			0.4		1.0	1.0		No hæmolysis (Positive reaction = 1 unit)	To determine quantitatively the degree of complement fixation.
	13	0.2	0.9	0.1			0.8		1.0	1.0		No hæmolysis (Positive reaction = 2 units)	
	14	0.2	1.3	0.1			0.4		1.0	1.0		Complete hæmolysis (Negative reaction)	Shows that there was no immune body present in the patient's serum with the aid of the antigen to fix the complement.
	15		1.5	0.1			0.4		1.0	1.0		Complete hæmolysis	Proves that the immune body itself will not fix complement.

streptococci, staphylococci, colon bacilli, or cornybacteria sufficed to fix complement. This does not jeopardize the specificity of the gonococcus antigen, since it is explained on the basis of supervention of a mixed infection.

2. In ten per cent of sera examined a weakly positive result was obtained with polyvalent micrococcus catarrhalis antigen; in these cases the reaction was much more marked with the various gonococcic antigens. Thus it may be inferred that the association between the gonococcus and the micrococcus catarrhalis is not positively and absolutely defined; and it is not unlikely, on the one hand, that a culture of the M. catarrhalis is occasionally included in a supposedly specific polyvalent gonococcus antigen, while on the other it is undoubtedly true that a mixed infection, often due to the M. catarrhalis, exists in patients suffering from gonorrhœa and its complications.

3. A negative gonococcus fixation test does not necessarily mean that the patient is not infected with the gonococcus; in a primary uncomplicated acute case we have never observed a positive reaction prior to the sixth week, nor have we obtained positive reactions where the anterior urethra or vagina alone were involved.

4. A positive result apparently is more specific than the Wassermann reaction, since thus far we have found no diseases other than

gonorrhœal in which a positive result was obtained.

5. A positive reaction has been discovered in 21 per cent of patients supposedly clinically cured; such patients, therefore, should not be discharged from treatment or observation until a negative response is obtained, provided the patient has not been the recipient of immunotherapy.

6. Thirty-three per cent to seventy-five per cent of patients with chronic posterior urethritis, prostatitis, and seminal vesiculitis, with recurrent exacerbations, demonstrate a positive reaction.

7. Sixty-six per cent of all stricture cases have shown a positive result.

8. Approximately one hundred per cent of cases of active epididymitis or arthritis, at least by the fifth week, have demonstrated a positive gonococcus complement-fixation reaction.

9. The test has proved of value in gynecology in the differential diagnosis of various pelvic inflammatory diseases from one another and from neoplastic formations; also in the differentiation of certain acute and chronic arthritides.

10. Sociologically, the gonococcus complement-fixation test seems destined, along with the Wassermann, to play an important rôle in the future in determining the matrimonial fitness of candidates.

STUDIES IN RENAL FUNCTION WITH SPECIAL REFERENCE TO NON-PROTEIN NITROGEN AND SUGAR CONCENTRATION IN THE BLOOD, PHENOLSULPHONEPHTHALEIN ELIMINATION AND BLOOD PRESSURE *

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Until the theories of nephritis are reduced to one and until that one has been conclusively proved to be correct, continued investigations, both clinical and experimental, seem to be demanded.

These studies in nephritis deal on the one hand, with the accumulation or retention in the blood of the end-products of protein metabolism and glucose concentration in the blood, and on the other hand, with the functional capacity of the kidneys in so far as the latter may be determined by the elimination of phenolsulphonephthalein. In short, the purpose of this paper is to study:

1. The relation of protein feeding to nitrogen retention in the blood.
2. The relation of nitrogen retention to renal function.
3. The relation of blood-sugar, blood-pressure, phenolsulphonephthalein elimination and nitrogen retention.

So far as we can ascertain, there have been no studies covering all of these phases simultaneously and the possible relationship existing between them; though quite recently Folin[1] and Frothingham and Smillie[2] have reported on somewhat similar studies, the latter presentation having been published shortly before the completion of this work.

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* From the William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa.

* Read before the Section of Medicine of the College of Physicians of Philadelphia, February, 1915.

† Aided by the J. Alison Scott Research Fund.

‡ Woodward Fellow in Physiological Chemistry.

1. Folin, Otto, Denis, W., and Seymour, Malcolm: The Non-Protein Nitrogen Content of the Blood in Chromic Vascular Nephritis (Arteriosclerosis) as Influenced by the Level of Protein Metabolism, THE ARCHIVES INT. MED., 1914, xiii, 224.

2. Frothingham, Channing, and Smillie, Wilson, G.: The Relation Between the Phenolsulphonephthalein Excretion in the Urine and the Non-Protein Nitrogen of the Blood in Human Cases, THE ARCHIVES INT. MED., 1914, xiv, 541.

I. THE RELATION OF PROTEIN FEEDING TO NITROGEN RETENTION IN THE BLOOD

With but two exceptions, these observations were made on ward patients in the University Hospital on the service of Dr. Alfred Stengel.

To study the effect of the level of protein diets on the retention of nitrogen in the blood, we first placed the patient on a diet of about 2,300 calories, which represented approximately 5 gm. of nitrogen; then on a diet of 2,150 calories, corresponding to 12 gm. of nitrogen, and finally, on a diet of 2,300 calories or about 18 gm. of nitrogen.

TABLE 1.—Influence of Protein Feeding on Retention of Nitrogen in Blood in Cardiorenal Disease

Case	Diagnosis	Age	Sex*	Date	Nitrogen per hundred c.c. of blood, mg.		
					Low Protein Diet	Ward Diet	High Protein Diet
1. J. H. B.	Chronic nephritis; chronic endocarditis	39	♂	4/1	23	18	30.8
2. J. McW.	Chronic nephritis; chronic myocarditis	48	♂	4/1	28	28	35.5
3. J. H.	Chronic interstitial nephritis; chronic myocarditis	34	♂	4/1	.....	28	33.6
4. J. W.	Chronic nephritis; chronic myocarditis	45	♂	4/2	26	28	
5. H. L.	Chronic interstitial nephritis; myocardial weakness; arteriosclerosis	75	♂	5/9	26	32	39
6. A. P.	Chronic nephritis; chronic myocarditis	49	♂	5/9	30	36	39
7. L. S.	Chronic interstitial nephritis; myocardial weakness	55	♀	7/21	23	30	37
8. S. C.	Arteriosclerosis.....	70	♀	7/21	18	29	35
9. F. G.	Chronic nephritis; chronic myocarditis; asthma	49	♀	10/19	29	28	

* In this and the following tables, ♂ denotes male, and ♀ female.

The duration of each period was three days, and in the afternoon of the third day the blood was withdrawn from a vein in the arm and analyzed according to the recent method of Folin and Farmer[3] with the modification that the ammonia liberated was collected in standardized fiftieth normal sulphuric acid and titrated with fiftieth normal sodium hydroxid. It is generally conceded that by this method normal values are those with a total nitrogen of less than 30 mg. per hundred c.c. of blood. We found that all but two cases revealed higher figures at the end of the high protein diet, as will be seen in Tables 1 and 2,

3. Folin, O., and Farmer: Jour. Biol. Chem., 1912, xi, 527.

which are good illustrations of the influence of protein feeding on the retention of nitrogen in nephritics.

In several cases of pure nephritis of the chronic interstitial type and in which there was an increase in non-coagulable nitrogen on a low protein diet, there was a striking reduction in tolerance for an increasing amount of proteid, as was shown by the marked rise in retention products as well as by nausea and vomiting in a few cases (Table 2). Clinically, these results indicate not only the importance of a low proteid diet in certain types of nephritis, but they also illustrate the advantages of this test for a more accurate diagnosis of these types.

TABLE 2.—Rise in Retention Products Following Increase in Protein Ingestion in Cases of Chronic Interstitial Nephritis

Case	Diagnosis	Age	Sex*	Date	Nitrogen per hundred c.c. of blood, mg.		
					Low Protein Diet	Ward Diet	High Protein Diet
1. W. P.	Chronic interstitial nephritis	65	♂	4/1	26	33	40
2. L. L.	Chronic interstitial nephritis	55	♀	4/1	29	30	32
3. D.	Chronic interstitial nephritis	55	♀	4/9	43	54	60
4. G. C. B.	Chronic interstitial nephritis	40	♂	2/26	28	25	43
5. M. W.	Chronic interstitial nephritis	28	♀	5/4	43	35	
6. Mr. R.	Chronic interstitial nephritis	27	♂	6/15	67	88	92
7. S. R.	Chronic interstitial nephritis	26	♂	6/28	56		
8. E. B.	Chronic interstitial nephritis	43	♂	7/6	30	46	56
9. E. H.	Chronic interstitial nephritis	21	♀	7/7	22	29	37
10. P. F.	Chronic interstitial nephritis	61	♂	6/13	39	33	42
11. M. L.	Chronic interstitial nephritis	53	♀	10/1	36	37	
12. S. B. J.	Chronic interstitial nephritis	43	♂	11/27	50		

In fact, observations on nitrogen retention are becoming more and more frequent in the literature. Macwitz, Rosenberg and Tscherkoff,[4] in an exhaustive study of the pathology of nephritis and its functional diagnosis by various tests, conclude that nitrogen retention is indicative of vascular disease and that, in chronic cases, it is a good index of the degree of insufficiency and gives a valuable clue for both prognosis and therapy.

With regard to the influence of chronic passive congestion on nitrogen retention in the "cardiorenal" symptom-complex, our results

4. Macwitz, Rosenberg and Tscherkoff: München. Med. Wchnschr., 1914, No. 23, p. 1268.

TABLE 3.—Result of Examination of a Series of Cardiorenal Cases

Case	Diagnosis	Age	Sex	Blood Nitrogen Mg. per 100 c.c. of Blood	Blood Sugar Gm. 100 c.c. of Blood	Phthalein Elimination % in 2 Hrs.	Blood Pressure	Eye-Grounds	Edema	Liver*	Albumin	Casts	Sp. Gr.
1. J. H. B.	Chronic nephritis; chronic endocarditis	39	♂	23	0.07	10	110/60	.....	++	10	+	+	1.007
2. J. McW.	Chronic nephritis; chronic myocarditis	48	♂	28	0.076	40	135/98	.....	++	4	++	++	1.025
3. J. H	Chronic interstitial nephritis; chronic myocarditis	34	♂	28	0.085	40	190/130	Early sclerosis	++	10	+	+	1.015
4. J. W.	Chronic nephritis; chronic myocarditis	45	♂	26	0.09	45	138/70	.....	++	4	++	++	1.021
5. H. L.	Chronic interstitial nephritis; myocardial weakness; arteriosclerosis	75	♂	26	0.131	30	155/90	Indentation of veins. Arteries thickened	—	5	+	+	1.015
6. A. P.	Chronic nephritis; chronic myocarditis	49	♂	30	0.159	15	150/90	.....	+	4.5	++	++	1.025
7. L. S.	Chronic interstitial nephritis; myocardial weakness	55	♀	23	.....	25	155/86	Moderate sclerosis	+	1.5	+	—	1.017
8. M. C.	Chronic interstitial nephritis; chronic myocarditis	41	♀	28	0.09	8	158/105	Incipient sclerotic changes	+	6	++	+	1.013
9. F. G	Chronic nephritis; myocarditis; asthma	49	♀	28	0.153	33	200/130	Normal.....	+	?	++	++	1.021
10. B. J. B.	Chronic nephritis; myocardial weakness	65	♂	67	.....	30	160/70	.....	++	10	++	++	1.019

* Centimeters below costal border in midclavicular line.

TABLE 4.—Result of Tests in Cases of Chronic Interstitial Nephritis with Hypertension

Case	Diagnosis	Age	Sex	Blood Nitrogen Mg. per 100 c.c. of Blood	Blood Sugar Gm. 100 c.c. of Blood	Phthalein Elimination % in 2 hrs.	Blood Pressure	Eye-Grounds	Albumin	Casts	Sp. Gr.
1. W. P.	Chronic interstitial nephritis	65	♂	26	0.120	35	180/130	.....	+	+	1.025
2. L. L.	Chronic interstitial nephritis	55	♀	29	0.082	43	225/110	.....	+	+	1.021
3. D.	Chronic interstitial nephritis	55	♂	43	0.106	..	210/150	.....	+	+	
4. G. C. B.	Chronic interstitial nephritis	40	♂	43	0.075	48	185/136	.....	+	+	1.015
5. M. W.	Chronic interstitial nephritis	28	♀	43	0.123	10	256/163	.....	++	+	1.013
6. Mr. R.	Chronic interstitial nephritis	27	♂	67	0.117	18	210/130	.....	++	++	1.009
7. S. R.	Chronic interstitial nephritis	26	♂	56	0.123	22	205/150	Disks edematous. Hemorrhages	++	++	1.013
8. E. B.	Chronic interstitial nephritis	43	♂	46	.....	16	155/110	.....	+	+	1.014
9. E. H.	Chronic interstitial nephritis	21	♀	35	0.07	40	210/115	Edema. Veins full and dark. Patches of exudate	+	+	1.008
10. P. F.	Chronic interstitial nephritis	61	♂	39	0.074	24	190/110	Neuritis, hemorrhages, sclerosis	++	++	1.019
11. M. L.	Chronic interstitial nephritis	53	♀	36	0.082	37	170/135	Vessels sclerotic. Senile choroidal changes	+	+	1.013
12. C. B.	Chronic interstitial nephritis	40	♀	....	0.146	10	250/150	Retinitis. Hemorrhages	++	++	1.015
13. B. W.	Chronic interstitial nephritis	22	♀	33	0.085	60	205/170	Sclerotic changes....	+	—	1.009
14. S. B. J.	Chronic interstitial nephritis	43	♂	50	0.088	37	242/152	Marked sclerosis....	+	+	1.017
15. McC.	Chronic interstitial nephritis	66	♂	58	0.08	75	178/95	Veins full. Hemorrhagic exudation	+	++	1.021

confirm those of Rowntree and Fitz,[5] in that there is practically no increase in waste nitrogenous products provoked by such congestion per se; although Strauss and Hohlweg[6] state that this factor is responsible for a moderate increase in these products. See Tables 1 and 3.

In our series of cardiorenal cases, in many of which chronic passive congestion was very evident, only one presented a rise in blood nitrogen. To be of real value in therapy and prognosis, we believe that the test should be repeated at intervals, that due consideration should be given the time and the amount of protein intake, and finally, that each test should be accompanied by a determination of the phenolsulphonephthalein elimination. When both the clinical picture and these details are observed, the value of the test cannot be disputed.

In Table 3, it will be noted that of the cardiorenal group investigated, the absence of retained nitrogen is striking when contrasted with Table 4, in which cases of pure chronic interstitial nephritis with hypertension are considered. In the latter group, eleven out of fourteen cases show evidence of retention, and it is this same group in Table 2 which illustrates a marked reduction in tolerance for proteins as noted above. Certain it is that excessive amounts of nitrogen in the blood afford an index of renal functional capacity. The significance of these results in the pure nephritic cases, however, both from the prognostic and therapeutic point of view, will be dealt with later.

II. THE RELATION OF NITROGEN RETENTION TO RENAL FUNCTION AS DETERMINED BY THE PHENOLSULPHONEPHTHALEIN ELIMINATION

The technic of the phenolsulphonephthalein test used was that originated by Rowntree and Geraghty.[7] Before discussing the results in our three groups of cases, it may be advisable to note certain conditions under which marked variations in the phthalein output occur. These are:

1. Chronic passive congestion.
2. Various stages of nephritis.
3. Hyperpermeability.

1. *Chronic Passive Congestion.*—Several workers have shown that marked chronic passive congestion greatly decreases the phenolsulphonephthalein output and that as the circulation improves, the output

5. Rowntree, L. G., and Fitz, R.: Studies of Renal Function in Renal, Cardiorenal and Cardiac Diseases, The Archives Int. Med., 1913, xi, 258.

6. Strauss and Hohlweg: Quoted by Rowntree and Fitz, The Archives Int. Med., 1913, xi, 278.

7. Rowntree and Geraghty: Jour. Pharm. and Exper. Therap., 1910, i, 579.

rises if there is no coexisting renal involvement.[8, 9] This fact may well be utilized in determining the relative responsibility of heart or kidney in the troublesome symptom complex, cardiorenal disease, as successful therapy in this condition rests largely on our knowledge of the underlying causative factor.

2. *Various Stages of Nephritis.*—Phenolsulphonephthalein elimination varies during the course of the illness (nephritis), a fact well established and one which we have frequently observed. Our most striking example of this is Case 4 in Table 5, in which the phenolsulphonephthalein rose from 17 per cent. to 70 per cent. in five weeks, and in which there was no marked evidence of cardiac disease. The patient's improvement paralleled this rise. We freely concede that a rise as marked as this in so short a time cannot be an index of the actual degree of change in renal tissue, but that it is rather an illustration of the fluctuation in functional capacity during the course of the illness. Thus again, if the phenolsulphonephthalein test is repeated at intervals, its value from the point of view of prognosis is evident. We believe that too much emphasis should not be laid on one phenolsulphonephthalein elimination, and in the differentiation of nephritis from chronic passive congestion, it would seem that a series of both phenolsulphonephthalein and nitrogen tests should be carried out in order to reach any definite conclusions.

3. *Hyperpermeability.*—In the past year, Pepper and Austin[10] have called attention to the existence of cases of nephritis in which the functional capacity of the kidney is normal or even above normal. Baetjer[11] has described such cases and considers that there may be a stage in nephritis when hyperpermeability exists at least to phenolsulphonephthalein and some other substances. We have also noted a few cases, but so far the opportunity for serial studies of them has not been presented. It may be that this so-called hyperpermeability is merely an illustration of the possible existence of damaged kidneys with normal functional capacity, at least for some substances. Cases of this type are usually accompanied by chlorid retention.

In our series of pure nephritis of the chronic interstitial type, several interesting facts may be noted (see Table 4). Nearly every case showed retention of nitrogen, decreased phenolsulphonephthalein elimination and high blood-pressure, while the blood-sugar was slightly

8. Farr, C. B., and Austin, J. H.: Jour. Exper. Med., 1913, xviii, 228.

9. Rowntree, L. G., Fitz, R., and Geraghty, J. T.: The Effects of Experimental Chronic Passive Congestion on Renal Function, The Archives Int. Med., 1913, xi, 121.

10. Pepper and Austin: Am. Jour. Med. Sc., 1913, cxlv, 254.

11. Baetjer, Walter A.: Superpermeability in Nephritis, The Archives Int. Med., 1913, xi, 593.

TABLE 5.—Result of the Study of a Series of Cases of Chronic Parenchymatous Nephritis

Case	Diagnosis	Age	Sex	Blood Nitrogen Mg. per 100 c.c. of Blood	Blood Sugar Gm. 100 c.c. of Blood	Phthalein Elimination % in 2 Hrs.	Blood Pressure	Eye-Grounds	Edema	Liver	Albumin	Casts	Sp. Gr.
1. A. S.	Chronic parenchymatous nephritis	22	♀	....	0.095	27	146/90	.....	++	—	++	++	1.013
2. W. M.	Chronic parenchymatous nephritis; myocardial weakness	24	♂	33	0.072	20	98/65	Related retinal veins	++	—	++	++	1.028
3. A. K.	Chronic parenchymatous nephritis	25	♀	74	.....	7	170/130	Normal.....	++	—	++	++	1.019
4. F. A.	Chronic nephritis.....	24	♀	36	.....	17	115/70	Normal.....	+	—	+	+	1.031
5. S. G.*	Chronic parenchymatous nephritis	20	♀	81	.....	5	145/110	.....	++	—	++	++	1.020
6. J.McV.	Chronic parenchymatous nephritis	47	♂	145	0.087	0	140/80	.....	+	4 cm.	++	++	1.012

* At necropsy the kidneys were found to be secondarily contracted.

above normal in six of the fourteen cases studied. It should be noted that nine of these fifteen patients ranged from 26 to 43 years of age, and that the prognosis was grave in most instances.

Less striking results were obtained in a series of cardiorenal cases as will be seen in Table 3. One case gave evidence of nitrogen retention while the phenolsulphonephthalein varied from 8 per cent. to 45 per cent. The blood pressure was high in three cases, as was the blood sugar.

The series of cases of chronic parenchymatous nephritis shown in Table 5 is too short to permit of definite conclusions.

III. THE RELATION OF BLOOD-SUGAR, NITROGEN RETENTION, PHENOLSULPHONEPHTHALEIN ELIMINATION AND HIGH BLOOD PRESSURE

The question of hyperglycemia in high-pressure nephritis is of considerable theoretical interest. Since Neubauer[12] first called attention to this, several workers have confirmed his findings while a few have failed to do so. Thus the present status of blood-sugar concentration in nephritis may be regarded as one of the many points at issue and for that reason is included in these studies. The blood-sugar was determined by the micro method of Bang[13] in which a small amount of blood is required for each test. The blood is obtained by finger puncture, which insures simplicity and the elimination of unnecessary discomfort to the patient. All food was withheld from the patient for at least six hours before the blood was withdrawn. Normal figures by this method lie between 0.06 and 0.1 per cent.

In a former publication, one of us (Hopkins[14]) reported on a study of the blood-sugar in twenty-eight cases of nephritis. Briefly stated, the conclusions to be drawn from that work and the cases here presented are as follows:

A slight hyperglycemia occurs in many high-pressure nephritics and frequently in those with low phenolsulphonephthalein elimination (Table 4). There is no relation between the height of the blood pressure and the degree of hyperglycemia. In most nephritics without high pressure, the blood-sugar is normal. Edema and hepatic congestion do not seem to influence the concentration of blood-sugar (Tables 3 and 4).

Of the eight cases in this series which showed a retention of over 40 mg. of nitrogen, four gave an elevation in blood-sugar. This con-

12. Neubauer: Biochem. Ztschr., 1910, xxv, 284.
13. Bang, I.: Der Blutzucker, Wiesbaden, 1913.
14. Hopkins, A. H.: Am. Jour. Med. Sc., 1915, cxlix, 254.

firms the recent work of Borchardt and Bennigson,[15] who also found nitrogen retention a frequent accompaniment of hyperglycemia.

Of the eight cases in which two or three estimations of blood-sugar were made after the various periods of protein feeding, no constant changes were noted.

Alimentary hyperglycemia was pronounced in three out of four cases in which 100 gm. of glucose were fed by mouth and serial tests of blood-sugar made each half hour for several hours.

CONCLUSIONS

1. Protein feeding in nephritis has a direct influence on the retention of nitrogen in the blood. This is most pronounced in the pure chronic interstitial type with hypertension.

2. The estimation of retention by blood analysis is of definite clinical value from the point of view of therapy, and though this series is too limited to permit of definite conclusions with regard to its prognostic value, our findings so far confirm those of other workers who advocate its usefulness in this field.

3. Chronic passive congestion does not cause an increase of waste nitrogenous products in the blood.

4. In the presence of nitrogen retention, the phenolsulphonephthalein output is usually low and the blood pressure frequently high.

5. Chronic passive congestion may greatly impair the phenolsulphonephthalein output. Variation in the elimination of this dye may also be noted in different stages of nephritis, and in cases with "hyperpermeability."

6. To be of value, the nitrogen retention and phenolsulphonephthalein tests should be repeated at intervals, the value of the former being increased when combined with clinical observations of the patient, diet, etc.

7. A slight hyperglycemia occurs in many high-pressure nephritics and frequently in those with retention of nitrogen and impaired phenolsulphonephthalein elimination.

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15. Borchardt, L., and Bennigson. W.: München. Med. Wchnschr., October, 1913, p. 2275.

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Studies in Diphtheroids. — I.*

The Nature and Clinical Importance of Pseudo-Diphtheria Bacilli.

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The frequent occurrence of organisms that are loosely called pseudo-diphtheria bacilli, in material examined at the Pepper Laboratory, has caused the writer to investigate the position of this group among the bacteria and the importance of the isolated strains in their respective cases, while Dr. S. S. Kneass has been working with their biometrical position.

The pseudo-diphtheria bacillus has been known since 1887, when isolated by Loeffler; Hoffman described it in the following year. It cannot be said that the descriptions and conclusions upon observations prior to the year 1904 are very satisfactory. They deal chiefly with the pseudo-form in diphtheria, the relation of it to the true Klebs-Loeffler bacillus, and the existence of the Neisser granules. In the year mentioned Graham-Smith and Hamilton cleared the atmosphere somewhat, the first by bacteriological, and the second by pathological observations.

It will become apparent to any one reviewing the literature that we are dealing with several varieties and that the name pseudo-diphtheria has been given to all the organisms which chance to have the morphology and staining characters

* Received for publication March 5, 1915.

similar to diphtheria bacilli but do not possess their typical pathogenic power for guinea-pigs.

The groupings of the organism of diphtheria and its relatives by Emerson puts the thing very clearly. He says there are (1) "Bacilli with typical morphology, typical cultural characteristics, especially the ability to form acid from glucose, and which produce the typical lesions in animals, which are in the opinion of all observers Bacillus diphtheriæ; (2) bacilli with typical morphology, typical cultural reactions, especially the ability to form acid from glucose, but which are not pathogenic to animals, may be called avirulent diphtheria bacilli; (3) bacilli with typical morphology, but which do not conform in their cultural reaction with the diphtheria bacillus and which are either non-pathogenic to animals or do not produce typical lesions, and may properly be called pseudo-diphtheria; (4) finally, there are a number of organisms which resemble pseudo-diphtheria in many ways, but whose morphology is not exactly the same, since they do not show the polar staining and are often shorter and a little thicker than the typical form and which have different cultural characteristics and differ in their pathogenicity. This group certainly includes the pseudo-diphtheria bacillus of Hoffman, the xerosis bacillus and others."

To those who are interested in the subject of the relation of the organisms of this group, the papers of Graham-Smith and Teomin will be profitable reading.

Pseudo-diphtheria bacilli are rods somewhat shorter and plumper than the typical bacilli, solidly staining or occasionally of double wedge shape, or barred; sometimes growing into longer curved, barred, and beaded forms; growing meagerly upon coagulated serum but much better than diphtheria bacilli upon agar. They produce no soluble toxin and animals are little if any affected by injections of fluid cultures or bacillary masses. This has been corroborated lately by Smyth, who showed that artificial tissue cultures were restrained by Klebs-Loeffler bacilli but not by pseudo-forms. They are able to ferment dextrose, saccharose and

maltose, while the true diphtheria bacilli acidify dextrose, dextrine, and maltose but never saccharose. The ability to ferment dextrose is denied by some observers, but this may be explained by the fact that alkalin medium was used with litmus as an indicator. All the true diphtheria bacilli and all but one pseudo-culture I have examined were able to produce acid upon neutral agar with one per cent glucose and litmus. The Hoffman type may not ferment glucose.

In order for us to understand the difficulties of interpreting the finding of pseudo-diphtheria bacilli, it will be necessary to consider briefly the distribution of these diphtheria-like organisms. The pseudo-diphtheria bacilli are frequently found without pathological change in the nose, throat, external ear, vulva, vagina, male external genitalia, urine, and even reported upon the skin of healthy persons, but more commonly in the presence of an eruption. Neuman found the organisms in question in nearly one hundred per cent of normal noses. The normal diphtheroid of the nose is called *Bacterium coryzae segmentosus.* It is said to be of some importance in common colds. Neufeld seems to have encountered rods, mildly pathogenic for pigs, in ozæna sufficiently often for him to consider them irritative but not a causative factor. In this regard one recalls Bergey's statement that the pseudo-diphtheria forms are more important in continuing an inflammation than in inciting it.

Graham-Smith reports diphtheroid organisms as common in normal ears, in ears in scarlatina and describes two varieties which he considers normal to the external auditory canal. He asserts that in order to decide whether an organism be of importance in the disease in the ear, its biology and immunology must be worked out.

Hamilton found pseudo-diphtheria forms in seventy-two per cent of scarlatinal otitis media cases, representing three biological types, for two of which there seemed to be reason to consider them the causative agents. Frosch, Conradi, Koch, and others have found organisms corresponding to the diphtheria bacilli in the urine during diphtheria. Kolmer, Ohlmacher, and Townsend have found pseudo-diphtheria

bacilli in the urine, anterior urethra, and upon the glans penis of normal males. The diphtheria-like rods frequently accompany the gonococcus, and Ohlmacher believes that a combination of them with gonococcus in vaccine is beneficial. These organisms isolated from normal individuals are, for the most part, non-virulent. In the female, the pseudo-diphtheria bacillus is asserted by Wigelius to be the commonest organism on the vulva and in the puerperal vagina, a statement corroborated by Wangott and Sitzenfrey, who assert that they are non-pathogenic. Cohn found no pseudo-diphtheria forms in localized puerperal infections, while in non-puerperal he has found them alone and in conjunction with staphylococci.

There is a large group of observers who believe that there is no essential difference between diphtheria and the pseudo-diphtheria bacilli, while there is an equally large group of dualists which consider them separate species. This is not only of importance from a bacteriological standpoint, but from the standpoint of mutation. If the pseudo-diphtheria bacilli are the results of mutation or degradation of true Klebs-Loeffler forms, when and where is the change accomplished? It is well known that the true diphtheria bacilli may penetrate the body, entering the lymph nodes principally, but also other organs. Many cases of septicemia with true diphtheria bacilli are on record. If the mutation theory be correct the finding of pseudo-diphtheria bacilli in various parts of the body and in various mixed infections might be explained on the basis of a mutation or a degradation in vivo. It seems that from the observations of Frosch, Trautmann and Gaehtgens that diphtheria bacilli isolated from septicemia cases are slightly changed in morphology and less active in biology, an alteration which would approach what has been outlined above as a pseudo-diphtheria form. These observations naturally lead to the thought that diphtheria bacilli enter the body and are changed into a pseudo-form,

During the early days when the monists outnumbered the dualists there were several observations of the mutation from

true to false and vice versa, chiefly by the passage of the organisms through experimental animals and by subjection to high temperatures. Graham-Smith and Clark have very conclusively shown that such passage experiments, in combination with toxin and anti-toxin, have failed to transfer a saccharose fermenting organism into a non-saccharose fermenting organism. The earlier observers (with possibly the exception of Bergey and Neisser) failed to take this fermentative power into consideration.

It is not trustworthy to base the conclusions of mutation upon morphology, but some observations of German workers are worth noting in this direction. Trautmann and Gaehtens observed a pseudo-diphtheria bacillus from an atypical angina, through many generations of passages, and saw a change from a stiff, solidly staining rod to rows of cocci or diplobacilli of a bent, beaded, granular form. The organism was mildly pathogenic for guinea-pigs after this " mutation," whereas before it had been non-pathogenic. These authors believe that the body fluids exert an influence upon the diphtheria bacillus and transform it into the pseudo-diphtheria bacillus.

The observations of Jacobsthal indicates that more than one variety of bacillus may be isolated from the blood, which observation has been corroborated by Bernhardt and Paneth.

The observations upon mutation in the throat in a bacteriological sense are hardly conclusive, as it is impossible to maintain that we are dealing with a culture which emanates from one organism. Moreover, the observations of Clark seem to indicate that diphtheria bacilli do not tend to assume the pseudo-diphtheria form as diphtheria improves, but that on the other hand the early forms of the diphtheria bacilli are of the pseudo-shape, and if growing upon favorable medium will develop into the typical picture. Furthermore, as the disease improves, the pseudo-diphtheria forms have a chance to develop and are not overgrown by the diphtheria bacilli. It may be said in summing up the foregoing that from the literature there is no proof that a mutation has ever

been accomplished artificially, but there does seem reason to think that diphtheria bacilli within the body fluid and tissues are atypical, lose in virulence, and vary in character. I shall cite cases having pseudo-diphtheria in blood and elsewhere simultaneously.

Pathogenicity. — The grouping of experiments upon pathogenicity in the pseudo-diphtheria group is rendered difficult by the fact that the authors have not described their strains sufficiently. There are very few articles which indicate that the original culture isolated from a patient was pathogenic for guinea-pigs. Even the organisms so well described by Hamilton and Reudiger merely produced a loss of weight and sometimes a bacteriemia when they had passed through a series of pigs. The organism described by Dudgeon produced a mild local lesion in its passage, and Rosenow's strain produced a fatal bacteriemia. Nearly all of the reports indicate that a slight increase of virulence was attained by several passages. The principal lesion caused by the pseudo-diphtheria bacillus is a slight indurated infiltration at the site of inoculation. Bergey is the only one who seems to attach any importance to aggressin formation by this organism. Strains that are allowed to grow saprophytically quickly lose whatever virulence they may have acquired. For the very virulent examples Hamilton and Horton note that there may be bacteriemia, marked congestion of the liver and kidneys, a slight ascites and a very mild congestion of the adrenals. No pseudo-forms seem to produce a large necrotizing, subcutaneous edema and hemorrhage in the adrenals.

Pathogenicity for human beings. — Having indicated that little is to be gained by a review of the pathogenicity of pseudo-forms for guinea-pigs we must now consider the infections of human beings ascribed to these bacteria.

The first to call attention to the virulence for human beings in a very definite and specific way was Reudiger, whose work was amplified by Hamilton and Horton. These authors show that cultures isolated from the scarlet fever and other forms of angina consist of, first — those which can

be mildly pathogenic to animals by passage and are neutralized by a serum made against them, and second — those which never become pathogenic. The former are believed to be of importance in the patient's condition. Hamilton reports these organisms in otitis media, the patient's serum having a low opsonic value for them, thus warranting the belief that they were of importance in the infection. These so-called virulent pseudo-diphtheria bacilli produce an antiserum specific for themselves. Cave, Hewlett, Knight, Priestly, Richmond, Salter, and the authors just cited believe that a mild, frequently unilateral tonsillitis may be caused by the virulent pseudo-forms. Beyer reports pharyngitis and tracheitis due to an avirulent diphtheria bacillus. There might be some question about this case but the author reports that diphtheria antitoxin was of no value. Councilman, Mallory and Pearce, and MacWaters report finding this organism in noma and facial ulcers. There is, however, not a great deal of evidence to prove that they were active germs. Dudgeon reports the finding of a diphtheroid bacillus in a cellulitis, following spontaneous fracture in a tabetic. Powlowski reports pseudo-diphtheria bacilli in post-operative suppuration in two amputations, one for tuberculosis and one for cellulitis. Townsend reports a case of pseudo membranous and fibrosing cystitis caused by the pseudo-diphtheria bacillus. The urine was alkaline, ropy, and full of mucous shreds, albumin, and phosphates. There was some blood and pus, but no kidney elements. There was little constitutional effect from this infection. Autogenous vaccines did little good. Rosenow reports an ulcerative cystitis of several months duration, caused by pseudo-diphtheria bacilli of the virulent type, which were agglutinated by the patient's serum. The organisms produced a bacteriemia in guinea-pigs when given in large doses. The clinical condition was improved by autogenous vaccines. Francioni describes a case of hemoglobinuria, cyanosis and jaundice without fever, thus corresponding to the Winckel's syndrome, from which during life he was able to isolate from the blood a pseudo-diphtheria bacillus non-pathogenic for guinea-pigs. These

organisms were also found on histological section of the kidney, liver, and spleen, particularly in blood collections and blood vessels. There were fatty degeneration of the heart muscle, sub-mucous hemorrhage in the bladder, hemorrhages in the spleen and congestion of all the viscera. Bergey found the pseudo-diphtheria bacilli frequently in superficial suppurating wounds pure and in mixture. McLeod and Klaer report a septicemia from which they were able to isolate a pseudo-diphtheria bacillus from the blood on two occasions. The case was probably endocarditis; the organism was non-virulent aside from a slight subcutaneous infiltration at the site of inoculation. In one instance a peritonitis without bacteriemia was produced by a large dose. Babes and Monolesco report an endocarditis from which was isolated directly a pseudo-diphtheria bacillus, non-pathogenic for guinea-pigs, slightly so for mice. Roosen-Runge reports the finding of a diphtheroid rod, producing acid in bouillon, in the pus and blood of a case of empyema and endocarditis. This organism, however, produced only a local lesion and some emaciation in guinea-pigs. Ohlmacher found the pseudo-diphtheria bacilli in conjunction with *Micrococcus aureus* in a sero-cellular leptomeningitis and ependymitis. In the ventricles the bacillary form was in pure culture.

The foregoing are all the credible cases of infection of the pseudo-diphtheria bacilli, and only three of them seemed sufficiently established. There is a dearth of evidence to convict the organism in nearly all cases. With very few exceptions there are no agglutination or absorption tests or experimental evidences of bactericidal power in the patient's blood. It seems to the writer that in view of the well-known difficulty in determining the exact relation of the pseudo-diphtheria forms to the diphtheria bacillus and the diseases it causes, it is only fair to demand that some of the immunity tests be presented. It is not to be expected that the organisms shall be isolated on every occasion and from every lesion in perfectly pure culture, but it can be required that the organism shall predominate and that the blood shall show some immunity reaction. I do not think

that it is necessary for the isolated germ to possess guinea-pig virulence. The work of Hamilton demonstrates that the virulent pseudo-diphtheria bacilli may be of importance in otitis by showing the low opsonic index in patients harboring the virulent form, and an increase of this index by autogenous vaccination.

By immunity tests and the sugar reactions I believe that we can separate the avirulent diphtheria bacilli, the pseudo-diphtheria bacilli, and the bacillus of Hoffman. I wish here to enter a plea for the continuance of this nomenclature, first advocated, I think, by Neisser. This observer, a frank dualist, expressed his opinion very clearly before the Freie Vereinigung für Mikrobiologie, in 1913, that we may have typical virulent and non-virulent and atypical virulent and non-virulent strains, if the strains in question differ in minor details from the clinical diphtheria bacilli. If, however, the strains differ in several characters the organism has nothing to do with the diphtheria bacillus. Whether or not this be true remains to be seen, but sure it is that from a clinical standpoint the pathogenicity of the various types mentioned above deserve separate names for the present.

In mixed infections the pseudo-diphtheria bacilli are very common, particularly in tuberculous processes. The frequency with which they have been found in conjunction with other organisms as indicated by the cases recited above would seem to bear out in another line the assertion of Bergey.

Their observation in the cases which I shall recite and the late observations in Hodgkin's disease open up the question as to their penetration into the body from one of its entrances at periods of lowered resistance. The localization of the pseudo-diphtheria bacilli in the pharynx is of importance, not only from the question of degradation from true diphtheria bacilli, but in the matter of transmission of angina from person to person. Graham-Smith thinks that they may be transmitted just as true diphtheria and Cave seems to believe that they are of importance in producing an inflammation with high fever, rapid pulse, and red face. He asserts that they may produce a membrane.

Organisms of the Hoffman type. — Under this I shall mention briefly Bacterium xerosis, the organism of Fraenkel and Much, and aberrant forms. The xerosis bacillus is present in many normal eyes and is common in mixed infection in conjunctivitis. It probably has no importance away from the eye. By some authors, Gilbert, Dernehl and others, it is looked upon with concern in operative cases, because its extracts seem to produce some irritative phenomena when instilled into the eyes of experimental animals. It probably has no relation to the diphtheria bacillus, but can assume forms surprisingly like that organism. Axenfeld asserts that it favors the growth of Koch-Weeks' bacillus.

In leprosy, diphtheroid rods have been found, according to Wolbach and Honeij. The work upon human and rat leprosy would suggest that four kinds of organisms, diphtheroid, pigmented acid fasts, non-pigmented acid fasts, and aërobes have been cultivated in different parts of the world. It would seem that they all may be implicated, some mutation having occurred or that they are variants of one organism. The diphtheroids may be secondary invaders. These organisms, for the most part, are short, heavy, broad rods. So far as the literature goes they do not seem to be of importance in the necrosis of leprosy.

Hodgkin's disease has lately been attributed to a pseudo-diphtheria bacillus. This organism, first seen by Fraenkel and Much, was isolated and studied by deNegri and Mieremet. They found a rod which varied according to the age of the culture and of the medium, from short, plump rods or cocci to quite long, heavy, blunt or sharply pointed bacilli. They vary somewhat in their staining property and are chiefly Gram negative, the granules only retaining the stain, as a rule. These authors were unable to produce Hodgkin's disease in lower animals, but Bunting and Yates, who believe they have the same organism in their cases of Hodgkin's, claim positive results by the inoculation of cultures and pieces of gland into monkeys. The most important support for the belief that these bacteria have something to do with Hodgkin's disease comes from Billings and

Rosenow, who isolated a similar organism from eleven out of twelve cases and used it as a vaccine, with which treatment, combined with X-ray, they claim curative results. Diphtheroid rods have been found in enlarged glands near chronically inflamed joints. There is as yet no one form of diphtheroid definitely connected with Hodgkin's disease or arthritis.

The bacteriology of pseudo-diphtheria bacilli has little to offer in the determination of the relation of the various types and as help in determining the importance of the organism in human pathology. So far as the morphology of the organism is concerned, the experienced observer has little difficulty in distinguishing between the typical long, granular and beaded rod of the true diphtheria bacillus in growth upon coagulated blood serum and the shorter, plumper, straighter, barred or double wedge-shaped rod of the pseudo form under the same conditions of growth. Under other conditions of growth, and in atypical cases, the bacteriologist will be obliged to rely upon the fermentation and animal tests. Most observers agree that the true diphtheria bacillus ferments glucose, maltose, dextrine, but never saccharose, whereas the pseudo-diphtheria bacillus usually ferments saccharose and maltose and dextrine. These observations are made upon media made with litmus. If the indicator be phtholphthalein the pseudo-diphtheria bacilli will be found to produce a slight acidity in glucose with its high limit barely reaching the lowest point shown by the true Klebs-Loeffler bacillus.

Fortunately in true infections with this organism, agglutinin is formed and the serum opsonin is decreased. A mild bactericidal power of the serum arises in patients and a very pronounced bacteriolysin can be produced in experimental animals. This antibody is thermostable and does not require the addition of fresh complement. No antibodies are formed by the various members of this group which will answer to separate them by the complement-fixation method as has been shown by Kolmer and others.

It appears from the work of Hamilton, Horton and others

that autogenous vaccination is profitable where the isolated strain of pseudo-diphtheria bacillus can be convicted of the infection.

The objects of my work upon the pseudo-diphtheria group were to discover their frequency in various infections, to decide upon criteria from which to judge of their etiological importance, to explain their occurrence in the blood and to test out the often suggested theory of a degradation within the body from true Klebs-Loeffler to avirulent diphtheria bacilli or to the Hoffman type.

I have given a list below which will show how frequently the atypical forms have been met. In Case 20 only have I been able to prove that they were the causative agents.

From my note-book I find the isolation of the pseudo-diphtheria bacilli from the following conditions:

(1) — A thoracic suppuration, pseudo-diphtheria bacilli associated with a streptococcus which failed to grow.

(2) — from an acute pleuritis and peritonitis with congestion of the lungs, pseudo-diphtheria bacilli associated with a Bacillus coli and Ps. pyocaneus.

(3) — from a broncho-pneumonia autopsy, the pseudo-diphtheria bacilli in pure culture, no smears were made.

(4) — an acute otitis ending in cellulitis at side of face, a direct operation culture, avirulent diphtheria bacilli in pure culture.

(5) — broncho-pneumonia of long duration, bloody sputum, a streptococcus of the mucosus variety, pseudo-diphtheria, and Micrococcus albus.

(6) — a blood culture upon acute lymphatic leukemia, pseudo-diphtheria bacilli in pure culture.

The records at the Pepper Laboratory supply the following cases:

(7) — An undiagnosed condition giving a slight, bloody discharge from the nose, two colonies upon a blood agar plate, Micrococcus aureus and pseudo-diphtheria bacilli (not segmentosus).

(8) — empyema, pus obtained by thoracentesis, pneumococcus, pseudo-diphtheria bacilli and Micrococcus candidus.

(9) — abscess of the abdominal wall, opened and drained, direct operation culture, Micrococcus aureus, pseudo-diphtheria bacillus.

(10) — (E) nephritis and sinusitis, from nasal swabs, Streptococcus pyogenes, Micrococcus albus and aureus and pseudo-diphtheria bacilli; from the urine on two occasions the pseudo-diphtheria bacillus in pure culture, of the same biological variety that was found in the nose.

(11) — post-partum septicemia, blood culture, pseudo-diphtheria bacilli in pure culture (non-virulent for guinea-pigs).

(12) — (C) perineal abscess, Bacillus coli, pseudo-diphtheria bacilli; mixed vaccine, improvement.

(13) — post puerperal typhoid fever, intra-uterine culture, pseudo-diphtheria bacilli.

(14) — (D) acute articular rheumatism blood culture, pseudo-diphtheria bacilli.

(15) — abdominal abscess, direct culture, Micrococcus aureus, pseudo-diphtheria bacilli.

(16) — (A) undiagnosed, blood culture, one of three flasks, pseudo-diphtheria bacilli.

(17) — (H) infected wound of hip, direct pus culture, Micrococcus albus, avirulent diphtheria bacillus.

(18) — corneal ulcer, Bacillus xerosis pure.

(19) — 7866 — endocarditis, blood culture in three of five flasks, pseudo-diphtheria bacilli, throat culture negative.

(20) — an example of the aberrant pseudo-diphtheria forms was reported by me in the Zentralblatt fur Bakt. V. 70 as the apparent cause of an obscure infection, probably endocarditis. The immunity reactions all resulted positively. Guinea-pigs were not affected in the slightest.

Three cases of angina have occurred in the University Hospital upon which the first clinical and bacteriological diagnosis was diphtheria. Upon close examination of the cultures it was found that they differed in granularity from the true Klebs-Loeffler bacillus and were of quite uniform size and arrangement. Sugar tests placed them at once in the pseudo class. One culture tested was not virulent for guinea-pigs. The cases from which these cultures came presented a rather limited and only lightly adherent pseudo-membrane. There was fever up to 103° F. and a very rapid pulse. The face was quite red and in one there was a general bodily blush. Two patients had received one thousand units of diphtheria antitoxin upon entering the hospital, while the third, a nurse, was not benefited by antitoxin. Although the patients were quite ill, recovery was rapid under local treatment. No sequelæ occurred.

Certain of these strains have been repeatedly injected into guinea-pigs, using cultures upon solid and fluid media both whole and after shaking with glass balls. The Case 10,

Culture E, was injected, the pig's blood drawn, its serum applied to the bacillary mass from a solid culture, incubated and the mixture injected into a fresh pig. This failed to show any virulence or anaphylactic effect.

Culture H, Case 17, was biologically a diphtheria bacillus, but failed to kill two guinea-pigs and could not be regained from the peritoneum.

The cases and the experimental work are cited to show the variety of conditions in which these pseudo-diphtheria organisms may be found. I wish to emphasize their isolation from the blood five times, in two of which, examined for this purpose, they were not to be found in throat swab cultures.

In the endocarditis case (19) there was no agglutination in greater dilution than normal blood and the phagocyte average was almost identical, 1.6 and 1.8. The agglutination test has been tried upon two of the cases from whose blood the pseudo-forms were isolated and upon three others (10, 12, and 13), all with negative results.

The existence of these microbes in the blood, in the absence of proof of their pathogenicity, deserves an explanation. It would seem that the only ways for them to penetrate would be from the upper air passages and the genito-urinary tract. From the data at hand one must conclude that the pseudo-diphtheria bacilli in these cases are not living parasitically but symbiotically or saprophytically within the body. Are they degraded diphtheria bacilli, or are they harmless dwellers on the mucous membrane, entering the blood during periods of lowered resistance? It is of course well nigh impossible to prove either hypothesis, especially the latter, but if these avirulent germs are "mutation" forms obviously they are acted upon by the blood or tissue, so that they lose virulence, change in morphology and some develop a new ferment. The work of Graham-Smith and others would indicate that the blood has little to do with the transition. This leaves the lymphatic tissue as a probable seat where the influence is exerted.

In reviewing the foregoing it would seem that there is nothing in the literature to show the origin of the non-virulent members of the group, but that they are degraded forms is suggested. Avirulent diphtheria bacilli and pseudo-forms are present in many infections and when of importance in their causation give rise to some immunity reactions. It is fair to demand these immunity reactions as criteria of their infectivity, since pseudo-forms are constantly present in the body openings and may at times be isolated from the blood when they seem to have nothing to do with the clinical condition. Aside from three cases of pseudo-diphtheria bacillus angina, these bacteria have been observed by the writer in twenty cases, in only one of which could they be shown to be causative. Excepting as a cause of otitis in which their position as the only etiological factor is not proved, and as the cause of an angina not favorably influenced by diphtheria antitoxin, only four definite cases of infection with pseudo-diphtheria bacilli are on record. The etiological importance of diphtheroids in adenopathies is not yet settled.

Studies in Diphtheroids. — II.

Source of Pseudo-Diphtheria Forms in the Body. Experiments on Mutation.

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In the previous paper it was repeatedly suggested that the pseudo-diphtheria bacilli may come from Klebs-Loeffler organisms by mutation, or as I prefer to call it, degradation. It seems fairly well accepted that true diphtheria organisms, when they penetrate the body, suffer some loss of virulence and biological activity. Possibly true or avirulent diphtheria bacilli are taken into the body and modified, or the transformation takes place in the mucosa. In the light of the findings of diphtheroids in Hodgkin's disease and leprosy and in glands near joints the seat of arthritis, the lymphatic system as a possible seat of the alteration, if such occur, deserves attention. In the previous paper I suggested this, as Graham-Smith's work seemed to rule out the blood as a factor. Nevertheless this fluid has been investigated also.

In judging whether my culture had undergone mutation or degeneration I decided to use, as criteria, loss of virulence for guinea-pigs, appearance of saccharose splitting enzyme in litmus media, and a permanent change in morphology.

Assuming the mutation dependent upon or associated with alteration of ferment action, I have begun with a biologically typical Klebs-Loeffler bacillus freshly isolated from clinical diphtheria, and submitted it to the following conditions: exposure to direct sunlight for varying periods, exposure to normal human and dog serum and dog immune serum, exposure to sodium oleate and to leucocytic extract, and lastly, to emulsions and culture medium made of lymphatic glands as nearly normal as I could procure them. Before beginning the experimental work this true Klebs-Loeffler organism (Culture K) was plated and isolated seven times, so that as far as possible by cultural methods I had a pure

culture. Injections of solid and fluid cultures killed guinea-pigs in forty-eight hours with typical pathological effect.

Exposure to sunlight occurred both upon cultures in blood serum agar and bouillon and dried upon filter papers, without effect upon their sugar fermenting power but naturally with a distinct loss of virulence. The growths from the exposed filter papers were made upon blood serum and exposed again when two days old. This experiment was also negative for saccharose fermentation. The morphology of this true Klebs-Loeffler strain was unchanged during this experiment.

Exposure to normal dog and rabbit serum, pure and in 1–10 dilution for one, three, six and twenty-four hours was without result, either upon sugar fermenting powers or guinea-pig virulence. Sodium oleate in dilution of 1–1000 was promptly bactericidal, but at 1–10000 reduced the dextrose fermenting power but did not develop a saccharose splitting enzyme. Only one experiment was done with leucocytes. Rabbit's leucocytes, washed once with salt solution, received the Klebs-Loeffler culture in proportions of one standard oese to one cubic centimeter of emulsion, allowed to stand over night in the ice-box after one-hour incubation at 37° C.; shaken fifteen minutes and injected into guinea-pigs with controls. The first generation did not ferment dextrose but the third did clearly. Saccharose was unchanged.

Lymph glands were obtained at post-mortem upon human bodies and a deer. A part was triturated with an equal weight of salt solution and mixed in equal parts with double strength agar. Other glands were seared with hot oil and opened by section with a sterile knife into Petrie dishes. Upon these, the lymph gland agar and lymph glands themselves, the true Klebs-Loeffler strain was cultivated for seven generations. There was a distinct loss of virulence of these strains as compared with cultures upon agar. In the third and fifth generations upon glands there was very early acidification of dextrose; upon the third day of these transplants a faint acidity appeared in saccharose. In the sixth and seventh generations from the glands, however, no

saccharose splitting was seen but the glucose acidification was early and marked. The two cultures, third and fifth day gland growths, were transferred daily from glucose to glucose and saccharose to saccharose for five days but in none of these transplants did acid appear in saccharose. The cultures upon lymph gland agar were entirely negative in the sugar tests.

A fully grown dog was immunized against this true Klebs-Loeffler strain by eight small, slowly increasing doses (twenty-four-hour bouillon, .05–.4 cubic centimeter) both subcutaneously and intravenously. Between the second and third, fourth and fifth, seventh and eighth injection blood cultures were taken. They remained sterile. After the fourth injection a gland was removed under ether from the groin and cultivated with negative results. After the sixth injection several mesenteric glands were cultured by laparotomy under ether, also with negative results. The dog had no gland large enough to remove and use for culture medium.

The culture, K, was grown in deep anaërobic dextrose serum agar, as nearly neutral in reaction as possible, for twelve generations, transferring every ten days always from anaërobic to anaërobic. The twelfth generation in this medium was almost exactly of the same virulence as the twenty-sixth generation of K upon Loeffler's blood serum. Injection material was obtained by growing the two cultures in bouillon for forty-eight hours. No saccharose fermentation appeared in the twelfth anaërobic culture.

Lymph gland medium was prepared from autopsy and operation material as near normal as possible (one gland sectioned showed only follicular hyperplasia). (Formula — 1 gram glands, 10 cubic centimeters salt solution, ice-box over night; agar 1 per cent, peptone 1.5 per cent, salt .5 per cent, sterilized at 50° C. four successive days in water bath.)

Culture K was planted from blood serum into the depths of this medium, as was also the transplant that had been grown in deep dextrose serum agar for twelve generations. Three generations of fifteen days growth were made of these two strains. At the end of the third fifteen-day period the

two series were transferred to bouillon for injection material and to blood serum for purity and morphological observations. These last having been controlled the bouillon cultures were injected into guinea-pigs. Control pigs were also injected with bouillon cultures made from the twenty-ninth generation of K upon blood serum.

There was no loss of virulence by the strains which had been subjected to the influence of lymph glands and anaërobiosis.

Tests upon glucose and saccharose with the two modified series showed no loss of glucose splitting power and no appearance of saccharose fermenting enzyme.

The morphology of this K upon Loeffler's medium has remained fairly constant save for a tendency in late generations to grow out in long, clubbed, mycelial forms with many metachromatic bodies. The final generation of each of the experimental anaërobic cultures has been a short, barred, and rounded end rod with a few red granules and many double wedge-shaped individuals showing an unstained median zone. The morphology of the original Klebs-Loeffler culture would reappear after three or four generations upon blood serum.

It seems that attempts at mutation by the influence of lymph tissue and blood have not been successful. The morphological change has undoubtedly given some observers the basis for such an assumption. The negative results of this work are not surprising, but it forms one step in an effort to explain the source and nature of the pseudo-diphtheria forms and the importance of the organism described as the cause of Hodgkin's disease. In a paper* on the latter subject will be found the morphology and biology of the true and pseudo-diphtheria bacilli used in my work, as well as of the organisms isolated from glands. It may be well to add here that these strains, all but three of which have been under observation nine months or more, have bred true to type as shown by at least three full observations.

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STUDIES IN DIPHTHEROIDS

III. BACTERIA ISOLATED FROM ENLARGED GLANDS, ESPECIALLY IN HODGKIN'S DISEASE *

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The object of this paper is to put on record observations on bacteriologic studies in adenopathies, especially Hodgkin's disease, in the light of the findings of Fraenkel, Much, de Negri, Mieremet, Rosenow, Bunting and others.

The descriptions given in literature of bacteria isolated from malignant lymphatic granulomas seem to indicate a considerable difference in character among the isolated strains. Early in my work on Hodgkin's disease, it became evident that while diphtheroids might be isolated from many cases of this disease, and indeed from other adenopathies, they varied in morphology and biology. Cultures were sent me by Dr. E. C. Rosenow of Chicago (marked R) and by Dr. J. A. Kolmer (marked K), and comparison of these two sets with those obtained at the Pepper Laboratory showed that the impression stated above was correct. It seemed well to tabulate all the diphtheroids at my command to see if any one form were common in pathologic or clinical Hodgkin's disease or in other adenopathies, and to compare their biology with related organisms. Previous studies with this group have shown the great variations in the biology of morphologically similar diphtheroids, and I did not look for much assistance from this side. Judging from de Negri and Mieremet's work, it seemed that Bordet medium and the morphology, especially in connection with Gram's stain, would enable one to define with some degree of certainty the organism to be considered most important in Hodgkin's disease.

It might be well at this point to state that as Hodgkin's disease I have considered cases with more or less generalized progressive glandular enlargement in which the individual members of the group did not fuse and soften, and showing under the microscope diffuse, mononuclear hyperplasia, prominence of large endothelioid cells and eosinophils, which elements are separated irregularly by a fine fibrosis. These glands should also show small necroses. This description is based on the disease picture as outlined in the work of Reed and

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Longcope. Search has always been made for tubercles and tubercle bacilli. The microscopic anatomy of Hodgkin's disease is by no means constant, and I doubt if the borderline between it and that of chronic adenitis and tuberculosis is clear in any one's mind. In citing my own cases below, the name "Hodgkin's disease" has been used only as a clinical term, pathologic diagnosis being made on the anatomic findings. The material for these studies was obtained by operation under local anesthesia or gas. In the arthritis cases, the most accessible gland or that near the principal joint focus was removed. Free glands of moderate size and firmness were selected from the Hodgkin's cases.

The following is the method used in studying the cases:

A section of a gland removed at operation was cut at once, one piece put into Orth's fluid or liquor formaldehydi, another dropped into hot oil, transferred to ether, then into salt solution and from that into a large sterile bottle; cut up with scissors within the bottle, pieces removed and planted on Bordet medium tubes and plates, serum dextrose agar, Loeffler's blood serum, serum water and rabbit blood agar. Smears made from a bit of the gland stained with Loeffler's, Gram's, carbolfuchsin and Wright's. In most of the cases the bits of tissue remaining after inoculating sufficient culture mediums were put into 15 per cent. antiformin, incubated at 37 C. over night and seeded on the same mediums. Two of the cases were fortunately exposed to antiformin only for an hour because cultures were positive then and negative after eighteen hours' exposure. All the cultures were plated on Bordet medium except two early ones before the necessity of this procedure was appreciated. It is necessary in this work to be sure that one has pure cultures. It is best to plate on Bordet medium and fish after three days' incubation, at which time the slightly varying colonies are differentiated. This medium has a good contrast surface.

The accompanying table was compiled from records made on cultures growing on mediums of the same method of preparation, and the cultures from Drs. Rosenow and Kolmer were tested on the same batch of mediums and at the same time, as Nos. 8772, 8916, 9144, 9265 and 9392, while the control, true diphtheria bacilli and pseudo forms 873-1 and 873-3 were grown on the same batch of culture material as 6638 and 6640. Observations on carbohydrates were made on litmus agar sugar mediums of reaction neutral to phenolphthalein. The other standard mediums were of 1+ reaction. Cultures isolated in the Pepper Laboratory are given numbers, while those from Drs. Rosenow and Kolmer are lettered. The generations from which these notes were made varied from the third to the tenth. Nos. 8772, 8916, 9265, 7144, 9392, the R's and the K's were all passed through three times and remained uniform in biology. The morphology has not been controlled by repetition to the same extent, but comparison of the original smears and those made during the cultural observations indicate that the staining characters remain the same. Culture 8772, now in its thirty-third generation, is the only one to change materially, having lost its beaded and segmented character and staining as long, slender, solid

rods. The drawings were made by the same person with the same equipment throughout.

Case 1 (6638).—A. S., a woman, aged 22, three months ago noticed that her neck began to swell on the left side, giving her no discomfort or pain. About two weeks later she noticed the same condition on the right side. One month later she noticed that she had lumps in the right axilla. There never was any pain or discomfort in these swollen glands. The glands on both sides of the neck have been steadily increasing in size; the most rapid growth shows in the glands in the right axilla.

Physical examination reveals a large mass of glands in the anterior of the neck on both sides. On the right the nodules are more discrete, although a number of them are massed together, on the left side they are at places definitely outlined, but the majority of the mass of glands are adherent. They are all very hard, smooth and noninflammatory, and in places are fixed quite tight. In the right axilla are a number of glands, one about the size of an egg, which is hard, smooth and discrete. It is also noninflammatory. Blood: R. B. C., 3,350,000; W. B. C., 14,200; Hb., 53 per cent.; P., 81 per cent.; L., 14 per cent.; M., 3 per cent.; T., 2 per cent.; E., 0 per cent. The axillary gland was removed under local anesthesia.

Section shows chronic lymph granuloma with an attempt to preserve lymphoid collections, but these and the chords are encroached on by diffuse round cell and endothelial leukocyte infiltrates and fine fibrosis. Eosinophils and multinucleated cells are numerous. Connective tissue is everywhere increased and hyaline. The focal necroses are missing; growth is active, as indicated by normal mitosis and many compression giant cells. Diagnosis, lymph granuloma.

Case 2 (6640).—L. P., aged 30. The patient first noticed a swelling in the right side of the neck one year ago. Twelve weeks ago cough and vomiting were present, eight weeks ago, fever, pain in abdomen, and sweating occurred.

Physical examination showed the lymphatic glands of the neck, axilla and right inguinal region to be greatly enlarged; the greatest enlargement was in the right cervical chains; here some were the size of small lemons; they extended from the angle of the jaw to the clavicle. There was one large one in the right submaxillary region. The enlargement was not so marked on the left; in the axillae they were about the size of horse chestnuts; they were not so large in the right groin; small ones were found in the left groin. No glands were palpable in the popliteal space or in the epitrochlear region. The glands were soft, movable, discrete and unattached to the skin; not tender. The chest showed pleural effusion; the abdomen, ascites, palpable liver and palpable spleen. Blood: Hb., 40 per cent.; R. B. C., 3,290,000; W. B. C., 4,100. Von Pirquet negative. Urine: Albumin and casts.

Section shows a tissue made up of large lymphocytes or endothelioid cells with a ground work of fine fibrosis and many scattered polynuclear cells. Eosinophils are numerous. There are many areas of necrosis, some wholly hyaline, others with a core of chromatin débris surrounded by a hyaline necrosis. Giant cells and multinucleated cells are numerous, most of which seem to be compression giant cells.

Case 3 (8916).—W. P. Two years ago the glands on the left side of neck began to swell, followed shortly by the left submaxillary gland. Previous to this for some time he had had enlarged glands or kernels in his neck, especially over the left mastoid. About a year ago a playmate of the patient died of Hodgkin's disease, which started in a similar manner to that of the patient. The blood count showed: Hb., 85 per cent.; R. B. C., 4,330,000; W. B. C., 8,300; Diff.; P., 15 per cent.; L., 38½ per cent.; L. M., 41 per cent.; E., 4 per cent.; B., 1 per cent. The group of large mononuclears here includes large lymphocytes and myeloblasts, large mononuclears and transitionals.

Physical examination showed the patient to be sallow or ashy. His neck was thin and suggested loss of weight. Considerable swelling was present in the left side of the neck involving the glands below the angle of the jaw and cervical region and postoccipital; but numerous small glands could be felt on both sides of the neck from the clavicle upward. There was doubtful enlargements in the right axilla, none in left. The inguinal glands were more distinct. A possible slight dulness was noted at the root of the right lung and harshness of inspiration. The sounds, however, were not altogether certain. The spleen was doubtfully palpable. No abdominal masses were noted.

Nov. 4, 1913: Marked improvement has appeared in the condition of the glands. Those of the neck have almost disappeared; one is still palpable just above the clavicle; none are discoverable in the axilla.

March 21, 1914: Some enlargement of the cervical glands still exists; these seem to be variable in size. A little while ago the patient noticed an enlarged gland in the right forearm or epitrochlear region, but this has disappeared.

March 28, 1914: Blood examination to-day shows a little improvement in hemoglobin and red cell count. Two series of Roentgen ray treatments have resulted in considerable improvement.

A gland about 5 by 4 mm. over the tip of the mastoid was removed under local anesthesia. The smears failed to show any rods. A section showed fairly well-encapsulated tissue of the general outline of a lymph node consisting of a disturbed follicular border and a medulla composed chiefly of fibrous tissue containing many dilated blood channels whose walls are imperfect and the endothelium of which is scanty. The follicular border shows loosely arranged lymphoid cells between which are red blood cells, and in the larger hyperplastic follicles, cells of the large mononuclear series. The endothelium of sinuses cannot be said to be increased. There are practically no eosinophils. There are no large phagocytes, no giant or ring cells. Here and there a fair number of polynuclears may be seen. The connective tissue of the medulla is well formed. The fibrils are clear and there seems to be no degenerative change in the section anywhere. It seems more like a chronic lymphadenitis than Hodgkin's disease or tumor.

The patient was continued on Roentgen ray and vaccine treatment and improvement has continued.

Case 4 (9265).—E. P., aged 24, colored, has had general adenopathy and rheumatic pains throughout the joints and extremities, for only about four weeks. The gland remained firm and discrete and the case seemed one of Hodgkin's disease. Histologic section showed chronic follicular enlargement with moderate intrafollicular and perifollicular fibrosis, compression of sinuses almost to obliteration, a notable increase in large mononuclears and fibroblasts of chords. Eosinophils and necrosis are absent; there was no tubercle formation, no giant cells. Cultivation produced a large micrococcus in pairs, each individual having a long transverse diameter but no rods. The patient refused to stay in the hospital and was lost to further observation.

Case 5 (3541).—McC., had adenopathy of both cervical and right axillary regions which began as an enlargement of the right epitrochlear gland. The right pectoral region was indurated like carcinoma *en cuirasse*. After removal of some glands for experimental purposes, the patient developed a general pneumococcus infection and died.

Section from operated glands showed a chronic adenitis with features of sarcoma, in places very like endothelioma. The cultures remained sterile except one Bordet medium tube and one blood agar tube and one blood serum tube on which grew a white coccus. No colonies of rods could be discovered.

Necropsy showed a sarcomatous growth of the lymph glands, probably endothelioma. As the cell was small, round or oblong with a small amount of acid-staining protoplasm and a large vesicular and hyperchromatic nucleus, the tumor possibly arose from the endothelial cells of the lymph spaces. The rest

of the postmortem indicated arteriosclerosis, fibrosis in the lung and the evidences were found of the acute infection through which the patient had passed.

Case 6 (8772).—L. W., a woman, aged 32, had bilateral cervical adenopathy of four months' duration with firm, discrete glands. At operation those removed showed a yellow-gray surface with a few minute points of hemorrhage. Near the margins there was apparently fine fibrosis. The gland section was dry. Microscopic examination revealed a rapidly growing lesion chiefly along the lymph channels, made up of large, palely staining, irregular cells; probably endothelioma. The fibrous tissue was abundant, but loose. There was no resemblance to Hodgkin's disease. Culture on Bordet and blood agar gave some colonies of cocci and one brownish colony on a Bordet tube, proving to be an irregular granular rod.

Roentgen ray and vaccine treatment was instituted with at first favorable results. The patient was pregnant on arrival at the hospital, and it was decided that termination of this condition was necessary for her good. After return from the maternity, treatment for the adenopathy was resumed. The patient left the hospital in a short time. The report is that she has died.

Case 7 (8604).—J., aged 19, had bilateral adenopathy with an acute exacerbation since the removal of adenoids and tonsils two months before operation. Large masses of discrete glands were found, the individual glands being soft and resilient. Operation showed soft, fatty, necrotic, gray-yellow glands with here and there precaseous necrosis. Caseous tuberculosis was confirmed by stain and section. *M. albus* appeared in pure culture in every tube. No diphtheroids were noted.

Case 8.—A. G. F., a woman, aged 33, was in good health until seven years ago, when she became pregnant and at the same time developed a swelling in the right side of the neck which lasted two months and then disappeared. After the birth of her child, a similar swelling developed in the right axilla which remained until five years ago when it was drained, at which time it was very much inflamed and contained pus. Two years ago glands were removed from the neck and axilla.

Smears and cultures made in the ordinary way showed no tubercle bacilli, two slender, solidly staining rods and one pair of cocci by Loeffler's stain. The small lymphocytes, large mononuclears and a few polynuclears were most numerous. Incubation of the gland on Bordet and blood agar for six days showed nothing. A section from the gland showed a disturbed follicular border, between the lymph-cell collections of which are irregular areas of epithelioid cells and an occasional giant cell. The margins of the epithelioid cell collections showed numerous fibroblasts toward the center of the gland. Areas of eosin-staining necrosis are present. No eosinophils are to be seen. A few tubercle bacilli may be noted.

Case 9 (9144).—M. W., a white woman, aged 22, had always been healthy until four months before admission, when she had an attack of tonsillitis lasting one week. Following this her left wrist became swollen, red, hot, tender and stiff, and later her left ankle, right wrist, knees, elbows and shoulders became involved in a similar manner. Finally the finger joints were involved and these were at the time of admission, giving her the most trouble. In the meantime she had been given twenty-four antirheumatic antitoxin injections without benefit, and, indeed, had lost 18 pounds in weight.

Physical examination showed a somewhat sallow complexion; a gland the size of a finger tip was palpable in the right axilla, and a few posterior and anterior cervical nodes were palpable. The tonsils and pharynx were somewhat congested. There were sordes on the teeth and a slight pyorrhea was present. The elbows were fixed in partial flexion, the wrists limited in motion. The proximal phalangeal joints of both hands were swollen, slightly reddened, hot and tender; these swellings were fusiform in shape. The power in the hands was decidedly diminished. The gonococcus fixation test and Wassermann were

negative. Roentgenoscopy of the hands showed beginning atrophic arthritis of the proximal phalangeal joints. May 21, Dr. Eliason removed a gland from the right axilla for culture and microscopic examination. On discharge she was greatly improved; the elbows were movable, the swelling of ankles and wrists had diminished, the power in the hands had increased.

A smear failed to show any bacteria. Cytology was negative. A section shows large loose follicles with quite definite increase in delicate connective tissue. The interfollicular sinus architecture is disturbed by the growth of the follicles, and fibrous tissue is definitely increased. The capsule is little, if any thickened. No especial kind of cell other than the small lymphoids is present. There is no necrosis, no eosinophils.

Case 10 (9392).—J. M., a woman, aged 41. Her present illness began seven years ago, coming on a few hours after delivery with pain and tenderness in spine; soon other joints were involved — knees, shoulders, elbows, wrists and fingers. Trouble then varied from time to time, never very severe, until two months ago, when she had an acute exacerbation in the knees, both becoming red and swollen.

Physical examination showed the right epitrochlear to be the only gland palpable. The tonsils were atrophic. The right shoulder and elbow were tender and motion was limited. Atrophic changes appeared in the finger joints. There was marked swelling of both knees, the right one being the larger and somewhat tender, and presented a distinct fluctuation. Roentgenoscopy of the wrist showed atrophic changes. Cultures from the throat were negative; from the fluid from the knee joint also negative; from the blood, negative. The right epitrochlear gland was removed and three organisms isolated. Autogenous vaccine was started. On discharge after only three injections, she was feeling much better, the anemia was improving and the pain in the knees had decreased and was entirely absent in the other joints.

Case 11 (4554).—G. P. Three years ago, following an attack of grip with tonsillitis, the patient developed acute arthritis in the small joints of the feet; later in the wrists and fingers. These early attacks were associated with chills and acid sweats. Deformity persisted and has gradually progressed. The patient has lost weight and there has been considerable atrophy and progressive weakness of the muscles of the upper extremities. There is at present involvement of the fingers, elbows, wrists and knees, the most active process being in the knees. The tonsils are small but diseased, no other focus of infection being discoverable. October 16, a small lymph node was removed from the right groin for culture with negative result. Section shows a mild chronic lymphadenitis.

Case 12 (4555).—J. E., two years ago, developed acute arthritis in the right wrist, gradually progressive; since then the process has extended to the elbows, both wrists, fingers and knees, and includes the temporomandibular joints. No focus of infection has been found. Repeated blood cultures have been sterile. The most active joints at present are the knees. October 15, a small lymph node was removed from the left groin for culture. Result was negative.

Case 13 (6639).—B. N., aged 40, white. Present illness began two weeks ago with pain in back, spreading to hands and knees, these joints becoming swollen. Then, in order, hips, ankles, shoulders and elbows were involved. At first the trouble went from one joint to another. Now all are involved.

Physical examination shows irregularity of pupils, pyorrhea, cervical adenopathy. The heart is negative. Both shoulders are limited in motion and crepitate. The left elbow is ankylosed and tender. The left wrist is tender, enlarged, motion limited. All finger joints are similarly affected. There is muscular wasting about the joints of the upper extremity. The right knee-joint is enlarged and tender. There is some effusion and motion is limited and associated with muscular atrophy. The ankle and toes are likewise affected. Von

Pirquet is negative. A roentgenogram shows no bone changes in knee and right hand. The Wassermann is negative, the Neisserian fixation-test is negative. The epitrochlear gland is sterile.

Section shows lymphadenoid tissue in a state of chronic follicular and interstitial formation. In the chords and sinuses one may see endothelial and large lymphocyte hyperplasia, and there is a prominence of the vascular endothelium. The connective tissue, both in the gland and in the capsule, is increased, hyaline and poor in cells. Diagnosis, chronic lymphadenitis.

The first two cases (6638, 6640) correspond to the picture accepted as that of Hodgkin's disease, and from them were isolated diphtheroid organisms of quite similar morphology but distinctly differing in biology (Figs. 1 to 6). They are similar in Loeffler's stain to R. B. 2 (Figs. 39 and 40), R. E. 1 (Figs. 50 and 51), and K. II-3 (Fig. 54). Vaccine treatment is now being given in both these cases but no report can be given yet as to its action.

The third case is one of Hodgkin's disease from its history and was so diagnosed by Dr. Stengel. The histologic section does not show the picture required but it should be remembered that Roentgen-ray treatment had been used for several months before the gland culture was made. This would probably alter the anatomy of the tissue. From this case no less than seven slightly varying bacteria were found (Figs. 7 to 23). Among these Nos. 1 (Figs. 7 to 10), 4 (Figs. 15 to 17), and 7 (Figs. 18 to 20) most resemble the cultures in the frank cases of Hodgkin's disease as given above. There are certain similarities between these and some of the cultures of Drs. Rosenow and Kolmer.

The fourth case was probably one of Hodgkin's disease although certainly far from typical; only cocci were culturable.

In the fifth case, Patient McC., unfortunately, the culture failed to give a growth and he died so soon after the operation that further work was impossible. It would have been interesting to have gotten a growth in his case because in the next case, another endothelioma, there was a prompt growth of a long rod (8772, Figs. 25 to 29). This organism bears no resemblance to any other of my own cultures but is quite like R. C. 3 (Figs. 45 to 47); Dr. Rosenow marks this case as one of Hodgkin's disease. This bacterium is not at all like those in my cases of frank Hodgkin's.

From the sixth and seventh cases, diagnosed as tuberculosis by the finding of the bacilli, no rods could be cultivated. The histologic sections from the case of A. G. F. were studied by Gram-Weigert, Giemsa and Much stains. Here and there a solidly staining gram-positive rod (probably a tubercle bacillus) was found, but no granules like the Much granules or rows of beads such as he has described in Hodgkin's disease were seen.

ILLUSTRATING FIGURES 1-4

Fig. 1.—1, 6638*a*, 48 hours agar—Loeffler; 2, 6638*a*, 48 hours Bordet—Loeffler; 3, 6638*b*, 48 hours agar—Loeffler; 4, 6638*b*, 48 hours Bordet—Loeffler; 5, 6640, 48 hours agar—Loeffler; 6, 6640, 48 hours Bordet—Loeffler; 7, 8916-1, 72 hours agar—Loeffler; 8, 8916-1, 72 hours Bordet—Loeffler; 9, 8916-1, 72 hours blood serum—Loeffler; 10, 8916-1, 72 hours agar—Gram; 11, 8916-2, 48 hours agar—Loeffler; 12, 8916-2, 48 hours blood serum—Loeffler; 13, 8916-2, 24 hours agar—Gram; 14, 8916-2, 24 hours Bordet—Gram; 15, 8916-4, 48 hours agar—Loeffler.

Fig. 2.—16, 8916-4, 48 hours Bordet—Loeffler; 17, 8916-4, 48 hours blood serum—Loeffler; 18, 8916-7, 48 hours agar—Loeffler; 19, 8916-7, 48 hours Bordet—Loeffler; 20, 8916-7, 48 hours bouillon—Loeffler; 21, 8916-9, 72 hours bouillon—Loeffler; 22, 8916-9, 72 hours Bordet—Gram; 23, 8916-10, 72 hours Bordet—Gram; 24, 9265, 48 hours blood serum—Gram; 25, 8772, 48 hours agar—Loeffler; 26, 8772, 72 hours blood serum—Loeffler; 27, 8772, 48 hours agar—Loeffler; 28, 8772, 72 hours Bordet—Gram; 29, 8772, 72 hours blood serum—Gram; 30, 9144-1, 48 hours Bordet—Gram.

Fig. 3.—31, 9144-2, 48 hours blood serum—Gram; 32, 9392-1, 48 hours agar—Loeffler; 33, 9392-1, 48 hours Bordet—Loeffler; 34, 9392-1, eighth day Bordet—Loeffler; 35, 9392-1, 72 hours Bordet—Gram; 36, R. A.-1, 48 hours Bordet—Loeffler; 37, R. A.-2, 48 hours Bordet—Loeffler; 38, R. B.-1, 48 hours Bordet—Gram; 39, R. B.-2, 48 hours Bordet—Loeffler; 40, R. B.-2, 72 hours Bordet—Gram; 41, R. B.-4, 24 hours agar—Loeffler; 42, R. B.-4, 48 hours Bordet—Loeffler; 43, R. B.-4, 48 hours Bordet—Gram; 44, R. C.-2, 24 hours agar—Gram; 45, R. C.-3, 24 hours agar—Gram.

Fig. 4.—46, R. C.-3, 24 hours Bordet—Gram; 47, R. C.-3, eighth day blood serum—Gram; 48, R. D.-1, 48 hours bouillon—Loeffler; 49, R. D.-2, eighth day agar—Gram; 50, R. E.-1, 72 hours Bordet—Loeffler; 51, R. E.-1, eighth day agar—Gram; 52, K. I.-1, 72 hours blood serum—Loeffler; 53, K. II.-1, 48 hours Bordet—Gram; 54, K. II.-3, 72 hours Bordet—Gram; 55, K. II.-4, 48 hours Bordet—Gram; 56, Klebs-Loeffler "K," 48 hours agar—Loeffler; 57, Klebs-Loeffler "K," 48 hours Bordet-Loeffler; 58, Klebs-Loeffler "K," 24 hours blood serum—Loeffler; 59, 873-1, Pseudo-diphtheria 48 hours Bordet—Loeffler; 60, 873-3, Pseudo-diphtheria 48 hours Bordet—Loeffler.

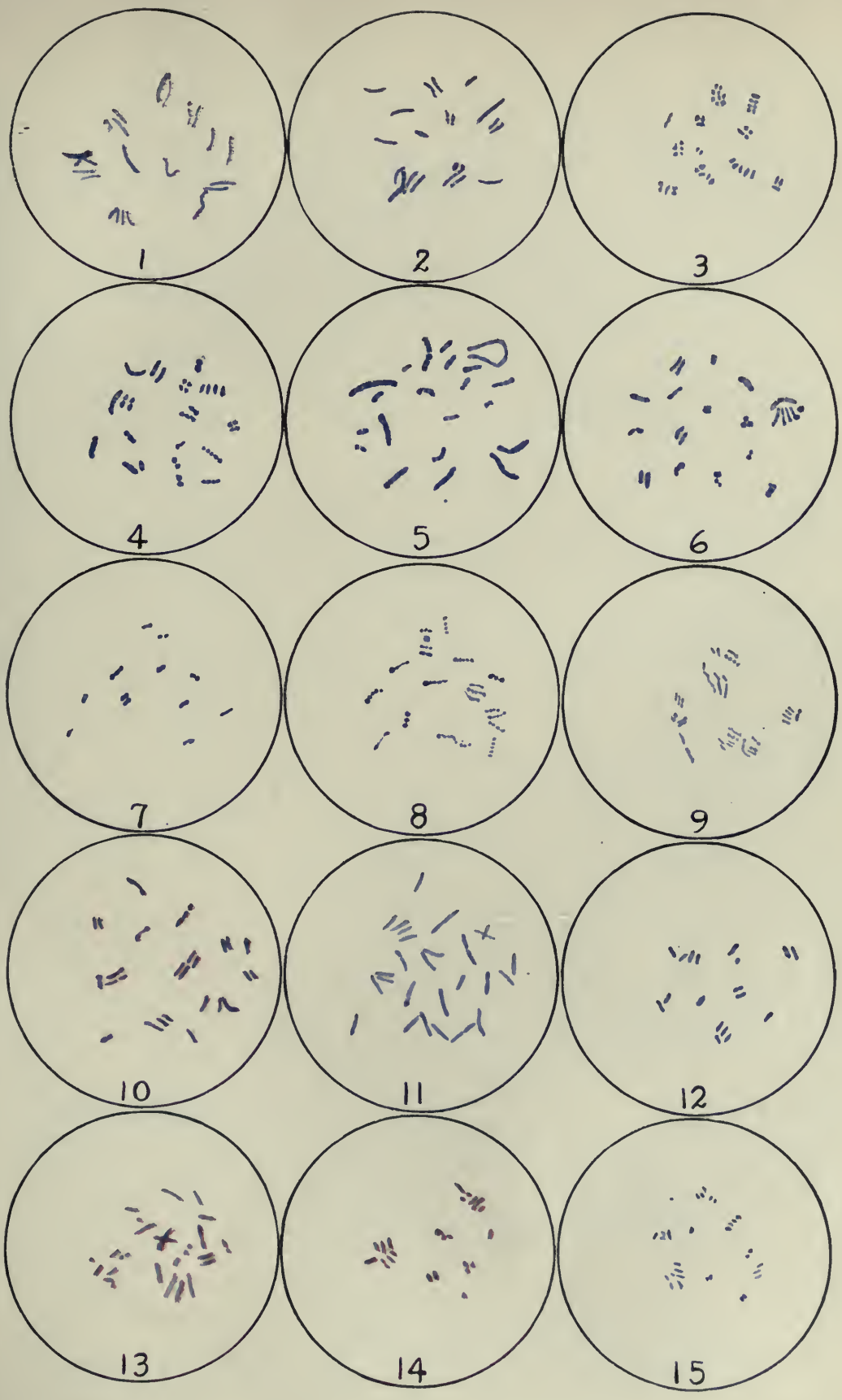

Figure 1

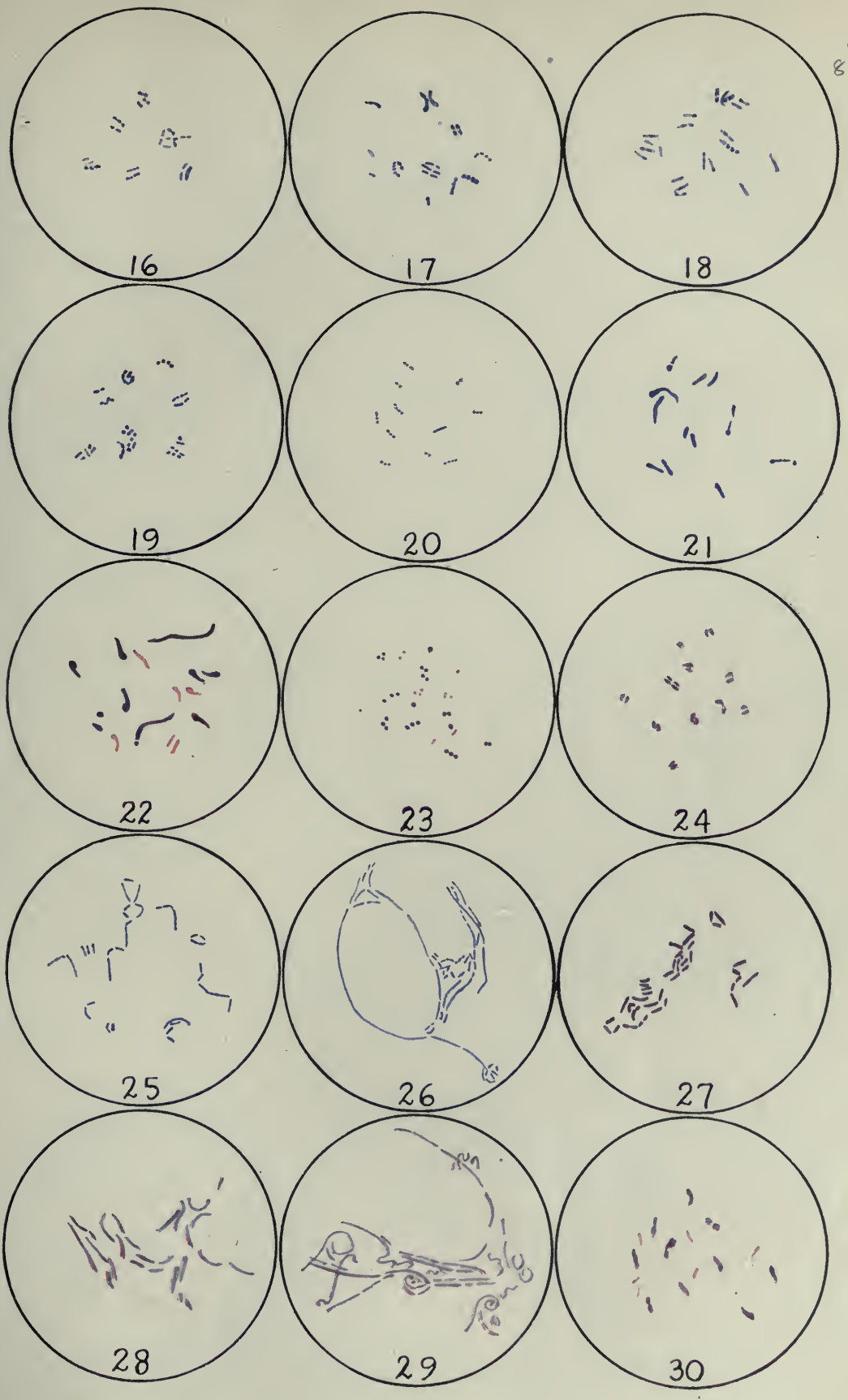

Figure 2

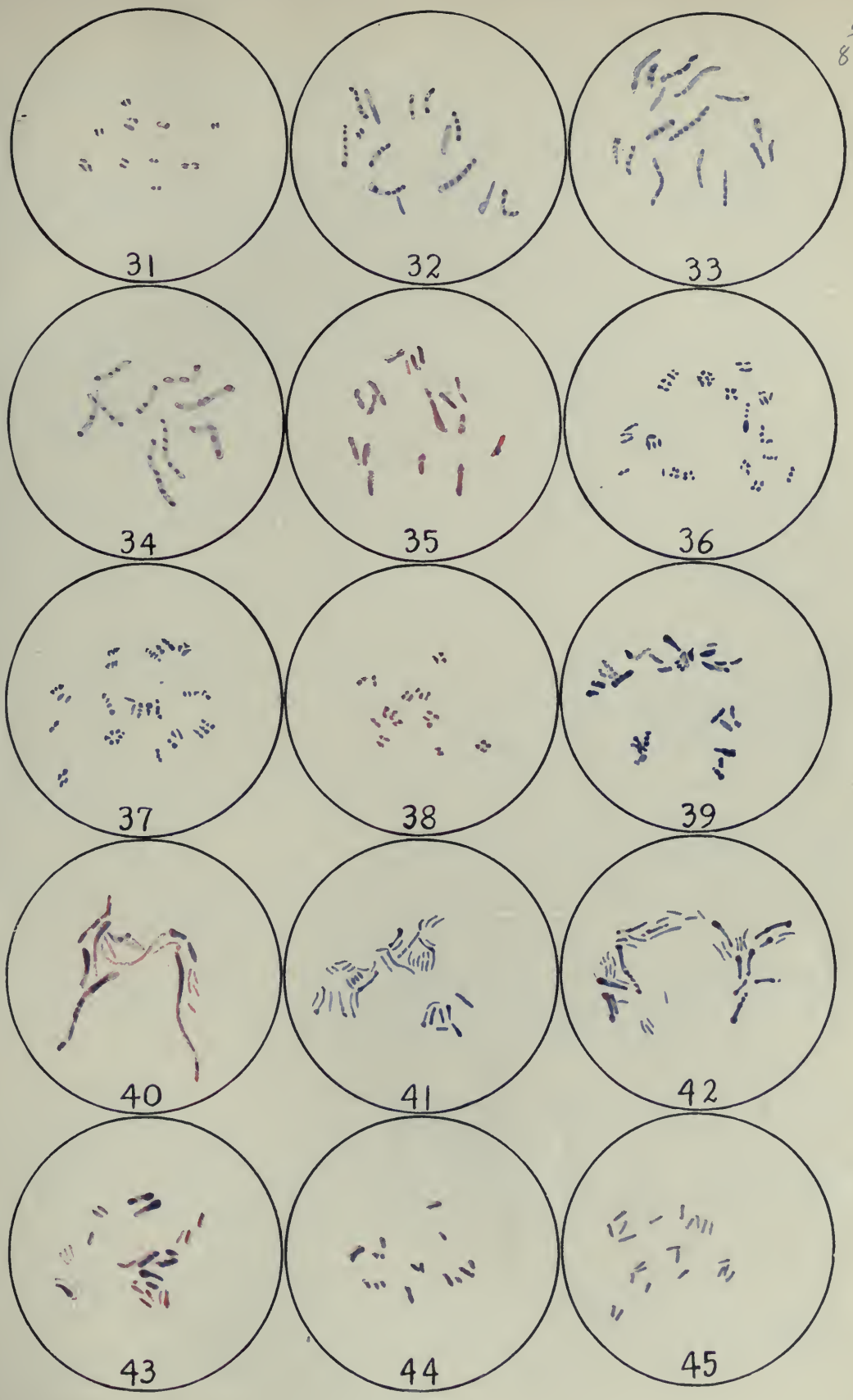

Figure 3

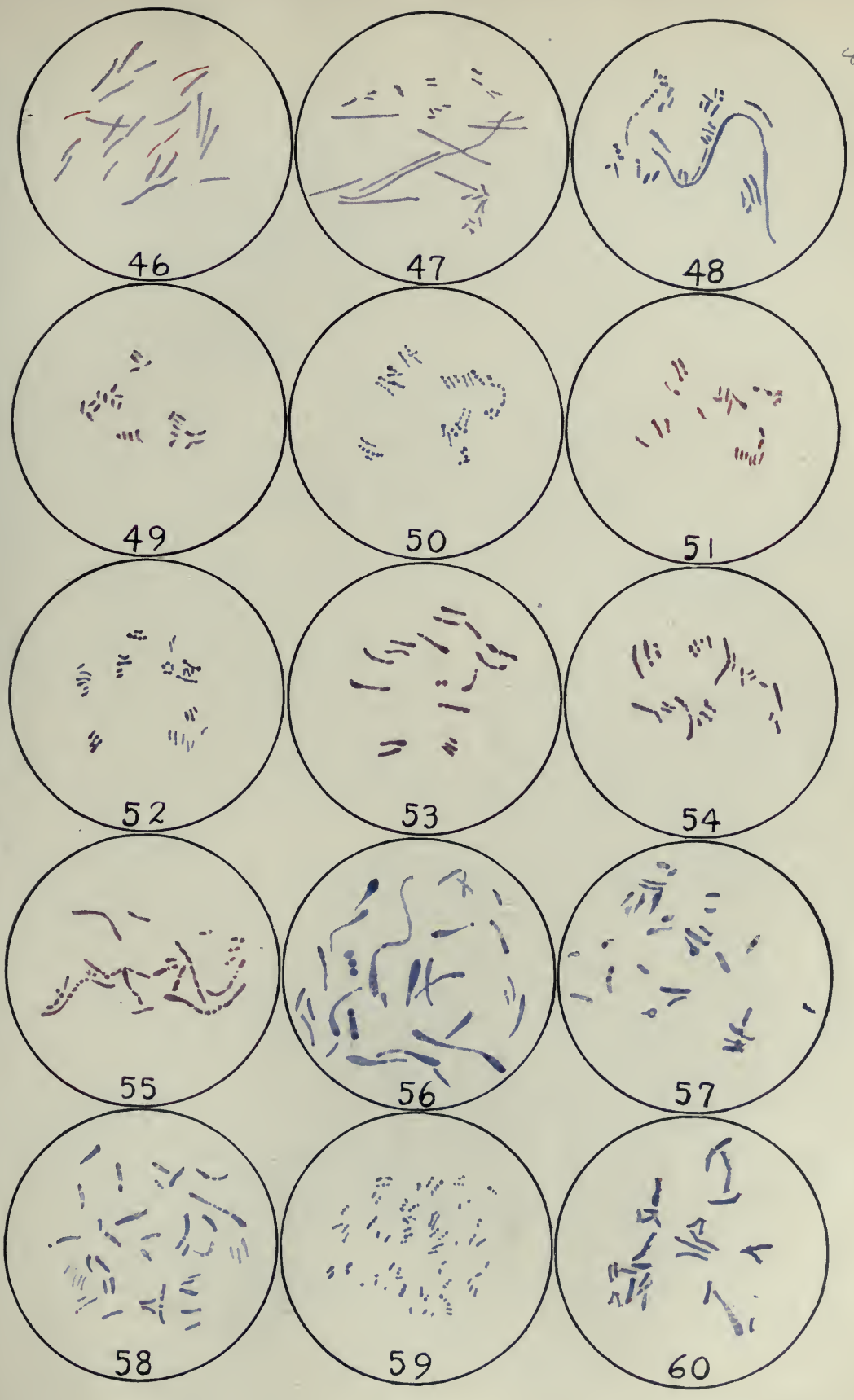

Figure 4

The last five cases are instances of arthritis, from two of which interesting cultures were obtained. 9144-1 (Fig. 30) resembles rather closely R. B. 2 (Fig. 40) and R. E. 1 (Figs. 50 and 51), and is like a small example of 8916-9 (Figs. 21 and 22), while 9392-1 (Figs. 32 and 35), although drawn with difficulty because of its poor staining properties, is similar to 6640 (Fig. 5). Neither of these cases even remotely resemble Hodgkin's disease and it is noteworthy that diphtheroids were found in them.

GENERAL DISCUSSION

Examination of the charted characters, the morphology notes and the pictures reveals a bewildering variety of organisms, closely related diphtheroids and a few cocci. To dispense with the latter it may be pointed out that they were *M. albus* in two cases (8772 and 8916), two slightly varying streptococci in an arthritis case (9392) and a large diplococcus in a possible Hodgkin's case (9265). In this instance the remark of Dr. Rosenow may be recalled, to the effect that cocci predominate in the more recently enlarged glands. This glandular enlargement was only of four weeks' duration.

Consideration of the diphtheroids fails to show any two alike, and as has been suggested in discussing the case groups there is no marked similarity between cultures isolated from closely related pathologic conditions, with the possible exception of the two frank Hodgkin's cases, 6638 and 6640. In the first of these two there were three pseudodiphtheria bacilli, one of which resembles morphologically the single bacterium isolated from 6640, being distinct in its cultural characteristics.

It does not seem profitable to attempt any conclusions from this series of 13 cases, even in conjunction with the material given me by Dr. Rosenow and Dr. Kolmer, further than to state that no one bacterial variety with definite morphologic and cultural characters has been isolated from eleven cases diagnosed as Hodgkin's disease (Rosenow 5, Kolmer 2, my own cases 4) to which may be added a case of endothelioma of the lymph nodes. Diphtheroid bacilli have been isolated from glands in at least two cases having no clinical or anatomic resemblance to Hodgkin's disease and from Case C. of Dr. Rosenow, an instance of mediastinal tumor. Much work will be necessary to establish the microbic cause of Hodgkin's disease especially in the direction of immunology. This I have already begun but I have not as yet sufficient data to make it worth discussing. Two of the cases have failed to show agglutination of the bacteria isolated in them, and one did not bind complement with the patient's serum.

A study of the duration of enlargement of the gland supplying the cultures and the bacteriologic result reveals nothing. The position of the gland seems to have nothing to do with the kind of organism

Case No.	Age and Sex	Duration	Clinical Diagnosis	Pathologic Diagnosis	Vaccine Therapy and Result	Forms of Bacteria Found	Acid and Antiformin Fast	Agar	Blood Agar	Blood Serum	Bordet
6638 a A. S.	22 ♀	3 mos.	Hodgkin's disease	Lymph granuloma of endothelioid type, fibrosis, necroses and eosinophils	Improved ?	Diphtheroids	0 Antiformin fast	Dull gray, flat band of discrete delicate colonies	As agar becoming opaque	As agar but glistening and confluent	As agar becoming confluent, spreading and brown
6638 b	22 ♀	3 mos.	Hodgkin's disease	Lymph granuloma of endothelioid type, fibrosis, necroses and eosinophils	Improved ?	Diphtheroids	0 Antiformin fast	Glistening, raised, yellow-white, smooth, entire band	As agar pinkish; hemolysis slight	As agar	Slightly waxy, flat, dirty gray - green band becoming brownish, medium brown-yellow, then greenish-brown
6638 c	22 ♀	3 mos.	Hodgkin's disease	Lymph granuloma of endothelioid type, fibrosis, necroses and eosinophils	Improved ?	Diphtheroids	0 Antiformin fast	Luxuriant, opaque, glistening, white, entire band	As agar more luxuriant; hemolysis + medium becomes brown-opaque	As agar more luxuriant	Dirty gray, raised, dull band with glistening edges, becoming brown, medium first, yellow-brown then green
6640 L. P.	30 ♀	1 yr.	Hodgkin's disease	Lymph granuloma of endothelioid and mononuclear type, fibrosis and eosinophilia	Yes, Improved ?	Diphtheroids Three slightly varying colonies with same morphology and biology	Antiformin fast (?) 1 hour + 18 hours 0	Delicate, dull, moist, translucent, dirty-white band	As agar hemolysis 0	As agar more luxuriant	Thin, moist, gray band
8916-1 W. P.	9 ♂	7 mos.	Hodgkin's disease	Lymphadenoma of chronic hyperplastic variety	Improvement with Roentgen ray, later bacterins added greater improvement	Seven closely similar diphtheroids. M. albus	0 Rods not destroyed but not acid-fast	Moist, flat, yellow, entire, discrete	Slimy, dirty-white, opaque	Faint orange-yellow, discrete, sunken colonies, liquefaction +	Slimy, moist, opaque, luxuriant, yellow becoming greenish
8916-2	9 ♂	7 mos.	Hodgkin's disease	Lymphadenoma of chronic hyperplastic variety	Improvement with Roentgen ray, later bacterins added greater improvement	Seven closely similar diphtheroids. M. albus	0 Rods not destroyed but not acid-fast	Delicate, flat, colorless streak	Delicate, flat, discrete colonies	Flat, even, delicate streak, becoming yellow	Glistening, moist, pale gray, spreading, becoming brown, medium chocolate
8916-4	9 ♂	7 mos.	Hodgkin's disease	Lymphadenoma of chronic hyperplastic variety	Improvement with Roentgen ray, later bacterins added greater improvement	Seven closely similar diphtheroids. M. albus	0 Rods not destroyed but not acid-fast	Faint, moist, glistening band becoming yellow, then gray	As agar, but becoming brownish-gray	Faint, dry, white band	Luxuriant, dull orange-yellow, becoming gray, flat and dry
8916-7	9 ♂	7 mos.	Hodgkin's disease	Lymphadenoma of chronic hyperplastic variety	Improvement with Roentgen ray, later bacterins added greater improvement	Seven closely similar diphtheroids. M. albus	0 Rods not destroyed but not acid-fast	Faint, dull, yellow-white band	Dull, yellow-white butyrous	Faint, dull-yellow, and dry brown	Thin, dull, flat, brown-yellow, becoming frosted gray
8916-8	9 ♂	7 mos.	Hodgkin's disease	Lymphadenoma of chronic hyperplastic variety	Improvement with Roentgen ray, later bacterins added greater improvement	Seven closely similar diphtheroids. M. albus	0 Rods not destroyed but not acid-fast	Glistening, moist, raised, yellow, becoming brownish	As agar	Slimy, yellow, depressed; liquefaction = +	Smooth, flat, limited, waxy, pale brown
8916-9	9 ♂	7 mos.	Hodgkin's disease	Lymphadenoma of chronic hyperplastic variety	Improvement with Roentgen ray, later bacterins added greater improvement	Seven closely similar diphtheroids. M. albus	0 Rods not destroyed but not acid-fast	Flat, moist, slimy, pale yellow	As agar but becoming brownish	Flat, smooth, glistening, lemon-yellow	Like agar but more luxuriant; distinct brown, chocolate
8916-10	9 ♂	7 mos.	Hodgkin's disease	Lymphadenoma of chronic hyperplastic variety	Improvement with Roentgen ray, later bacterins added greater improvement	Seven closely similar diphtheroids. M. albus	0 Rods not destroyed but not acid-fast	Faint, dirty-white, slimy, translucent band	Faint, smooth, brownish-yellow	Limited orange-yellow smear	Faint, flat, orange band becoming brown-gray

Bouillon	Potato	Litmus Milk	Gelatin	Inulin	Acidity Reduction								Hemolysis	Indol	Pigment	Medium Discoloration	Pathogenic for Guinea-Pigs
					Glucose	Lactose	Saccharose	Maltose	Mannite	Glycerin	Dextrin	Galactose					
Clear sand-like granules	No visible growth	Faintly acid	No visible growth	Alkaline	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0	0	0	0	0
Clear sand-like granules and flocculent sediment	Spreading glistening smear, later slightly raised	Faintly alkaline	Faintly turbid liq. 0	Alkaline	+ +	0 0	0 0	0 0	0 0	0 0	0 0	0 0	+	0	See blood serum	Bordet, greenish-brown	
Turbid granules; sand-like, flocculent sediment	Dirty white smear, becoming raised and yellowish	Faintly alkaline	Faintly turbid liq. 0	Alkaline	+ +	0 0	— 0	0 0	0 0	— +	0 0	0 0	+	0	0	Bordet, greenish; blood agar, brown	
Mucoid sediment	No visible growth	No change	No visible growth	0	+ 0	0 0	+ 0	0 0	0 0	0 0	0 0	0 0	0	0	0	0	
Faint turbidity, flocculent sediment	Luxuriant, spreading, orange	Faintly acid	Luxuriant growth liquefaction +	0	+ 0	0 0	+ 0	+ 0	+ 0	+ 0	0 0	0 0	+	+	Agar, yellow; Bordet, greenish; potato, orange	0	0
Clear, little flocculent sediment	No visible growth	Faintly alkaline after 12 days	Faintly turbid at top; liquefaction 0	0	— 0	— 0	— 0	— 0	— +	— +	— 0	— +	0	+	Blood serum, yellow	Bordet, chocolate-brown	0
Flocculent sediment	No visible growth	Faintly alkaline	Liquefaction 0	0	+ 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0	+	Faint yellow	0	0
No growth	No visible growth	No change	Liquefaction 0	0	+ 0	0 0	0 0	0 0	0 0	0 0	+ 0	0 +	0	0	Faint yellow	0	0
Faint general turbidity; slimy sediment	Glistening, flat, spreading, yellow, becoming brown	Acid coagulation digestion	Liquefaction +	0	+ +	0 0	+ 0	0 0	+ 0	0 0	+ 0	+ +	+	+	Yellowish	0	Septicemia in 2 of 3 guinea-pigs
Faint turbidity; fluorescent sediment	Yellowish stain	No change	Liquefaction 0; faint turbidity	0	+ +	+ 0	+ 0	0 0	— 0	+ +	+ 0	+ 0	Slight	+	Yellow-brown	Bordet, chocolate-brown	0
No growth	No visible growth	Coagulation; acidity reduction	Liquefaction 0	0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0	0	Yellow-brown	0	0

Case No.	Age and Sex	Duration	Clinical Diagnosis	Pathologic Diagnosis	Vaccine Therapy and Result	Forms of Bacteria Found	Acid and Antiformin Fast	Agar	Blood Agar	Blood Serum	Bordet
9265 E. P.	22 ♂	4 wks.	General adenopathy, probably Hodgkin's disease	Lymphadenoma of chronic hyperplastic variety; no eosinophils or necroses	No.....	Cocci do not grow into bacillary forms	.....	Scanty, slimy, dirty-white	As agar	No perceptible growth	As agar
8772 L. W.	32 ♀	4 mos.	Hodgkin's disease	Endothelioma rapidly growing in lymph channels	Improvement with Roentgen ray, later death	M. albus diphtheroid	0 Rods not destroyed but not acid-fast	Tiny, delicate, colorless, discrete, round colonies	As agar, but growth and medium becoming brown	Flat, dull, yellowish streak	Dull, brown-green streak of tiny, discrete colonies, medium beneath, chocolate brown
9144-1 I.M.W	22 ♀	6 mos.	Multiple infectious arthritis with adenopathy	.....	Improvement	Diphtheroids	0 Rods not destroyed but not acid-fast	Faint, dirty-white, slimy band	As agar	As agar but whiter	Luxuriant, opaque, otherwise like agar
9144-2	22 ♀	6 mos.	Multiple infectious arthritis with adenopathy	.....	Improvement	Diphtheroids	0 Rods not destroyed but not acid-fast	Moist, slimy, opaque, raised, orange to salmon to brown	As agar	As agar; but salmon-yellow becoming pink coral	Just as agar
9392-1 J. M.	41 ♀	7 yrs.	General arthritis, anemia with adenopathy	.....	Improvement	Diphtheroid	0 0	Delicate, raised, moist, opaque, glistening, becoming salmon-pink	As agar, except color pinkish-brown	As agar, except color pale pink	As agar, but color rose pink
9392-2	41 ♀	7 yrs.	General arthritis, anemia with adenopathy	.....	Improvement	Diphtheroid coccus	0 0	Raised, moist, white, tiny, discrete colonies	At first as agar, then hazy gray, then colonies becoming brown	As agar	Dry, brown, discrete colonies tending to coalesce; medium chocolate brown
9392-3	41 ♀	7 yrs.	General arthritis, anemia with adenopathy	.....	Improvement	Diphtheroid coccus	0 0	Yellowish-white, small, discrete colonies	Coalescing, grayish growth becoming brown and scanty	Pearl-white, discrete colonies	Dark, dry, greenish-yellow band, causing brown-green discoloration of medium
RA-1 767	54 ♂	4 yrs.	Hodgkin's disease	Hodgkin's disease	Marked improvement	Diphtheroids	0 Rods not destroyed but not acid-fast	Discrete to confluent, moist, dirty, orange-yellow becoming canary-yellow	As agar, color changes from yellow to dirty brown, with methemoglobin color of medium	Flat, dry, dirty, orange-yellow	Smooth, flat, slightly waxy, spreading, green-brown band; medium brown
RA 2 767	....	.....	.....	.....	.....	Diphtheroids	0 Rods not destroyed but not acid-fast	Raised, moist, glistening, slimy, canary-yellow band	As agar, but orange-yellow to greenish becoming brown	As agar	Dry, flat, smooth, opaque, spreading, yellow-brown, medium chocolate
RB-1 907	48 ♀	1½ yrs.	Mediastinal tumor	?	No improvement	Diphtheroids	0 Rods not destroyed but not acid-fast	Raised, moist, slimy, glistening, opaque band becoming brown-yellow	As agar, medium dark and opaque	As agar but growth going salmon to orange-yellow to coral-pink	As agar, growth salmon and then red-brown
RB-2 907	....	.....	.....	.....	.....	Diphtheroids	0 Rods not destroyed but not acid-fast	Smooth, moist, glistening gray-white band	As agar; hemolysis at top, becoming pale and slimy	As agar, but band becoming pale yellow; medium dark under growth	As agar, but growth dirty brownish gray
RB-4 907	....	.....	.....	.....	.....	Diphtheroids	0 Rods not destroyed but not acid-fast	Moist, glistening, dirty-white band	As agar; hemolysis +	As agar but porcelain white	As agar but yellowish becoming dirty yellow-brown

Bouillon	Potato	Litmus Milk	Gelatin	Inulin	Acidity Reduction								Hemolysis	Indol	Pigment	Medium Discoloration	Pathogenic for Guinea-Pigs
					Glucose	Lactose	Saccharose	Maltose	Mannite	Glycerin	Dextrin	Galactose					
Faint turbidity	No perceptible growth	Slightly alkaline; no other change	Liquefaction 0	0	+ 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0	+	0	0	
No visible growth	No visible growth	Very faintly acid	Faint growth liquefaction 0	Faintly acid	+ 0	0 0	+ 0	+ 0	0 0	0 0	+ 0	0 0	0	0	Dull greenish-brown	Chocolate-brown; green directly under colonies	0 Rabbits' glands 0
No growth	No visible growth	Faint acidity	Liquefaction 0	0	+ 0	0 0	+ 0	+ 0	— 0	+ 0	+ 0	+ 0	0	0	0	Bordet, gray-brown	0
Turbid with slimy, yellow sediment	Salmon yellow	Alkaline reduced	Mucoid sediment liquefaction 0	0	0 +	— 0	0 0	— 0	— 0	— 0	— +	— +	0	+	Yellow; salmon pink	0	0
Delicate pellicle and flocculent sediment	As agar	Alkaline; no other change	Liquefaction 0	0	0 +	— 0	— 0	— 0	— 0	0 0	— 0	0 +	0	0	Pink	0	0
No growth	No visible growth	Acid, coagulated, reduced	Liquefaction 0	0	+ 0	+ 0	+ 0	— 0	0 0	0 0	+ 0	+ 0	0	0	0	Bordet, chocolate-brown	0
Faint turbidity; flocculent sediment	No visible growth	Acid, coagulated, reduced	Flocculent sediment liquefaction 0	0	+ 0	+ 0	+ 0	+ 0	0 0	0 0	+ 0	+ 0	0	0	Agar, yellow; Bordet, yellow-brown	Bordet, brown-green	0
Turbid flocculent sediment	No visible growth	Alkaline; no other change	Heavy flocculent sediment; liquefaction 0	—	+ 0	— 0	+ 0	+ +	— 0	— 0	— +	— +	Methemoglobin	+	Agar, orange-yellow; Bordet, green-brown	Bordet, brown	0
Faint cloudiness	No visible growth	Alkaline	Turbid, heavy, mucoid sediment	—	+ 0	— 0	+ 0	+ 0	— 0	— 0	— +	— +	0	0	Agar, yellow; Bordet, yellow-green-brown	Bordet, faint chocolate-brown	0
Turbid, mucoid sediment	No visible growth	No change	Faint cloudiness, liquefaction 0	—	0 +	0 0	0 0	0 0	— 0	— 0	— 0	— +	0	0	Yellow, orange, pink, brown	Blood agar, dark brown	0
Turbid, flocculent and granular sediment	No visible growth	Alkaline reduced	Liquefaction 0	0	+ +	— 0	+ 0	+ 0	— 0	— 0	— 0	— +	+	+	Blood serum, yellow	Blood serum, brown	0
Turbid, flocculent sediment	No visible growth	Alkaline reduced	No change; liquefaction 0	0	+ 0	— 0	+ 0	+ 0	— 0	— 0	0 0	— +	+	+	0	0	0

Case No.	Age and Sex	Duration	Clinical Diagnosis	Pathologic Diagnosis	Vaccine Therapy and Result	Forms of Bacteria Found	Acid and Antiformin Fast	Agar	Blood Agar	Blood Serum	Bordet
RC-1 934	? ♀	?	Hodgkin's disease	Hodgkin's disease	Marked improvement	Diphtheroids	0 0	Smooth, flat, moist, glistening spreading, pale blue-gray	As agar, but at first pink, then brown, then green; hemolysis +	As agar, later liquefying	Flat, limited, discrete colonies tending to coalesce; gray-brown to red-brown
RC-2 923	....	.....	.....	.....	.....	Diphtheroids	0 0	Slimy, moist, raised, opaque, faint orange band	As agar, but brownish	As agar, but salmon color becoming coral pink	Moist, glistening, deep orange-yellow, becoming ochre
RC-3 923	....	.....	.....	.....	.....	Diphtheroids	0 Rods not dissolved	Moist, raised, smooth, glistening limited, white	Yellowish-white, smooth, flat, glistening, discrete to coalescing	Early as minute, yellow, depressed, discrete colonies; liquefaction +	Raised, moist, glistening, discrete to confluent, raised luxuriant, dirty gray-brown
RD-1 936	29 ♂	1½ yrs.	Hodgkin's disease	Hodgkin's disease	Marked improvements	Diphtheroids	0 Rods not dissolved	Raised, moist, glistening, slimy, dirty white	As agar; pale; hemolysis +	Dry, delicate, dirty white	As agar, rather gray, then pink, with greenish fluorescence in medium
RD-2 936	....	.....	.....	.....	.....	Diphtheroids	0 0	Irregular, discrete to confluent, granular, dirty yellow-white	As agar; hemolysis slight	Smooth, flat, moist, becoming yellowish	Flat, granular, discrete to confluent, waxy, opaque, gray, lusterless
RE-1 C	....	.....	.....	.....	.....	Diphtheroids	0 0	Faint, moist, glistening, opaque band	As agar	As agar, but yellow white	Uniform, flat, confluent, gray, with reflection of reddish-brown
KI-1	24 ♂	6-8 wks.	Hodgkin's disease	Hodgkin's disease	No continued improvement, patient died	Diphtheroids	0 0	Moist, raised, glistening, opaque, slimy, salmon-yellow band	As agar; medium brown	As agar	As agar; medium opaque brown
KII-1	49 ♂	Auto'y 3 mos. duration	Hodgkin's disease	Hodgkin's disease	.....	Diphtheroids	0 Rods not dissolved	Faint, almost invisible band seemingly made up of discrete colonies	As agar; medium opaque	As agar	As agar; growth faint grayish-yellow somewhat more luxuriant
KII-3	....	.....	.....	.....	.....	Diphtheroids	0 0	Delicate, dull, moist, dirty white, translucent	As agar	As agar	As agar, becoming more luxuriant, flat, dry, grayish-green
KII-4	....	.....	.....	.....	.....	Diphtheroids	0 Rods not dissolved	Faint streak of discrete, yellow colonies	As agar	As agar	As agar, becoming confluent, dirty gray-brown; medium chocolate brown
K*	....	.....	.....	.....	.....	.....	0	Glistening, pale gray discrete colonies, becoming confluent and whiter	Similar but luxuriant and raised, medium hemolyzed and then darkened	Glistening, confluent, moist, raised band becoming slightly yellowish	Dull, waxy, brownish band; medium becoming brown and without contrast with growth
873-1†	....	.....	.....	.....	.....	.....	0	Luxuriant, glistening, smooth, raised, opaque, dirty white	As agar, more luxuriant and greenish sheen; hemolysis slight	As agar, more luxuriant, raised	Pale salmon-pink, raised, glistening, medium becoming dark brown
873-3‡	....	.....	.....	.....	.....	.....	0	Glistening, smooth, flat, opaque, greenish-white	Glistening, raised, slimy, greenish, hemolysis slight	As agar, more luxuriant becoming lemon-yellow	Somewhat flat, slightly waxy, brownish-yellow, medium darker

Bouillon	Potato	Litmus Milk	Gelatin	Inulin	Acidity Reduction								Hemolysis	Indol	Pigment	Medium Discoloration	Pathogenic for Guinea-Pigs
					Glucose	Lactose	Saccharose	Maltose	Mannite	Glycerin	Dextrin	Galactose					
No visible growth	No visible growth	Alkaline reduced	Faintly turbid; liquefaction 0	0	+ 0	— +	+ 0	+ +	+ 0	+ 0	+ 0	— +	+	0	Greenish, brownish, reddish tinge	0	0
Faint turbidity	No visible growth	Alkaline	Turbid; liquefaction 0	0	0 +	0 0	0 0	0 0	0 0	0 0	0 0	0 +	0	0	Orange-yellow to pink	0	0
Turbid; mucoid sediment	No visible growth	Coagulation, faintly alkaline (?), reduction digestion	Liquefaction 0	Acid reduced	+ 0	— 0	+ 0	0 0	+ 0	+ +	+ 0	0 0	0	0	Yellowish-brown	0	0
Turbid, mucoid sediment	Slimy, dirty white	Alkaline	Turbid; liquefaction 0	0	0 +	— 0	0 0	— 0	— 0	0 0	0 0	— +	+	+	Bordet, fluorescent	See Bordet	0
Flocculi and granules at bottom	No visible growth	No change	0	0	0 0	0 0	+ 0	0 0	0 0	0 0	+ 0	0 0	+	0	Faint yellow	0	
Mucoid sediment	No visible growth	Faint acidity	Mucoid sediment; liquefaction 0	Alkaline	+ +	0 0	0 0	0 0	0 0	0 0	0 0	0 +	0	0	Faint yellow	0	
Turbid, mucoid sediment	Faint salmon - yellow smear	Alkaline	Mucoid sediment; liquefaction 0	0	— 0	— 0	— 0	— 0	— 0	— +	— 0	— +	0	0	Salmon	Browning of blood and Bordet	
Faintly turbid; slight granular sediment	No visible growth	Faintly alkaline	Faintly turbid; liquefaction 0	0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0	0	0	0	
Faintly turbid; mucoid sediment	No visible growth	Faintly alkaline	Faintly turbid; floating flocculi, liquefaction 0	Alkaline	+ 0	0 0	0 0	0 0	0 0	0 0	+ 0	+ +	0	0	0	0	
Turbid, flocculent sediment	No visible growth	Faintly alkaline	Turbid at top; liquefaction 0	Alkaline	+ 0	0 0	0 0	0 0	0 0	0 0	+ 0	+ 0	0	0	0	Bordet, chocolate-brown	0
Turbid, heavy, flocculent sediment	Delicate smear	No change	Faintly turbid, liquefaction 0	Acid then neutral	+ 0	0 0	0 0	+ 0	0 0	0 0	+ 0	? 0	+	0	0	Bordet, brown	Virulent for guinea-pigs
Turbid, heavy, flocculent sediment	Spreading smear, becoming raised and yellowish	Faintly alkaline	Faintly turbid; liquefaction 0	Alkaline	+ 0	0 0	+ 0	0 0	— 0	0 0	0 0	+ 0	+	0	0 See Bordet	Darkens blood mediums	0
Clear, thick, tenacious, membrane-like sediment	Faint smear, becoming deep yellow	Faintly alkaline	Faint growth; liquefaction 0	No change	+ 0	— 0	+ 0	0 0	0 0	0 0	0 0	0 0	+	0	Yellowish on several mediums	Blood mediums darker	0

* From this patient the true Klebs-Loeffler bacillus has reached the twenty-sixth generation on Loeffler's blood serum.
† In this case the bacillus was of pseudodiphtheria (Winslow, No. 9).
‡ In this case the bacillus was of pseudodiphtheria (spinal fluid, case of cerebrospinal meningitis. Service not explained).

isolated. In the Hodgkin's and similar cases material was removed from the inguinal region twice, axilla and mastoid region each once. The endothelioma was in the cervical glands. The arthritis cases giving positive cultures supplied respectively an axillary and epitrochlear gland. The position of these last two glands is such that drainage from the throat and genital tract, the common habitats of diphtheroids, is probably excluded as a means of their recent reception. However, they probably entered through one of these places at some time.

The glands of the patients in the two frank Hodgkin's cases and the tuberculous adenitis case, A. G. F., have been studied by the Much, Gram-Weigert and Ziehl-Nielson stains. Tubercle bacilli have been found only in the last case. No Much granules, either single or in rows were found in the first two. By the Much method a few diphtheroids were found in 6640 as pale rods with poorly staining, large, blue granules. No metachromatic bodies were found. Only one rod was found in the Gram-Weigert preparations and it was nearly solidly blue.

The material presented here may be summed up as follows:

Diphtheroid rods may be isolated from Hodgkin's disease and other adenopathies, but there is no uniformity in biology and morphology among the strains isolated by three observers from clinical and pathologic Hodgkin's disease. Diphtheroid rods, similar in biology and morphology to those found in Hodgkin's disease, may be found in enlarged glands in cases of chronic atrophic arthritis and other conditions.

Diphtheroids have been found in glands the seat of a neoplasm (8772), and in the enlarged glands near a neoplasm (R. C.). There is great similarity in all respects between the gland diphtheroids and those found in normal and pathologic seats in the body, the so-called pseudodiphtheria bacilli. More facts are demanded to show the exact relation of the diphtheroids to Hodgkin's disease.

In one case, in which autogenous vaccination was given a fair trial, the patient has died.

Another patient, already improving under the Roentgen ray, continued to improve with autogenous vaccination and no Roentgen ray. In arthritis cases the patients receiving vaccine consisting wholly or in part of diphtheroids, showed some improvement.

Much granules have not been found in sections of lymph granulomas of the Hodgkin's type.

The cases used in this work were in the services of Dr. Stengel, Dr. Frazier, and Dr. A. C. Wood at the University Hospital. I wish to thank these gentlemen for the use of the material.

I am indebted to Mrs. V. C. Brogdon for much assistance in running through cultures and for the preparation of the chart, and to Miss L. H. Irwin for the drawings.

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A STUDY OF HYDROPS UNIVERSALIS FETUS, WITH THE REPORT OF A CASE[1]

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The study of antenatal disease, from the purely clinical standpoint, is as old as medicine, Hippocrates himself having recorded a diverse series of cases. The study of such conditions from the scientific and experimental side is, however, recent, and indeed, may be said, like the condition it investigates, to be at the threshold of birth.

By far the most notable contribution to the subject has been made by Ballantyne,[1] of Edinburgh, whose published works contain a wealth of information presented in most scholarly fashion.

The particular form of antenatal disease which has come under the notice of the writer, general edema of the fetus, or better hydrops universalis fetus, is one which, while fortunately of rare occurrence, has been a fairly frequent theme of medical essayists.

A survey of this literature reveals the fact that there is, as yet, but little attempt at uniformity in the classification of the cases and a most marked variance of opinion as to the causative factors.

This confusion is probably due to the fact (as held by many of the writers themselves) that most observers have seen but one, or at most a very few cases and the opportunity has not been given to one man to study a large series.

[1] From the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania.

For a definition of hydrops universalis fetus, Ballantyne cannot be improved upon; he defines it as "a morbid condition of the fetus, characterized by general anasarca, by the presence of fluid effusions in the peritoneal, pleural and pericardial sacs, and usually by edema of the placenta, and it results in the death of the fetus or infant before, during, or very soon after birth."

From the foregoing description it is apparent that the features of great interest in this condition are the pathogenesis and the mechanism of its causation and it is here that opinions differ so widely.

The history of the case coming under the observation of the writer was as follows: Mrs. S., American, I-para, aged twenty-three years. She was of healthy parentage, had measles, chicken-pox, etc., in childhood, and there was a somewhat indefinite history of an attack of nephritis in infancy. When seventeen years old she had a severe and prolonged attack of jaundice, the cause of which was not determined. With these exceptions she had always been in excellent health.

Menses were established at thirteen years, were regular, the flow excessive for the first year, then normal in amount and there was some dysmenorrhea during the first day.

Her first pregnancy was marked by a kidney insufficiency of moderate degree. There was hyperemesis during the first three months, after which there developed albuminuria, together with the presence of hyaline and granular casts and erythrocytes.

Pregnancy was terminated by induction of labor at the thirty-sixth week, by reason of a moderate degree of pelvic contraction, and a healthy child was spontaneously delivered. The patient slowly recovered from her kidney lesion, the urine showing some albumin for months after delivery. She then remained in perfect health for a year when she again became pregnant (last menstruation, October 15, 1913). This pregnancy was marked by severe vomiting

and nausea for the first four months, after which she was fairly comfortable until the seventh month. At this time after some physical fatigue, she suffered an acute and lancinating pain in the right iliac fossa, whence it radiated to the umbilicus and toward the back. The temperature was never above 99°; the pulse rose to 120°; there was a leukocytosis of 12,500. The picture was that of an acute appendicitis, and the patient was treated upon that assumption, by absolute rest, starvation, and cold to the abdomen.

The pain subsided after five days and she again felt well until with some return of the abdominal pain there developed a rapid increase in the girth of the abdomen, with great distress from pressure. This condition continued, the hydramnios steadily increasing, until on June 10 she fell into labor. Upon rupture of the membranes an estimated amount of one gallon of liquor amnii escaped. The fetus lay in the L. O. A. position of the vertex, labor was long and tedious, but eventuated in the birth of a female child, which did not breathe, although the heart action was feebly perceptible for a few moments. The pulmotor was used for one-half hour, without result.

The mother made an uneventful recovery. During this pregnancy she had shown a very faint trace of albumin in the urine on occasions, but had not been ill, or shown any evidence of serious disturbance of the kidney, though she gave a clinical impression of suffering from some toxemia.

The child was a female, weighing 7.5 pounds. It was the seat of a marked general edema, involving the head, trunk, and extremities. The facial edema was so great as to almost obliterate the features; there was a large ascites, hydropericardium, and hydrothorax. Complete autopsy was not performed. The placenta weighed 4.5 pounds. It was soft, friable, pale, and enormously edematous. On section serous fluid freely escaped from all parts.

Microscopically the villi showed a great edema and some degeneration of the syncytial cells (Figs. 1, 2, 3). The villi

presented vacuolation, there was separation of the connective tissue by edema, and the syncytial cells were swollen, their nuclei pale, and in many instances shrunken.

The cord was thick and edematous, but showed no other change.

In order to draw deductions from the histories of as many of these cases as possible, all those recorded since Ballantyne's work in (1902) have been tabulated and are appended to this paper.

An analysis of these cases finds them readily divisible into two great groups, each of which possesses certain common and integral features. These two divisions are: (1) those cases in which the edema is due to some mechanical or structural defect in the fetus or its membranes, and (2), those due to a toxemia of the mother and secondarily of the fetus without any morphological defect necessarily present.

Two intricate and elaborate classifications have been suggested, that of Broekhuizen[2] and that of Croizier.[3] The Broekhuizen system is as follows:

FETAL CAUSES

1. Heart and vessels.
   - (*a*) Fetal endocarditis.
   - (*b*) Congenital absence of a chamber of the heart (Pott).
   - (*c*) Small foramen ovale (Lawson Tait, Osler).
   - (*d*) Obliteration of the ductus Botalli (Nieberding, Ribemont, Desaignes).
   - (*e*) Displacement of the heart and vessels following diaphragmatic hernia (Damann, Behn, Fuhr).
2. Kidneys.
   - (*a*) Congenital cystic (Schürenk, Herinenier).
   - (*b*) Hypertrophy (Gueniat).
   - (*c*) Hyperplasia (Opitz).
   - (*d*) Fetal nephritis (Kuesch).

3. Liver.
   (*a*) Hypertrophy (Sculen, Protheroe, Smith, Nachtigäller).
   (*b*) Atrophy and cirrhosis (Snow, Beck, Russell Andrews, Berther).
   (*c*) Hypertrophy of the liver and spleen (Potocki, Porak, Rainier).
4. Blood.
   (*a*) Fetal leukemia (Sanger, Lahs, Siefart).
5. Fetal syphilis (Osiander, Cruveilhier, Spiegelberg, Strassmann).
6. Fetal peritonitis (Simpson, Opitz, Vecchi).
7. Absence of the thoracic duct (Smith, Birmingham, Ballantyne).

CONDITIONS OUTSIDE THE FETUS

8. Thrombosis of the umbilical veins (Ballantyne, Betchler, Pollnow, Opitz).
9. Edema and hyperplasia of the placenta (Guise, Krieger, Bassett, Ruge, Longaker).
10. Hydrops of the entire ovum.

REMOTE MATERNAL CAUSES FOR ANASARCA

11. Malaria (Höuck).
12. Endometritis (Fuhr).
13. Hydremia (Betschler, Küller, Ritter).
14. Leukemia (Ahlfeld).
15. Chronic nephritis and kidney of pregnancy (Strauch, Kohn, Weber).

Croizier in his classification divides the causative agents of fetal edema into the following groups:

FETAL

1. Lesions characterized by the presence of neoplasms, including cystic degeneration of the kidney.

2. Anomalies of development.
   (*a*) Cardiac lesions, hydropericardium, etc.
   (*b*) Absence of the thoracic duct.
   (*c*) Diaphragmatic or umbilical hernia.
   (*d*) Hypoplasia of the kidney.
3. Inflammatory lesions.
   (*a*) Fetal peritonitis.
   (*b*) Fetal nephritis.
   (*c*) Fetal syphilis.
4. Histological lesions of non-inflammatory type.
   (*a*) Suprarenal disease.
   (*b*) Disease of the blood.

PLACENTAL LESIONS

1. Edema of the placenta.
2. Inflammatory lesions of the placenta.

LESIONS OF THE CORD

1. Velamentous insertion.

CASES WITHOUT AUTOPSY

On considering these classifications, it will be seen that both the toxic group and the group of mechanical defects are very prominent, and the cases easily fall into one or the other division.

It is the opinion of the writer that those cases due to inflammatory disease or fetal peritonitis should not be included in this subject, but should be regarded as secondary to the specific form of inflammation causing the lesion.

In order not to duplicate work done, Ballantyne's analysis of cases reported prior to 1900 will be freely quoted in connection with those collected here, all of which have been published since that time. The clinical histories show some rather constant features. In Ballantyne's 68 cases the mother was nearly always well advanced in her child-bearing life, and in only 7 out of 65 cases was her age less than thirty.

This is not at all in accord with the statistics of cases reported since 1900, as in a total of 30 wherein the age was recorded, 17, or over one-half, were between the ages of twenty-five and thirty, of which number 6 were under twenty-five; 9 were between thirty and thirty-five and only 4 were over thirty-five years. Taking the recorded cases as a whole then, the majority have occurred in women over thirty, though if the more recent cases alone be considered, these statistics must be reversed.

In only one of Ballantyne's cases was the mother a primipara, in all the others she was a multipara and had generally had a large number of pregnancies. As might be supposed from the greater youth of the patients, the mother in the cases collected by the writer had had fewer pregnancies; 5 of them were primiparæ, 8 had borne between one and three children, and 17 were multiparæ of more than three pregnancies.

The previous health of the mother appears to play no great role in the development of a dropsical infant. In some instances it was described as "weakly, delicate," etc., but in general no stress is laid upon this portion of the history.

The previous obstetric history was bad in 12 of the writer's cases as against 9 in which it was good. If this proportion, almost 66 per cent., be compared with an ordinary series of obstetric histories, it will, obviously, be found to be enormously increased, the usual ratio being certainly not over 10 per cent. at a very high estimation.

The maternal health during the pregnancy which resulted in the birth of the dropsical infant was, in general, much impaired, and herein lies an important factor in determining the cause and mechanism of production of these cases. The mothers were usually toxic, and in the 34 cases reviewed 20 had marked edema during pregnancy, 21 had albuminuria of various grades, and 11 suffered from hydramnios. In any series of cases where 62.5 per cent. suffer with albuminuria, almost as many with edema, and a third with hydramnios,

it is, to say the least, highly suggestive that some active agent in producing a pathological result is present.

Syphilis was conspicuous by its absence, six patients giving a positive reaction.

The pregnancies were frequently terminated prematurely, but when full maturity of the fetus was reached, the labors were usually tedious and difficult, owing to the swollen condition of the child, and in many instances were terminated by destructive procedures involving the fetus.

The child was usually stillborn or at most lived but a few minutes. None survived.

The morbid anatomy of the fetus and membranes is exceedingly varied and the lesions noted have been the basis of the greater part of the literature on this subject.

The general edema of the subcutaneous tissues and the serous cavities was the constant and invariable condition found. The edema varied from small swellings to enormous distention, affected different specimens in different body regions, but was usually general and diffuse in character. The effusion was serous in type, occasionally thick and resembling pseudomucin.

The edema was most marked in the subcutaneous tissues, there were usually hydropericardium, hydrothorax, ascites and commonly hydrocephalus.

The extremities were in general involved in the swelling, though occasionally there is special mention of the fact that they were not affected. There was commonly an anemia of greater or less degree. The visceral lesions varied almost with the individual case, there being nothing constant in the pathology, and in the 37 cases analyzed, these lesions were distributed as follows: No lesions found 11, heart and vessels 2, urinary apparatus 6, liver 8, spleen 4, suprarenal 2, blood 4, spine 1, tumors 1, no autopsy 2.

Such a wide range of morbid anatomy, so many different forms of disease, such vague conditions as "enlarged spleen," "degeneration of the erythrocytes," and so on, point only

to the conclusion that there is no specific pathology for general dropsy to be found in the fetus itself, and it is apparent that the cause must be sought elsewhere.

The one significant point in studying these infants is that in all of them the lesions may well be ascribed to defects in nutrition or errors in the circulation, a statement of great importance, as will be developed later. In Ballantyne's cases the morbid anatomy also had a wide range, anemia, diaphragmatic hernia, liver and kidney change, leukemia, and transposition of the organs being all noted.

The placenta, however, had almost constant characteristics. It was markedly edematous, very large, friable, soft, and pale, and where studied microscopically there was shown edema of the villi or the vessels were obliterated and the lumina filled with cells and debris. In other words, there was a blocking of circulation and fluid interchange between the mother and the fetus, the block taking place at the point of most delicate balance, the placenta. In 37 cases the placenta was described as edematous and diseased in 28, negative in 7, and not studied in 2.

The cord was in general thick and edematous, and in some instances showed a round-cell infiltration of its connective tissue. It has not been given especial attention by writers.

The pathogenesis and etiology of hydrops universalis fetus, as may be supposed from the different conclusions presented, is obscure. The older investigators considered the condition as due to maternal disease alone, nephritis and cirrhosis of the liver being accepted as the underlying causes. Later the disease was thought to reside in the fetus itself. Osler described a cardiac anomaly and considered it responsible. Leukemia, congenital nephritis and many other conditions have been regarded as causative. The best explanation from the writer's standpoint was that of Fuhr,[4] who summarized the mechanism of the edema thus: (1) Chronic maternal endometritis, intensified by nephritis; (2) hyperplasia of the chorionic villi due to

decidual increase following upon the endometritis; (3) excessive absorption of fluid blood into the fetal circulation (partly from maternal hydramnios), overfilling of the circulation in the fetus, with resulting obstruction and edema; (4) hydramnios due to increased secretion from the fetal kidneys, an increase not, however, sufficient to overcome the obstruction; (5) edema of the placenta due to secondary obstruction in that organ.

Schridde[5] believes that the entire complex is due to changes in the cellular elements of the blood and the blood-making organs.

The general status of the entire situation is very well summed up by Ballantyne, who says: "Provisionally it may be supposed that general edema of the fetus may arise in the later months of fetal life, from maternal causes; possibly conditions which increase the blood-pressure in the placenta by causing structural changes in the maternal and (secondarily) in its fetal parts, may thus lead to backward pressure and transudation of serum in the fetal body. Again it may be supposed that in the early fetal or late embryonic period, structural anomalies may arise in the fetus (heart, kidney, liver, blood), which will directly produce the dropsy as it is produced in the adult; although with slight modifications and exaggerations on account of the peculiarities of the intra-uterine environment. These fetal conditions it may yet be found possible to trace back again to morbid maternal states; and it may even be that maternal or paternal conditions existing in the sexual cells before impregnation may be potent to direct the life of the impregnated ovum into abnormal manifestations. Let us here leave this subject; it is clear that it is obscure; this alone is clear."

When a large series of these cases is analytically surveyed, there appear certain elements constant for all the cases, or nearly so, which seem to point toward certain lines of investigation.

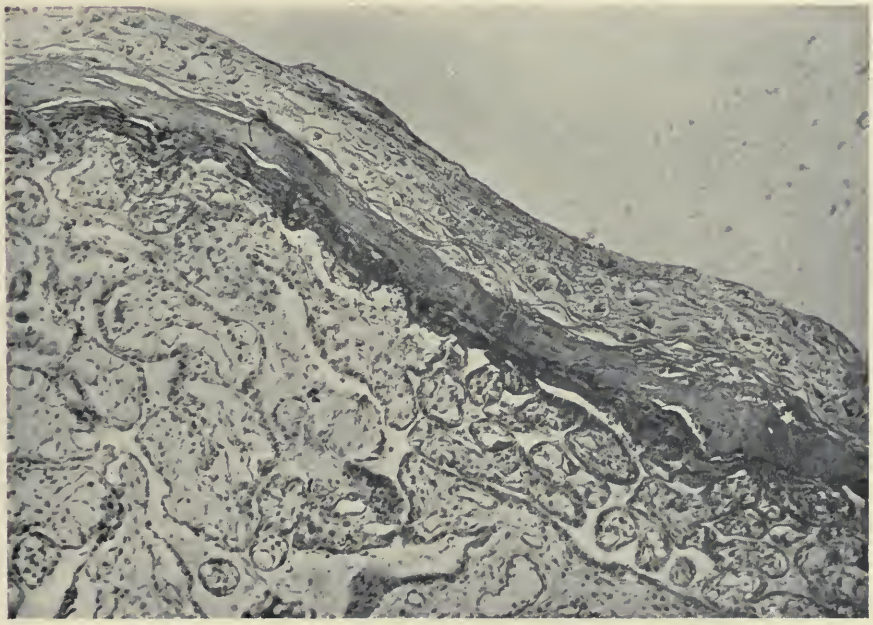

FIG. 1.—Section of placenta, showing the marginal sinus at *a*, sections of villi at *b*, and the fetal free surface at *c*.

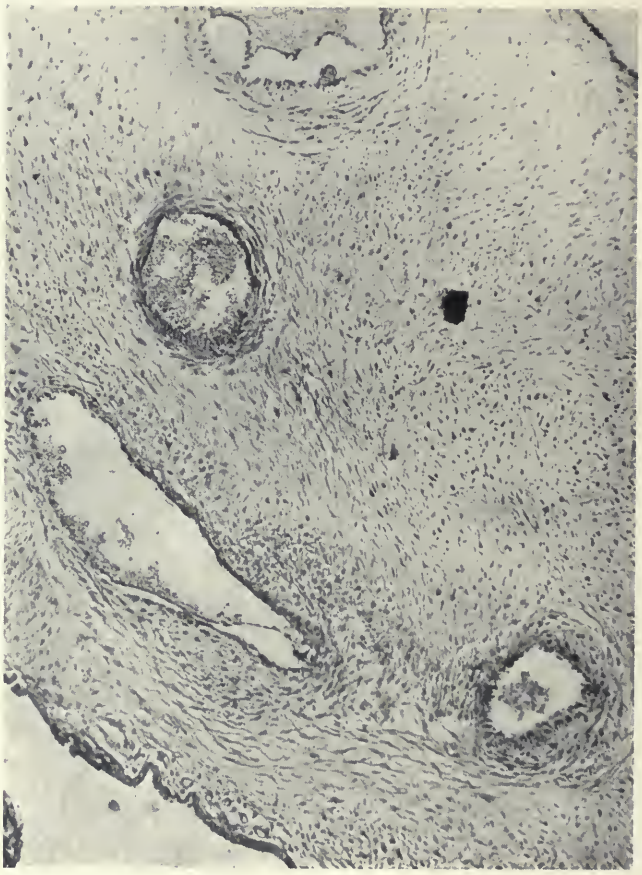

FIG. 2.—Section of placenta, showing a bloodvessel and several lacunæ much distended.

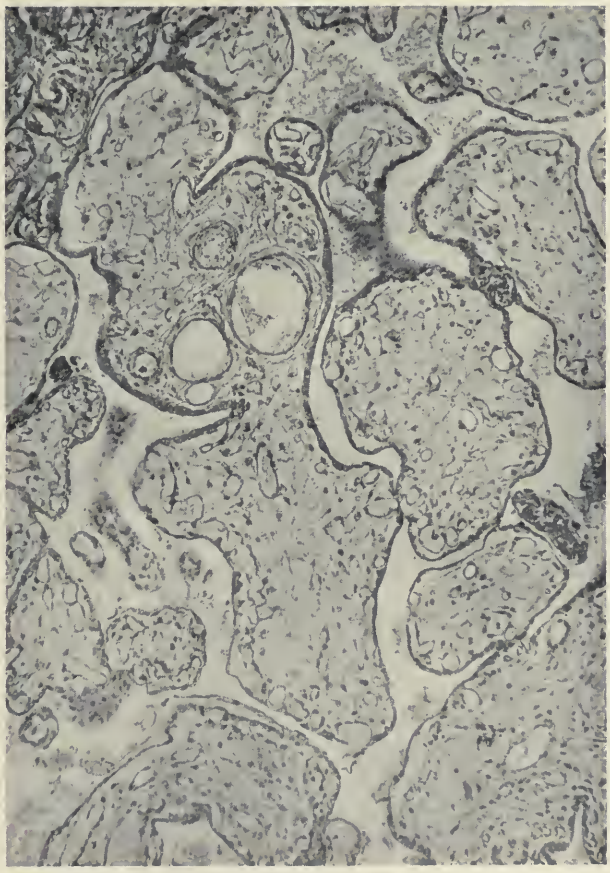

FIG. 3.—Placenta (high power), showing swollen and edematous syncytial cells, wide separation of the villi, vacuolation, and degeneration of the syncytium.

The pathogenesis and the morbid anatomy of the specimens, permit general edema of the fetus to fall naturally into two great groups, as has been stated. The first or mechanical group, including such fetal diseases as blood dyscrasias and so on, may be dismissed with the comment that such morphological defects may or may not cause a general edema, as they interfere with normal circulation or not. The pathogenesis of the developmental errors themselves is not at all understood, and in the present state of biological knowledge, it must remain as a problem unsolved.

The cases due to toxemia, however, offer opportunity for speculation, deduction, and experiment. It is the opinion of the writer that the development of this variety, comprising many more than half the cases, may be reasonably considered as due to the orderly sequence of a chain of factors, each one of which may be adduced from a careful study of the case histories, from the mechanism of the production of edema in general and from a study of the facts concerned in placentation.

Such chain of factors would include the development in the mother of one or another of the forms in which toxemia is manifested; as a result of this the production of a tendency toward edema in general, the edema being most marked at the point where blood and other body fluid interchange is most specialized, *i. e.*, the stratum spongiosum of the placenta and the tufts of the villi. There would then ensue an edema of the placenta and a decrease in its functionating capacity with the secondary alterations in nutrition and circulatory disturbance in the fetus, culminating in a general edema of that organism.

Taking up the essential features of this pathological sequence in detail, maternal toxemia of pregnancy is so usual and ordinary event that the fact of its occurrence requires no further discussion here. The influence of any toxemia in the production of edema in general is also obvious

though the exact manner of the escape of fluid into the tissues is not so clear. A concise and acceptable analysis is given by Stengel,[6] who states that the causes of an increased amount of fluid in the tissues are (1) an increase of blood-pressure; (2) a decrease of tissue elasticity and pressure; (3) alterations in the blood, making it more diffusible; (4) alterations of the liquid in the tissues, increasing the osmotic quality of these; (5) increased permeability of the walls of the bloodvessels; (6) obstruction to the flow in the lymphatic vessels. Any one of or a combination of such conditions may be responsible for the appearance of edema in the presence of the toxemias of pregnancy.

If edema in general is present, it follows that the pathological increase in tissue fluids will be most marked in the region where the mechanism for fluid interchange between tissues is the most delicate and specialized. Such a region is the cortical zone of the placenta, where the fetal and maternal blood meet and exchange their products of waste and repair. The placenta, to briefly review its anatomy, consists of two parts, separated by the intervillous space. The fetal side, or chorion frondosum, comes into close contact with the maternal side or the decidua serotina. This latter structure has as its base the uterine musculature and the deep layer of the mucosa; then follows the stratum compactum and lastly the stratum spongiosum, just beyond which is the intervillous space, occupied by the villi of the chorion frondosum from the fetal side. From the surface of the compactum, processes arise, termed septa, which project into the intervillous space, grouping the villi into cotyledons and giving fixation to some of the roots of the villi. These septa do not reach the surface of the chorion throughout the body of the placenta, but at the periphery, around a given zone, they do come into contact with the chorion and unite beneath it to form the closing plate (Fig. 4).

It appears true that the intervillous space is filled with maternal blood derived from the uterine vessels, without

any restraining membrane being present, though Hertwig and others maintain that the space must be lined by a layer of endothelium.

However this may be, a free circulation of maternal blood occurs through the intervillous space, and the villi being completely immersed in this constantly renewed blood, an osmotic interchange takes place between the maternal blood in the space and the fetal blood contained in the vessels of the villi (McMurrich[7]).

This, then, is the point where toxins developed in the maternal blood, no matter what their origin, may exercise their activities upon the endothelium of the space and upon the syncytial cells of the chorion and cause a block of the normal osmotic interchange, and, what is of equal importance, may interfere with the metabolic, absorptive function of the chorionic epithelium.

It must be remembered that the nutrition of the embryo takes place in two ways, as well described by Keibel and Mall,[8] who state that, on the one hand, growth takes place by the transference of nutritive material from the blood of the mother to that of the child, and, on the other, by the direct absorption by the chorionic epithelium of products of the maternal mucous membrane, these products frequently being subjected to a kind of digestive process before they pass into the embryonic circulation.

These maternal substances are partly products of secretion, partly waste products, together with extravasated maternal blood, and have been included by Bonnet under the name embryotrophe and by English writers have been designated pabulum. In the hemochorial type of placenta there may be distinguished two stages of nutrition, an embryotrophic phase at the commencement of development, and a later hemotrophic stage, not sharply differentiated from the former in time, but during which the nutritive material is received from the maternal blood exclusively.

Writer.	Age.	Para.	Obstetric history.	Wassermann reaction.	History of present pregnancy.	Pathology, fetus, membranes, placenta, etc.
Ludwig[9]	32	II	First pregnancy normal	..	Rapid enlargement of the abdomen, pain, dyspnea, hydramnios, edema of legs, albuminuria, spontaneous birth, dead infant.	Weight, 2050 gm., general edema, trunk, head, extremities, endocarditis, mitral stenosis.
Opitz[10]	32	XII	.....	..	Induced labor eighth month, marked edema of the trunk and legs, marked albuminuria. Hydramnios. Delivery by craniotomy and dismemberment.	Fetus, enormous edema. The entire urinary apparatus was very hypoplastic. Placenta enormous, edematous, weighing 2280 gms.
Vecchi[11]	32	0	.....	..	Edema of legs, genitals and face; marked albuminuria; premature delivery with forceps. Fetus stillborn. Mother developed puerperal eclampsia; recovery.	Fetus showed polydactyliaplastic peritonitis; some degree cystic kidney. Placenta and cord large and edematous.
Sitzenfrey[12]	30	IV	Previous pregnancies normal, labor spontaneous.	—	At six months had influenza followed by edema, albuminuria and casts; marked nephritis, hydramnios. Fetus stillborn.	Female, 2410 gms. Extreme edema, acute nephritis. Placenta edematous, weight 2000 gms., diameter 38 cm., and showed edema of villi. Cord showed edema.
Commandeur[13]	40	I	Spontaneous delivery of a healthy infant.	..	Spontaneous delivery of a six months' dead fetus.	General edema. Weight 1890 gms. Ascites (450 c.c.); acute nephritis. Placenta normal.
Kreisch[14]	..	IV	First three pregnancies normal	—	Nephritis, marked albuminuria, edema, hydramnios. Difficult destructive delivery of a dead infant.	Fetus, general edema. Weight 3050 gms. No autopsy. Placenta enormous edematous, weight 2025 gms.
Rauchu and Rigaud[15]	20	0	.....	—	Delivered at eighth month; labor long and tedious. Fetus showed beginning maceration. Marked albuminuria.	General edema. Liver, spleen and kidneys normal. Placenta 620 gms. markedly edematous.
Charles[16]	42	VI	Three normal pregnancies, one stillborn, one child died a few hours after birth. Fifth child showed general edema.	..	Edema of legs and abdomen. Hydramnios, marked albuminuria, spontaneous delivery at seven months. Child died ten minutes after birth.	Fetus, weight 2750 gms., general edema. No autopsy. Placenta, weight 1850 gms.; very edematous.

Andrews[17]	28	VI	No abnormal children.	+ —	No edema, no hydramnios. Slow labor. Child breathed for a few moments.	General edema of the child, all organs normal except liver which showed well-marked cirrhosis. Placenta and cord show no changes.
Andrew[17] Case II	36	V	.....	—	Swelling of the legs, dyspnea and headache, albumin and casts in urine. Turn pregnancy; a healthy child followed by one, seat of general edema; died immediately after birth.	Child, general edema, weight 3 pounds 10 ounces. No lesions in the organs. Placenta very large, pale, edematous. The chorionic villi have their lumen filled with cells and the vessels are obliterated.
Jatho[18]	26	II	First birth a dropsical infant, lived two days.	..	Much edema of legs, labia and abdominal walls, excessive albuminuria. Spontaneous birth; dead child.	Child, male, very large and edematous, some endocarditis in right ventricle. Placenta very large and edematous.
W. Fischer[19]	25	V	Third child stillborn.	—	No edema, a trace of albumin, no casts. Spontaneous delivery; dead child.	Child, male, 41 cm. long, weight 1830 gms. Moderate general edema, enlargement of spleen and liver, many small hemorrhages; spleen, liver, and kidneys. Placenta 1730 gms. markedly edematous.
W. Fischer[19] Case II	26	II	.....	+	Edema of the legs, much albumin, a spontaneous birth followed by puerperal sepsis with recovery. Child dead.	Child, male, 45 cms. long; enlargement of spleen, many small hemorrhages in kidney, spleen, and liver. Placenta edematous 1220 gms. General edema of the child.
King[20]	32	X	First three children healthy eight healthy; the rest either stillborn or miscarriages or lived but a few days.	—	No edema, no albuminuria. Antepartum bleeding, spontaneous delivery of a dead edematous fetus.	General edema, features obliterated. Suprarenals ¾ in. long, both cortex and medulla were disorganized, marked small round cell infiltration. Other organs normal. Placenta large, white, and friable.
King[20]	29	II	First child syphilitic; second died at three months.	+	Spontaneously delivered of a living fetus which immediately died.	Fetus generally edematous more marked in abdomen and legs, subcutaneous hemorrhages were present. Placenta normal; cord edematous.
MacWalters[21]	30	0	.....	—	Albumin and casts; delivered of a dead fetus.	Fetus generally edematous; heart, kidney and liver normal. Placenta soft, friable, and enlarged.

Writer.	Age.	Para.	Obstetric history.	Wassermann reaction.	History of present pregnancy.	Pathology, fetus, membranes, placenta, etc.
Fleischmann and Wolff[22]	27	IV	Two previous children stillborn and edematous; one not edematous, lived 24 hours.	++	Normal amount liquor amnii. Spontaneous delivery. Child died in 24 hours.	General edema; many diffuse small hemorrhages. Hemorrhagic diathesis. Placenta 820 gms. not remarkable.
C. Sauvage[23]	44	III	First child living and well.	—	Marked albuminuria, edema of lower limbs and abdomen. Difficult labor. Child died after a few breaths.	Child 3200 gms. 46 cms. long. Marked general edema, organs show no change. Much edema of placenta.
C. Sauvage[23]	31	I	First pregnancy terminated by abortion at three months.	—	Marked hydramnios, albuminuria, edema of legs and abdominal wall. Infant delivered with forceps, died after a few inspirations.	Child weighs 4880 gms. Marked general edema, liver, and kidney congested, spleen hypoplastic. Placenta pale and edematous.
Bourret and Lathoud[24]	23	0	.....	+	Erysipelas, marked edema of the legs and albuminuria, hydramnios. Spontaneous delivery. Child lived six days.	Child, male, marked edema. Liver showed sclerosis of portal spaces. Polycystic disease of the kidneys. Placenta normal, 720 gms.
Commandeur[25]	24	II	Both children living at birth, but dying within twenty-four hours of toxemia.	—	Albuminuria, spontaneous labor, but difficult. Child dead.	Child, male, weight 2250 gms. Enormous general edema of body and head. Suprarenals showed degeneration of medullary portion. Placenta edematous, enormous weight 1560 gms..
O. Fischer[26]	31	I	Normal labor; normal child	—	Much pain in right side of abdomen; occasional slight bleeding from vagina, no varices or edema. Spontaneous delivery of a dead child. No hydramnios.	Fetus, 21 cms. long, weight 600 gms., general edema, most marked in trunk and head. Kyphosis and scoliosis present. Placenta edematous, 500 gms.; cord also edematous and showed round cell infiltration of its connective tissue.
White[27]	33	X	One child well; one lived two years; four stillborn; four miscarriages.	—	Thirty-two weeks pregnant; edema of feet; no hydramnios. Spontaneous delivery; child died in a few moments.	Child, female, 16½ in. long, 6 pounds. General edema. Viscera were normal. Placental very large, weight 3 pounds 9 ounces. Villi edematous.

W. Lahm[28]	23	0	.....	++	Eight months pregnant, labor spontaneous. Child died immediately after birth.	Child, male, 36 cms. long, weight 1500 gms. General edema. Liver and spleen much enlarged; the liver showed a marked, mixed cell infiltration as did the spleen and kidney. The cells were mostly erythroblasts, myeloblasts and myelocystes. Leukocytes were practically absent. Placenta edematous, 750 gms. Spirochetæ were demonstrated in the liver.
Rautmann[29]	33	V	Three children living, second child stillborn; third child died in three weeks	—	Edema of legs, headache and nosebleed; marked albuminuria; many casts. Spontaneous birth of dead child.	Child had enormous general edema; weight 530 gms. Liver, spleen and kidney showed a massive growth of large lymphoid cells. There were degenerative changes in the erythrocytes.
Schridde[5]	32	VII	Four normal births then three macerated children in succession.	—	Eight months pregnant, albuminuria and edema of the legs. Spontaneous delivery of a dead infant.	Child showed some liver changes, marked icterus, general edema. Placenta and cord edematous.
Schridde[5] Case II	28	IV	Children living and well.	..	Albuminuria, no edema. Placenta previa (seventh month).	Child, male, 32 cms. long, weight 1100 gms. Much general edema. Liver, weight 130 gms. and showed brownish pigmentation. Placenta edematous.
Teuffel[30]	30	Mult.	.....	..	Much icterus and edema; delivered spontaneously at sixth month.	Child generally edematous except hands and feet which were normal in size. Placenta enormously edematous.
Lieven[31]	29	V	Former pregnancies normal.	—	Suffered from marked albuminuria and edema of the legs and vulva. Large hydramnios. Difficult labor, dead child.	Child, female, enormous general edema. Organs normal except kidneys which showed an acute nephritis. The huge placenta was edematous, weight 1900 gms.
Davies[32]	30	V	Two children dead of pneumonia; one of jaundice; one stillborn.	—	Varicose veins, edema, hydramnios, dead child.	Child, female, 17½ in. long, 7¼ pounds. Marked general edema; spleen enlarged 2½ times normal size. Placenta edematous and showed vascularity of villi.

Writer.	Age.	Para.	Obstetric history.	Wassermann reaction.	History of present pregnancy.	Pathology, fetus, membranes, placenta, etc.
Nyhoff[33]	..	..	History wanting.	—	.....	Massive general edema; high leukocytosis. Placenta edematous, weight 1240 gms. Cord edematous.
Nyhoff[33] Case II	..	..	History wanting.	..	.....	A macerated, premature child very marked general edema. Organs normal. Placenta weighed 1080 gms., many necrotic villi.
Case III	..	..	History wanting.	..	.....	Stillborn female of 1800 gms. General anasarca of moderate degree. Heart unusually small and with the liver showed some round cell infiltration. Placenta enlarged not otherwise abnormal.
Case IV	..	..	.....	+	Mother had some edema during pregnancy.	Premature female, marked anasarca, weight 2300 gms., spleen and pancreas showed hypertrophy of the connective tissue. Placenta weighed 900 gms.
Case V	..	..	.....	—	No edema during pregnancy; very marked hydramnios; child lived 12 hours in an incubator.	Premature male, weight 1640 gms. Marked general edema. The liver was enlarged. Placenta thick and pale.
Case VI	..	..	.....	—	Mother had edema of legs, albuminuria, hydramnois. Child lived 18 hours.	Child, female, 2920 gms. Marked general edema. Organs showed no lesions. Placenta not edematous.
Case VII	..	..	.....	—	Mother was well, no edema nor albuminuria.	A dead female, 2020 gms. Anasarca of head, trunk, and thighs. A sarcoma involved the intestines, pancreas and left kidney. The liver and gall-bladder were free. Placenta edematous, 980 gms.

It follows, then, that from the contact of fetus and mother at the placenta there is transmitted not only the blood with its function of oxidation and removing the end products of oxidation, but also a distinct metabolic output takes place from the maternal tissues.

Both these phenomena are absolutely dependent upon the presence of cells fully capable of osmosis and of secreting and absorbing the nutritive elements. Any change in these cells brought about by toxins with edema and degeneration would at once disturb the metabolic balance and produce such resulting disorder of nutrition and of circulation in the fetus as readily to account for the changes found in hydrops universalis. The edema and cell degeneration of the placenta are well shown by Figs. 1, 2, 3, illustrating the case here reported.

The entire problem may be briefly summarized in the statement that hydrops universalis fetus is a condition characterized by a general edema of the fetus, associated with a great variety of visceral change and diseased states of the body fluids, or it may present no organic change whatever even to the most painstaking pathological examination, except, of course, the presence of abnormal amounts of serous fluid.

The condition is usually present in women who have shown the existence of some form of gestational toxemia or in whom the presence of such toxic factor is suspected but cannot be confirmed.

It almost always is associated with edema and degenerative changes in the placenta, the microscopic picture of which is very comparable, in general, to that shown to exist in the present case.

In the opinion of the writer, hydrops universalis fetus is due to a maternal toxemia impairing the circulation and the nutritive function of the placental cells, and thereby causing secondary circulatory and nutritional defects to ensue in the fetus.

An abstract of the cases reported since 1901 is appended.

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The Treatment of Diabetes Mellitus with Special Reference to Allen's Method.

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A review of the history of diabetes reveals a gradual development in our dietetic measures dependent upon an increasing knowledge of the physiology of metabolism and of its perversions in this disease. The first step in this evolution was taken by Rollo[1] in 1796, when he advocated the restriction of the diabetic diet to meat, eggs and milk with an avoidance of beer, potatoes, bread and other starchy foods. This dietary regulation was essentially qualitative and concerned the type of food permitted without definite attention to quantities involved. As early as 1820, however, Prout[2] noted that the dietary treatment of diabetes must be considered rather from the point of view of the amounts of the various foods permitted than merely of the kinds of food. Cantani[3] in 1880 was the first to lay particular stress upon the importance of restricting not only the amount of vegetable foods, but also the amount of proteins and to emphasize the value of the fats in replacing the carbohydrates of the diet. With Rubner's[4] determination a few years later of the caloric equivalents of the various classes of food stuffs, there was opened the possibility of placing on a more scientific basis the replacement of carbohydrates in the diet by an isodynamic amount of fat.

In 1883, Stadelman,[5] a pupil of Naunyn, discovered betaoxybutyric acid and formulated the theory that acid poisoning is the cause of the coma and of the increased ammonia excretion in diabetes. He then inaugurated the second important step in the treatment of diabetes; namely, the control of the acidosis by the use of alkali. Opponents of the view that diabetic coma is the result merely of an increased acidity produced in the blood or tissues arose however, among the early leaders of whom was Klemperer. But definite evidence in support of the views of these opponents of the acid-intoxication theory was not forthcoming until in 1904, when Wilbur[6] showed experimentally that oxybutyric acid has a toxicity out of proportion to its mere acidity, and that the neutral sodium salt of the acid is likewise toxic. Thus, definite support was given to the view that oxybutyric acid and probably other substances accumulating from the disordered metabolism in the diabetic are responsible for a specific toxic action quite independent of their acid

[1]Rollo: Abhandlung des Diabetes mellitus, 1796.
[2]Prout: Krankheiten des Magens und der Harnorgane, 1820.
[3]Cantani: Der Diabetes mellitus, *Il Policlinico*, 1820, 379.
[4]Rubner: *Zeitsch. f. Biologie Festschrift zu Voit*, 1901, XLII., 261. (Quoted Lusk, Science of Nutrition, 1909, p. 41.)
[5]Stadelmann: Ueber die Ursachen der path. Ammonia Ausscheidung beim Diabetes, *Exper. Archiv.*, 1883, XVII., 419.
[6]Wilbur, R. L.: Acidosis. *Jour. A. M. A.*, 1904, XLIII., 1228.

properties and are possibly thus a cause of diabetic coma. The benefit derived from sodium bicarbonate in staving off coma was attributed by this school largely to the more rapid elimination of the ketone bodies which is induced by this drug. In consequence of this point of view, which has not however been universally accepted, attention was directed to the importance of lessening the production of ketone bodies when coma seemed imminent rather than merely of neutralizing them, and stress was laid upon the necessity of curtailing the fats in the diet and of adding carbohydrates when coma threatened. The discovery that acidosis is an accompaniment of starvation in the normal individual and that such acidosis is promptly controlled by comparatively small amounts of carbohydrates, gave support to this treatment for incipient coma in the diabetic.

In 1906, Benedict and Török[7] showed that acetone excretion as well as the output of nitrogen and dextrose in the diabetic could be reduced by administering alcohol. This effect of alcohol is due to the fact that it can be oxidized by the diabetic, that in its oxidization it gives rise to no ketone bodies, and that since each gram yields seven calories, its utilization affords considerable sparing of the protein, fat and carbohydrate metabolism. Alcohol has accordingly become recognized as an important therapeutic agent in the treatment of diabetic acidosis. Meanwhile, von Noorden[8] had called attention to the value in the handling of severe diabetes of alternating periods of high fat and protein feeding, with periods of oatmeal feedings rich in fat, protein, and a particular carbohydrate, and with periods of very restricted feeding consisting of little more than green vegetables. The rationale of this treatment was, however, not clearly defined.

At the time of Allen's work, therefore, the therapy of diabetes consisted essentially of a combination of these contributions. Restriction of carbohydrate and protein intake was insisted upon in accordance with the carbohydrate tolerance of the patient. Fats were added to the diet to replace calorically the deficiency from withdrawal of the carbohydrates. When coma threatened, the fats were reduced, carbohydrates were permitted and alcohol and alkalies were given. The oatmeal and green vegetable days (practically starvation days) of von Noorden were often interpolated into the series of ordinary carbohydrate-free days.

Allen's[9] treatment is the direct outcome of experimental studies upon pancreatic diabetes in the dog. Thiroloix and Jacob had shown in 1910, that after partial pancreatectomy in the dog with removal of most, but not all, of the pancreas, a condition is produced such that the dog's urine will remain free of sugar if he be fed solely upon meat, but that he becomes glycosuric if fed upon carbohydrates; furthermore, that if such a dog be kept for some time upon a carbohydrate diet, his diabetes becomes severer, so that even upon a meat diet he now remains glycosuric. Allen conducted similar experiments and found that by varying the amount of pancreas removed from about three quarters up to about nine tenths of the whole, he could produce all grades of diabetes. In the mildest, the animal became glycosuric when fed carbohydrates freely, but, even after long-continued carbohydrate feeding, the urine became sugar free when the animal was returned to a meat diet. In the intermediate grades, the condition paralleled that observed by Thiroloix and Jacob; the animal was at first glycosuric only on a carbohydrate diet, but if kept for some time upon such a diet the animal would then remain glycosuric even when placed upon a meat diet. In the severest grades, the animals were from the outset glycosuric even on a meat diet. Allen next observed that an animal exhibiting this last, severest type of diabetes or an animal that from excessive carbohydrate feeding had become persistently glycosuric, might, if starved absolutely for a few days, exhibit a marked amelioration in its sugar tolerance and come to react like the animal in the milder intermediate grade. Further that following such improvement, the animal might if fed upon an exclusively meat diet, be kept alive and at constant weight for months, whereas the animals of the severer type if not fasted exhibited a constant decline in weight and strength to an early exitus.

On the basis of these experiments, Allen out-

[7]Benedict and Török: *Zeitsch. f. klin. Med.*, 1906, LX., 329.
[8]v. Noorden: Die Zuckerkrankheit und ihre Behandlung, 1901.

[9]Allen, F. M.: Studies concerning Diabetes. *Jour. A. M. A.*, 1914, LXIII., 939.

lined a treatment of severe human diabetes which consists essentially of placing the patient at rest in bed on absolute fasting except for alcohol, water and sodium bicarbonate. The alcohol is given in the form of whisky in hourly doses up to from 50 to 250 cubic centimeters daily. This fast is continued until from twenty-four to forty-eight hours after sugar has entirely disappeared from the urine. According to the severity of the case this requires from about one to eight days of fasting. Associated with the decrease in the glycosuria, there occurs an equally striking diminution in ketonuria. Thus it appears that the tendency of our ordinary carbohydrate-free diet to cause acidosis, ketonuria and coma in the severe diabetic is due not to the absence of carbohydrates but quite probably to the abundance of fats. The alcohol is not an essential feature of the fasting period.

During the fasting period, the patient, as a rule, experiences pronounced relief from the headache, heaviness and general discomfort often present before the treatment is instituted, and there is, as a rule, only the most trifling discomfort from hunger. Indeed, the patient frequently bears fasting with greater comfort than he does a limited diet. Following the fast, Allen places the patient upon green vegetables alone, without butter, oil or other food stuffs, and gradually increases the amount of the former up to reappearance of sugar in the urine. At the same time, he stops the alkali and gradually removes the alcohol.

Allen, in the form of what he calls green vegetables, gradually allows his patient from ten to eighty or one hundred grams of carbohydrate per day as the patient's tolerance permits. The green vegetables which we have been accustomed to use in our carbohydrate-free diets have been those that have a total carbohydrate of only about five per cent. or less and much of this is cellulose and, of the remainder, a considerable part was extracted in the cooking. Allen, however, prepares his green vegetables in steamers instead of boiling them so that the soluble carbohydrate is not lost, and he gradually makes use of the non-starchy vegetables of higher carbohydrate content, ten per cent., fifteen per cent., and even twenty per cent., such as turnips, carrots, parsnips and, finally, corn. The increase in the proteins and fats of the diet is made *pari passu* with this increase in the carbohydrate content in the form of green vegetables. If at any time, sugar reappears in the urine, he immediately introduces another fast day, thereafter gradually increasing the diet again as before. He has shown that the reappearance of sugar in the urine may result not only from increasing the carbohydrate or the protein of the diet, but also from an unduly rapid increase of the fats without change in the other constituents. This fact we have confirmed.

Finally, as the patient improves and his tolerance increases, Allen avoids permitting him to bring his weight up to normal, preferring rather to keep the patient about ten pounds below this point.

Turning now to our own results in the treatment of diabetes, we wish first to call attention to the fact that for the majority of cases presenting themselves, Allen's treatment is unnecessarily severe, and would probably be undesirable. Indeed, it is noteworthy how promptly most of our diabetic patients are rendered sugar free upon a calorically very rich carbohydrate-free diet. This diet is shown in Table I. (Carbohydrate Free Diet). Of the last thirty-four cases treated in our wards, fifteen were rendered sugar free on this diet within a week and five more became sugar free in the second week. This is the more striking in that most of these patients had been for some time previously under treatment for their condition without having become sugar free.

TABLE I.

CARBOHYDRATE-FREE DIET (DAILY RATION).

Eggs	3		
Bacon	15 gms.		
Meat	120 gms.		
Ham	60 gms.		
Butter	60 gms.	Proteids	74 gms.
Cheese	30 gms.	Fat	138 gms.
Olive Oil	15 c.c.	Carbohydrates	2 gms.*
Cream	45 c.c.	Calories	1570
Coffee	300 c.c.		
Tea	150 c.c.		
Broth	150 c.c.		

Lettuce and green vegetables.

*Plus the carbohydrate of the particular green vegetables permitted.

GREEN DIET (DAILY RATION).

Eggs	3	Proteids	35 gms.
Bacon	30 gms.	Fat	49 gms.
Coffee	150 c.c.	Carbohydrates	1 gm.
Olive Oil	15 c.c.	Calories	600

Lettuce and 5% green vegetables.

The most important factors in securing these results are, we believe, absolute rest in bed and the absolute exclusion from the diet of bread of any kind. Gluten bread appears to be one of the

chief obstacles in ridding the diabetic of glycosuria. After a variable period upon the carbohydrate free diet, the tolerance of most of these patients was considerably increased, so that often from fifty to one hundred grams of bread, potato, or other carbohydrate could be taken without inducing glycosuria. It is in the severer cases, especially in young adults, that Allen's treatment has its value. How promptly relief of the glycosuria and probably still more important of the ketonuria may be secured by this method, the presentation of a particular case will illustrate.

TABLE II.

Day of Treatment.	Diet.	Glucose.	Ketones.
2	C.F.	138 gms.	19 gms.
9	C.F.	66 gms.	19 gms.
11	C.F.	125 gms.	
12	80 c.c. whisky	100 gms.	
13	90 c.c. whisky	30 gms.	3 gms.
14	200 c.c. whisky	9 gms.	2 gms.
15	240 c.c. whisky	0	
16	Greens plus 80 c.c. whisky	0	
17	Greens plus 90 c.c. whisky	0	1 gm.
62	C.F. plus 30 gms. bread	0	2 gms.
67	C.F. plus 40 gms. bread	0	

A patient, J. U. (See Table II.) was admitted to the hospital last March. He was a boy of eighteen years, who for four months had noticed polyuria, thirst and increasing weakness. For a month, he had been under treatment but on a diet that included gluten bread. During the two weeks before admission, his symptoms had become rapidly worse, and he had been passing as much as three gallons of urine daily. The second day after admission, on the carbohydrate-free diet, the patient passed 138 grams of sugar and 19 grams of ketone bodies. After a week of treatment, continuously on carbohydrate-free diet, he still passed from 50 to 125 grams of sugar in the urine and still 19 grams of ketone bodies. On the tweifth day, he was therefore put on Allen's treatment. In two days of fasting, the ketones were reduced to three grams, and in four days of fasting, the sugar was gone from the urine. His diet was then gradually increased until, on the sixty-seventh day of treatment, he was discharged taking the full carbohydrate-free diet with forty grams of bread in addition, the urine being, on this diet, free from sugar and showing only two grams of ketones. After leaving the hospital in May, the patient continued upon this diet and returned to work. His urine remained sugar free until the middle of August. At this time his occupation took him to an institution where he could not obtain the proper diet, and a relapse resulted. He returned to our ward in September with about the same glycosuria and ketonuria as at the first admission, but this time two days of fasting sufficed to make him sugar free and to reduce the ketones to six grams. On the forty-fifth day of treatment, he was taking again the full carbohydrate-free diet and ten grams of bread without glycosuria or increase of the ketones.

At the first admission, the patient's weight was 117 pounds. During the fast this fell to 113 pounds, but upon discharge, had risen to 120 pounds. Upon his readmission, it was 121. During the second fast, it did not fall, and upon the second discharge it was 118. There was therefore only moderate loss of weight occurring during the fast and a quick restoration upon resuming feeding.

Similar satisfactory results have been obtained in about seven other cases.

During the period following the fast, our method has been to give first green vegetables alone; then the green diet shown in Table I. If the patient is still sugar free, this is gradually increased by about three stages to the full carbohydrate-free diet and when this is borne without glycosuria the ten, fifteen and twenty per cent. of green vegetables and then bread or potato are added in measured quantities. Reappearance of glycosuria at any time is immediately treated by a return for a few days to green vegetables. In one case, we obtained increased tolerance by resorting to a second six-day fast and, in other cases, an increased tolerance has apparently resulted from the use of von Noorden's oatmeal diet followed by green vegetables alone until the patient is again sugar free (usually one or two days) for these severer cases always develop glycosuria during the oatmeal days.

In the treatment and study of diabetes, a factor of great importance is the coöperation of an efficient nurse in charge of a ward devoted to metabolic studies. This combination we have fortunately available at the University Hospital, and it may be recommended as very desirable, wherever diabetics are to be cared for.

In conclusion, we feel that while Allen's treatment is desirable in only a small proportion of all diabetics, it affords a means of shortening the first stages of the treatment of those diabetics that do not promptly become sugar free when placed on a carbohydrate-free diet, and that it is the most effective treatment for the severer cases of diabetes exhibiting high ketonuria, cases that with other methods of treatment probably either would fail to become sugar free at all or would pass in a short time into coma.

AN EXTRAORDINARY POLYMORPHONUCLEAR LEUKOPENIA IN TYPHOID FEVER*

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This case is reported because of the most unusual degree of polymorphonuclear leukopenia which occurred during the third week of typhoid fever.

The patient, J. S., man, aged 19, was admitted, Sept. 26, 1915, to the service of Dr. Alfred Stengel, University Hospital. He complained of weakness, headache, diarrhea, and fever of gradual onset about September 12, persisting with varying intensity up to admission. Physical examination showed an emaciated young adult male, evidently toxic and

TABLE 1.—BLOOD COUNT

Day of Disease	W. B. C.	Poly.		Lymph.		L. M.	T.	E.	B.
		Rel.	Abs.	Rel.	Abs.				
17	7,600	0.5	38	98	7,448	1	0.25	0	0.25
20	5,400	1.5	81	94.5	5,103	3	1	0	0
23	11,900	33	3,927	58	6,902	4	5	0	0
24	12,000	58	6,960	41	4,920	1	0	0	0
25	8,400	58	4,872	39	3,276	2	1	0	0

heavily infected. The pulse was dichrotic; the posterior cervical, epitrochlear and inguinal lymph nodes were slightly enlarged; the spleen was not palpable on admission, but became so three days later; the abdomen was moderately distended and exhibited a few characteristic rose-spots; the thorax was negative except for the evidences of a mild acute bronchitis. The temperature ranged in the first twenty-four hours from 103.8 to 99.4 F., the pulse from 120 to 96, and the respirations from 28 to 24. The Widal reaction taken on the day of admission (considered the seventeenth day of the

* From the Medical Wards of the University Hospital.

TABLE 2.—ARNETH COUNT

	Nuclear Divisions				
	1	2	3	4	5
J. S., twenty-fourth day	48	38	10	4	0
Normal	5	35	41	17	2

disease) was strongly positive. A second Widal reaction four days later was the same. Blood culture was sterile, but a culture of the urine yielded *Bacillus typhosus*. The Wassermann reaction was negative.

The unusual leukocytic picture observed is shown in Table 1.

There was, therefore, present on admission and for a few days thereafter, a most extraordinary reduction in the number of the polymorphonuclear neutrophils. Less striking, but still pronounced, was the increase of the lymphocytes. In character, about one half of the latter were the typical small lymphocytes, the remainder were somewhat larger with paler nuclei and were of the type described as mesolymphocytes. Eosinophils were absent as usual in typhoid fever. One week later, the absolute polymorphonuclear count had returned approximately to normal. An Arneth count on the twenty-fourth day, contrasted with the normal Arneth count, is shown in Table 2, and indicates clearly the great predominance of young forms at this period. We must conclude, therefore, that the previous reduction of the polymorphonuclears was the result of a destruction of these cells and not of their mere withdrawal from the peripheral circulation.

On the twenty-fourth day, a persistence of the lymphocytosis gave an abnormally high total leukocyte count. During the further course of the case, which was one of uncomplicated typhoid fever, the total leukocytes varied from 6,100 to 15,900; the polymorphonuclears ranged from 44 to 68 per cent., and the lymphocytes from 28 to 55 per cent. The temperature remained approximately normal after the fifty-first day.

The possibility of the condition being one of aleukemic lymphatic leukemia received due consideration. The generalized enlargement of the lymph nodes was extremely trifling, but Pappenheim has reported two cases of lymphatic leukemia without enlargement of the lymph nodes, in one of which there were found in the blood 20,000 leukocytes per cubic centimeter, of which 96 per cent. were small lymphocytes. The character of the leukocytes found, the absence of any associated changes in the erythrocytes, however, and the further course of the case made the diagnosis of leukemia untenable.

An investigation was made to discover any drug or toxic substance to which the patient might have been subjected before admission and which might have been responsible for the destruction of the polymorphonuclears, but it was fruitless. It seems proper, therefore, to regard the blood picture in this case as an extreme exaggeration of the tendency characteristic of the blood in typhoid fever, namely, a diminution in the polymorphonuclear neutrophils to a minimum about the time of defervescence, a gradual increase in the lymphocytes persisting somewhat longer than does the diminution in the polymorphonuclears, and an absence of eosinophils during the febrile period. These alterations in the blood picture in typhoid fever have been noted in the extensive studies of Türk,[1] of Naegeli[2] and of Thayer.[3] The reduction of the polymorphonuclears to such a degree as in this case, however, has not previously been reported in typhoid fever, so far as we are aware. Naegeli mentions a boy with typhoid fever with an absolute polymorphonuclear count of 900, and Thayer notes a polymorphonuclear percental count of 24.6 as the lowest observed in studying 832 cases. Of leukopenias occurring in other diseases, there are three cases in the literature of a grade comparable with ours. Vacquez and Ribierre[4] reported the case of a young man with extensive tuberculosis of the mediastinal and peribronchial lymph nodes who exhibited shortly before death a leukocyte count of 2,300, of which 8 per cent., or 184 cells, were polymorphonuclear neutrophils. Brown[5] reported the case of a young woman with staphylococcic septicemia who, six days before death, had a leukocyte count of 1,000 with only 1 per cent. of polymorphonuclears. Türk's[6] was likewise one of staphylococcic septicemia in a young woman who three days before death had a leukocyte count of 940 with no polymorphonuclear

1. Türk, W.: Klinische Untersuchungen über das Verhalten des Blutes bei acuten Infectionskrankheiten, Vienna and Leipzig, 1898.
2. Naegeli, O.: Die Leucocyten beim Typhus abdominalis, Deutsch. Arch. f. klin. Med., 1900, lxvii, 279.
3. Thayer, W.: Observations on the Blood in Typhoid Fever, Johns Hopkins Hosp. Rep., 1900, viii, 487.
4. Vacquez and Ribierre: Lymphocythémies leucémiques et aleucémiques, Bull. et mém. Soc. méd. d hôp. de Paris, 1900, Series 3, xvii, 914.
5. Brown, P. K.: Fatal Case of Acute Primary Infectious Pharyngitis with Extreme Leukopenia, Am. Med., 1902, iii, 649.
6. Türk, W.: Septische Erkrankungen bei Verkümmerung des Granulozytensystems, Wien. klin. Wchnschr., 1907, xx, 157.

neutrophils in 532 cells examined. Two days later, the leukocytes were 1,950, of which 0.28 per cent. were polymorphonuclear leukocytes. At necropsy in this case, the bone marrow showed complete loss of the leukocytogenic series of cells, although the erythropoiesis appeared normal.

All three of these cases differ, however, from ours in that they were in an agonal state, and the reduction of the polymorphonuclears was, in the last case at least, associated with a degree of injury to the bone marrow which can scarcely have existed in our case in view of the rapid subsequent rise of the polymorphonuclear count to normal or, indeed, above normal.

We are inclined to regard the case, therefore, as merely an extreme exaggeration of the characteristic blood change in typhoid fever, the mechanism of which is still unknown.

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ENDOTHELIAL PHAGOCYTES IN PLEURAL EXUDATE DUE TO THE BACILLUS TYPHOSUS.

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The important part played by phagocytic endothelial cells in the pathology of typhoid fever has been recognized since Mallory,[1] in 1898, after a careful histological study of a large mass of material, came to the conclusion[2] that "the typhoid bacillus produces a mild type of inflammatory reaction consisting almost entirely of endothelial leukocytes which accumulate in large numbers where the typhoid toxin is strongest and thus form the lesions characteristic of typhoid fever. These endothelial leukocytes are strongly phagocytic for other cells, incorporating and digesting large numbers of them. In the intestinal lesions they take up chiefly lymphocytes, in the spleen, red blood-corpuscles, and in the blood-vessels, especially of the portal circulation, polymorphonuclear leukocytes and red blood-corpuscles." These endothelial phagocytes were demonstrable not only in the lymph nodules of the Peyer's patches and of the spleen, but also in the mesenteric lymph nodes, the liver, bone-marrow, clotted blood in the heart and in the alveolar exudate of pneumonia complicating typhoid fever. Mallory, moreover, quotes Eichhorst as having seen similar cells in the blood drawn from the tip of the finger of a typhoid patient during the second week of the disease. In all the localities mentioned the phagocytic cells have the same size and appearance while the engulfed cells vary in type according to the locality. The endothelial phagocytes are uniformly large, with more or less round, lightly staining, eccentrically placed nuclei, and a protoplasm which stains with varying intensity. The formation of phagocytic cells by proliferation from endothelial cells was not claimed by Mallory to be peculiar to

[1] Jour. Exp. Med., 1898, iii, 611.
[2] Mallory, F. B., Principles of Pathological Histology, 1914, p. 165.

typhoid except in regard to location, extent, and degree. He believes that their formation in large numbers may be the result of any mild toxin which acts diffusely and causes proliferation and not necrosis. Under these conditions it is clear that there is a close relationship histologically between tuberculosis and the typhoid process, and this becomes of considerable diagnostic interest when the reactions to infection of a serous surface, such as the pleura, are studied.

The pleura is covered throughout by a single layer of endothelial cells, and these cells appear in small numbers and are readily recognized in stained preparations of the sediment from almost all collections of fluid in the pleural cavity. By some they are referred to as endothelial cells and by others as large mononuclears, and it is claimed that similar forms may arise from connective-tissue cells, from the perivascular lymph spaces, or from the blood stream. They resemble very closely the endothelial phagocytes which proliferate in various localities in typhoid fever.

In mechanical effusions the endothelial cells are seldom numerous, but often occur in groups of two or more. Such groups are spoken of by the French writers as "placards" or placques, and have long been considered as indicating a mechanical or non-inflammatory effusion. Widal, Ravaut, and Dopter,[3] in 1902, emphasized the fact that in a mechanical effusion the endothelial cells are desquamated in groups and remain joined together and typical in appearance, while in an infectious pleurisy with effusion the endothelial cells may at first be joined in placques, but they soon separate and then become markedly altered in appearance. Thus they become swollen and stain poorly, vacuoles appear in the protoplasm, and the cell outline becomes indistinct. Also, it is only after the cell masses separate into single cells that they may exhibit phagocytic properties. This, however, is unusual in mechanical effusions. In all pleural effusions due to organisms other than the typhoid or tubercle bacillus the endothelial cells play but a small part and are usually lost sight of in the great polymorphonuclear reaction.

When the tubercle bacillus is the etiological factor the pleural effusion might be expected to show in stained preparations at least a moderate number of endothelial cells and lymphocytes. Some observers, however, claim that while numerous endothelial cells may be present early in a tuberculous effusion, yet once the pleura becomes covered by a fibrinous membrane this variety of cell will cease to be found, and lymphocytes will become the sole or at least the predominating form. As a result of this the finding of endothelial cells in an effusion of some duration has been considered as a strong argument against the effusion being of tuberculous origin, and Naunyn goes so far as to state that the presence of endothelial

[3] Compt. rend. d. l. Soc. de biol., 1902, liv, 1005.

cells excludes tuberculosis. On the other hand, Koster[4] found endothelial cells in the effusion of almost 50 per cent. of the known cases of tuberculosis he investigated, and other observers corroborate this. In no case, however, has the endothelial cell been described as the predominating form, nor is phagocytosis mentioned.

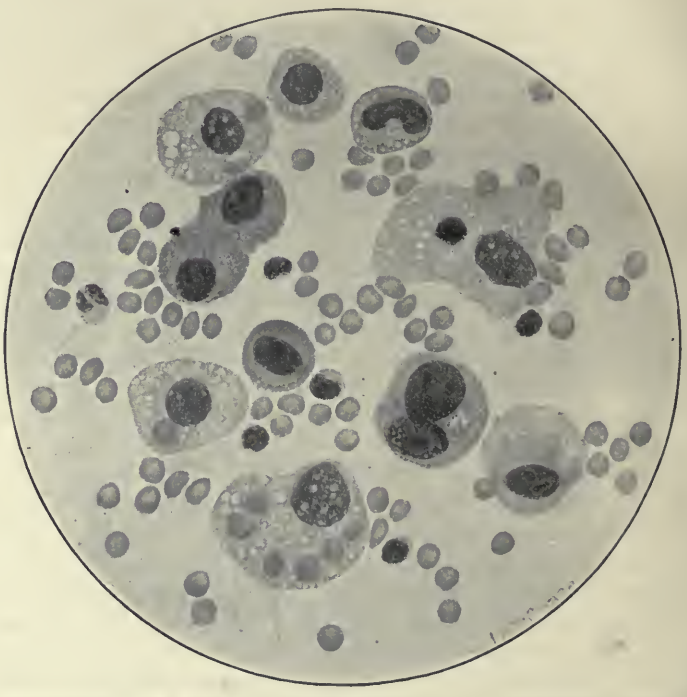
Typhoid pleurisy is not common (an incidence of about 1 to 2 per cent., in large series of reported cases), and there are but a few records of the cytology of effusion due to this infection. The cases reported indicate considerable variation in the cell picture. Widal and Ravaut,[5] to whom we owe the first systematic study of cytodiagnosis in puncture fluids, state that typhoid pleurisies are often hemorrhagic and contain a relative abundance of large mononuclear cells. Vincent[6] in each of two cases of pleurisy in typhoid fever, found that the effusion, although purulent, contained a considerable number of endothelial cells. He makes no mention of phagocytosis nor did cultures show the Bacillus typhosus; on the other hand, one effusion produced tuberculosis when injected into a guinea-pig. Widal and Lemierre[7] report a case in which endothelial cells predominated in the effusion. Some of these cells were vacuolated and of very large size, but no mention is made of phagocytosis. In this case the effusion yielded the typhoid bacillus on culture and also a positive agglutination test for the typhoid bacillus. The pleural effusion observed by Levi[8] in the course of typhoid fever, was sterile, but contained numerous polymorphonuclear cells and a moderate number of large mononuclears. Phagocytosis is not mentioned. Earl[9] states that the typhoid bacillus calls forth a polymorphonuclear excess of from 50 to 80 per cent. of the cells of an effusion.

The variations in these findings are probably to be explained by the fact that, clinically, typhoid pleurisy appears in two more or less distinct forms, and also that the effusions studied were obtained at different stages of the process. Pleurisy may complicate the onset of typhoid fever, and this is the form which has led to the use of the term pleurotyphoid by the French. The pleuritic symptoms may be marked but little or no effusion develops, and the whole process usually disappears after a few days. On the other hand, pleurisy may develop late in the disease at the time when purulent complications commonly occur. This form goes rapidly on to empyema, and is comparable in every way to the other purulent complications of the disease. The cells in such purulent collections are almost wholly polymorphonuclears, and a pyogenic coccus is sometimes obtained upon culture either alone or with the

[4] Nord. Med. Ark., 1905, xxxviii, H. 3, p. 1.
[5] Compt. rend. d. l. Soc. de biol., 1900, lii, 648.
[6] Semaine méd., 1903, s. 370.
[7] Ibid., 1913, s. 385.
[8] Ibid., 1903, s 370.
[9] Dublin Jour. Med. Sci., 1903, cxvi, 409.

Bacillus typhosus. It is impossible to say whether in these purulent effusions the polymorphonuclear reaction is to be attributed to a greater intensity or concentration of the typhoid toxin or to a superadded pyogenic infection.

In the transitory pleurisies occurring early in the course of typhoid fever the typhoid toxin is apparently not present in any great concentration, and one would expect in view of Mallory's work to find that the cells of the effusion were mostly of endothelial origin. Further, it might be expected that these endothelial cells would show active phagocytosis of other cells in the pleural effusion



Endothelial phagocytes from pleural effusion due to Bacillus typhosus.

just as do the endothelial leukocytes arising in other parts of the body in response to the typhoid infection. A careful search of the literature, however, does not reveal any record of such a finding, and the present example of endothelial phagocytosis in pleural exudate due to the Bacillus typhosus is therefore reported.

Case.—The patient, an adult male, with no previous medical history, entered the hospital of the University of Pennsylvania, stating that he had been ill with fever and chills for three weeks. He complained of no localizing symptoms, but upon questioning admitted the presence of a slight pain in the chest for a day or two.

The temperature and pulse were elevated. Physical examination revealed nothing abnormal other than the signs of a small effusion at the base of the right lung. Upon aspiration a few cubic centimeters of hemorrhagic serous fluid were withdrawn, and from the cytology of this a diagnosis of pleurisy due to the typhoid bacillus was ventured. This diagnosis was promptly verified by finding that both the blood serum and the effusion gave a positive agglutination test with typhoid bacilli and later by obtaining the Bacillus typhosus in pure culture from both the blood and the effusion. The titre of agglutination was 1 to 1000 in the blood, and 1 to 800 in the effusion. A second aspiration several days later gave similar fluid, but all signs of effusion then disappeared and no fluid was obtained on a third thoracentesis. The patient ran a typical typhoid course, without complications, to complete recovery, and at no time could any evidence of pulmonary disease be found either by physical examination or by stereoscopic roentgenographs.

The fluid obtained by aspiration was distinctly hemorrhagic but did not clot upon standing. Spreads made from the sediment and stained with Wright's stain showed the unusual cytology which is well illustrated in the accompanying drawing of selected cells. Excluding erythrocytes, about 60 per cent. of the cells of the effusion were large mononuclear cells with more or less round nuclei which were usually placed eccentrically and did not stain as deeply as did the nucleus of the small lymphocyte. The protoplasm of these large mononuclear cells varied in appearance; sometimes it was stained uniformly and deeply, while in other instances it was vacuolated and stained palely. In every respect these cells resembled the endothelial leukocytes or macrophages described by Mallory. A moderate number showed phagocytosis and had engulfed one or more erythrocytes. Several had engulfed lymphocytes and one instance of phagocytosis of a polymorphonuclear cell was observed. Some of the cells ingested showed evidence of digestion, while others appeared unaltered. Occasionally the endothelial cells were grouped together in plaques and these cells never showed phagocytosis. Through many gradations these endothelial cells merged into the typical mononuclear of the circulating blood and it was impossible to draw any distinguishing line. The effusion contained these cells in great excess of the white-blood cell count of the circulating blood and a careful search of stained preparations of the latter failed to reveal any large endothelial cells or any phagocytosis by the usual mononuclears.

This observation of the endothelial character of the reaction of the pleura to the typhoid bacillus and of the phagocytic power of such cells in a pleural effusion is of interest not merely from its correlation of typhoid pleurisy to the general pathology of typhoid fever, as described by Mallory, but also from its possible diagnostic significance. In the case here reported the diagnosis was ventured

with hesitation, but it would be warranted with greater confidence in the future upon finding a similar cytology. Pleurisy with effusion early in the course of typhoid fever is uncommon, but in any suspicious case an effusion should certainly have its cytology determined, if by this simple step a diagnosis may even occasionally be reached.

Conclusion. Pleurisy with effusion due to the Bacillus typhosus may occur early in the course of typhoid fever. The effusion is apt to be hemorrhagic and to contain a large number of endothelial leukocytes similar to those found elsewhere in the lesions of typhoid fever. These endothelial macrophages show phagocytosis of other cells, especially the erythrocytes of the effusion.

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THE VALUE OF THE AMBARD QUOTIENT IN THE ESTIMATION OF RENAL FUNCTION.

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In this study are reported the results obtained from the determination of Ambard's quotient of renal efficiency (as modified by McLean) in a series of clinical cases with and without nephritis. Ambard's quotient was the outcome of a series of studies beginning with that of Ambard and Papin,[1] in 1909, upon the laws governing the concentration of urea in urine. Previous workers had noted the difficulty of securing a constant urea concentration in the urine of either man or animals even on carefully controlled diets. Ambard and Papin found, however, that in the dog an exclusive meat diet with as much water as the animal desires produces a urine of remarkably uniform urea concentration. This concentration of urea under these conditions is independent of the protein content of the diet and is the maximal urinary urea concentration for that animal. Any factor which unduly increases the animal's thirst, such as the feeding of bread or of salt, a diarrhea, or an inadequate supply of food, will, if water is freely available, disturb this relation in the urea excretion and lower the concentration of the urea in the urine. These authors further observed, however, that the kidney can eliminate a much larger amount of urea in a unit time if the secretion be accomplished at a lower concentration. When the renal parenchyma was sufficiently reduced experimentally the animal could eliminate urea only at a lower concentration. At this lower concentration, however, a very large amount of urea could be excreted as the result of a polyuria, only following the stimula-

[1] Étude sur les concentrations urinarires, Arch. internat. de physiol., 1909, viii, 437.

tion from an accumulation of urea in the blood. Ambard[2] showed that both in the dog and in man, if a constancy of the urea concentration in the urine is maintained, the square root of the urea eliminated in the urine during any unit of time is closely proportional to the concentration of the urea in the blood in any given individual, and that this law (Ambard's first law) holds for nephritis as well as for the normal kidney. The unit of time used for measuring the urea excretion must, however, be short (about an hour or less), since the continual variations in both the rate of urea excretion and the concentration of the blood urea will otherwise cause apparent discrepancies in their interrelations. Variations in the concentration of the urea in the urine alter, however, the relation between the rate of urea excretion and the urea concentration of the blood. An effort was made by Ambard[3] to define mathematically the effect of this factor of concentration. To do so, however, was not easy, because of the extreme difficulty of obtaining subjects in which, with varying urinary urea concentration, the blood urea concentration remained constant. After many examinations, two subjects in which these conditions were realized were observed, and from them Ambard concluded (second law) that if the blood urea remains at a constant concentration, the rate of urea excretion is inversely proportional to the square root of the urea concentration in the urine. The validity of this second law was by no means so well established by Ambard's work as was his first. On the basis of these two laws, Ambard and Weill[4] developed a formula into which was introduced with these two factors, in the relations stated, an additional factor designed to compensate for the variations in weight between different individuals arbitrarily selecting 70 kilos as the standard weight. They also adopted in this formula 25 gms. of urea per liter as the standard concentration of urea in the urine. In its complete form, Ambard's quotient is as follows:

$$\text{Constant} = \frac{\text{Ur.}^2}{\text{D} \times \frac{70}{\text{wt.}} \times \sqrt{\frac{\text{c}}{25}}}$$

Ur. – gm. of urea per liter of blood.
D. – gm. of urea excreted in twenty-four hours.
Wt. – wt. of individual in kilos.
c. – gm. of urea per liter of urine.

For the sake of simplifying the calculation, and in order to make the quotient rise and fall with the renal efficiency instead of rising as the renal efficiency falls, and *vice versa*, as is the case with Ambard

[2] Rapports entre le taux de l'urée dans le sang et l'elimination de l'urée dans l'urine, Comp. rend. Soc. de biol., 1910, lxix, 411.

[3] Rapports de la quantité et du taux de l'urée dans l'urine, la concentration de l'urée du sang étant constante, ibid, 506.

[4] Les lois numériques de la secretion rénale de l'urée et du chlorure de sodium, Jour. de physiol. et de path. gén., 1912, xiv, 753.

and Weill's constant, McLean[5] has transposed the original formula without altering its essential principle, so that it stands as follows:

$$\text{Index} = \frac{\text{Gm. urea per 24 hours} \sqrt{\frac{\text{gms. urea per liter urine}}{}} \times 8.96}{\text{Wt. in kilos} \times (\text{gm. urea per liter of blood})^2}$$

When Ambard and Weill's quotient = 0.080, the standard normal, McLean's index = 100. According to McLean this index should be above 80 when renal function is normal, and indices below 80 indicate more or less impairment of renal function.

It must be remembered that Ambard's formula is constructed on a purely empirical basis. The particular functions, the square and square root, being chosen not on any logical grounds but merely because, in a number of instances, under certain conditions, the use of these functions gave calculated results agreeing with the observed findings. Even though each of Ambard's laws hold independently under the partially constant conditions of the original studies, it might be questioned whether the combination of these two laws as expressed in their formula would necessarily hold under varying conditions. That their formula does frequently accord with the observed facts has been shown by Ambard and Weill, by McLean and others. On the other hand, McLean, using his modification, sometimes obtains indices in normal individuals exhibiting the wide range of from 80 to 250. Addis and Watanable[6] conclude that the rate of urea excretion in man varies under physiological conditions, and that these variations cannot be completely explained by the variations in the amount of urea in the blood and urine. Pepper and Austin,[7] in dogs (using, however, total nitrogen instead of urea), found enormous variations in the quotient in different animals and in the same animals under different conditions. The question probably arises, therefore, as to whether Ambard and Weill's formula or its modification is the precise expression of the fundamental physiological law that governs the relation between the concentration of urea in the blood and in the urine and the rate of urea excretion, or whether it is merely a crude approximation to the actual quantitative relation, a sort of diagram that indicates the direction in which changes in one of the factors concerned will influence the others. This question can best be answered by an investigation of the quotients obtained in the same normal individual at different times under different conditions and by a comparison of the quotients obtained from different normal individuals. Only

[5] The Numerical Laws Governing the Rate of Excretion of Urea and Chlorides in Man, Jour. Exper. Med., 1915, xxii, 212.

[6] Rate of Urea Excretion; a Criticism of Ambard's and Weill's Law of Urea Excretion, Jour. Biol. Chem., 1916, xxiv, 203.

[7] Experimental Studies of Urinary and Blood Nitrogen Curves after Feeding, Jour. Biol. Chem., 1915, xxii, 81.

if these quotients consistently approximate a constant can we accept the formula as representing anything more significant than a sort of diagram of these relations.

A study of the quotient derived by applying Ambard's formula as modified by McLean to a number of individuals with presumably normal kidneys shows at once that the quotient obtained is anything but constant.

CHART I.—CASES WITH PRESUMABLY NORMAL KIDNEYS.

No.	Name.	Date.	Age.	Sex.	Diagnosis.	Urine urea per 24 hours.	Urine urea per liter.	Blood urea per liter.	Weight (kilo).	Index.	Blood-pressure.
1	Kelly	Jan. 17, 1916	37	M	Perihepatitis; pleurisy	17.90	9.23	.23	70	145	
		Jan. 25, 1916				20.98	11.90	.30	72	113	
		Jan. 21, 1916				20.08	5.22	.22	70	116	
2	Wa.	Jan. 5, 1916	24	M	Gastric neurosis; constipation	20.62	10.31	.19	56	280	90–68
		Jan. 8, 1916				27.67	2.82	.24	54	132	
		Jan. 10, 1916				20.20	4.80	.16	54	267	
3	VanS.	Feb. 1, 1916	29	M	Endothelioma of lymph nodes	20.9	12.0	.21	61	242	126–76
		Feb. 5, 1916				28.27	12.61	.24	61	255	
4	Ma.	Feb. 1, 1916	46	M	Angina pectoris	17.18	7.68	.21	75	128	104–70
		Feb. 8, 1916				29.46	6.71	.27	75	124	
		Feb. 15, 1916				16.78	8.16	.24	75	99	
5	Ki.	Jan. 27, 1916	..	..	Epilepsy	23.04	7.68	.23	70	153	
		Jan. 21, 1916				23.22	5.53	.33	70	64	
6	Li.	Nov. 8, 1915	50	M	Sciatica	11.46	17.40	.19	75	147	115–85
		Nov. 18, 1915				9.18	22.97	.22	77	79	112–74
		Nov. 30, 1915				24.25	26.25	.25	77	219	112–68
7	Gr.	Dec. 20, 1915	26	M	Bronchial asthma; subacute bronchitis	34.80	14.04	.27	72	222	
		Dec. 23, 1915				31.78	10.45	.24	71	224	
8	Em.	Nov. 21, 1915	42	M	Syphilitic hepatitis	24.52	26.7	.30	75	185	117–65
9	Mi.	Jan. 5, 1916	18	M	Chronic arthritis; mitral regurgitation with compensation	9.60	20.87	.22	63	124	122–70
10	At.	Jan. 5, 1916	22	M	Pulmonary infarction; subacute mitral and aortic regurgitation with compensation	14.16	19.15	.31	58	96	116–54
11	Ka.	Feb. 29, 1916	63	M	Headache	14.66	22.88	.31	54	120	115–75

In this study, which was made on patients in the medical wards of the University Hospital, periods of 72 minutes were employed (or in a few instances slightly larger periods up to 160 minutes) and the blood withdrawn from an arm vein 36 minutes after the period began. The urea was determined by the urease method described by Van Slyke and Cullen.[8]

The cases may be divided into three groups: First, cases in which there is no clinical or laboratory evidence of nephritis, nor of marked cardiovascular disease, nor of cardiac decompensation; the findings in these cases are tabulated in Chart I. Second, cases with definite evidence of more or less severe nephritis; tabulated in Chart II. Third, a few cases in which there is no definite neph-

[8] A Permanent Preparation of Urease and its Use in the Determination of Urea, Jour. Biol. Chem., 1914, xix, 211.

CHART II.—CASES WITH NEPHRITIS.

No.	Name.	Date.	Age.	Sex.	Diagnosis.	Urine urea per 24 hrs.	Urine urea per liter.	Blood urea per liter.	Weight.	Index.	Blood-pressure.	Eye-grounds.
1	Br.	Feb. 25, 1916	44	M	Advanced glomerulonephritis; cardiac decompensation	12.17	10.35	1.43	77	2	180 to 140	Sclerosis; retinitis.
		Mar. 4, 1916				10.83	8.20	1.05	80	3		
		Mar. 8, 1916				10.51	5.88	1.09	80	2		
2	McC.	Nov. 30, 1915	53	M	Advanced chronic nephritis	27.15	8.58	.94	66	12	210 to 150	Retinal exudate and edema.
		Dec. 15, 1915				20.93	17.16	1.14	66	9		
		Dec. 17, 1915				33.00	13.85	.98	64	18		
		Dec. 20, 1915				15.25	13.45	1.17	64	5.7		
3	Jo.	Nov. 29, 1915	35	M	Chronic glomerulonephritis	20.73	19.20	.75	62	23	180 to 112	
		Dec. 31, 1915				9.78	13.59	.51	62	20		
4	St.	Nov. 4, 1915	24	M	Advanced glomerulonephritis	18.02	4.00	.45	64	25	172 to 126	Neuroretinitis; exudate and hemorrhage.
		Nov. 15, 1915				18.01	4.02	.42	59	27		
		Nov. 29, 1915				16.23	2.75	.24	61	23		
		Dec. 3, 1915				23.75	5.11	.30	60	140		
5	O'D.	Apr. 17, 1916	39	F	Advanced glomerulonephritis	21.02	8.08	1.93	112	1.3	250 to 160	Marked neuroretinitis.
6	Wa.	Feb. 1, 1916	24	M	Chronic endocarditis; chronic nephritis; intermediate	55.4	16.10	.90	62	40	160 to 120	
7	An.	Jan. 5, 1916	47	F	Advanced glomerulonephritis	8.07	12.60	.83	68	5	272 to 141	Sclerosis; retinitis.
8	Ba.	Mar. 6, 1916	15	M	Glomerulonephritis; intermediate	16.38	13.76	.49	47	60	165 to 115	Negative.
9	Hi.	Nov. 27, 1915	55	M	Chronic glomerulonephritis	20.23	26.63	.48	64	64	210 to 110	
10	Ya.	Mar. 11, 1916	26	F	Chronic nephritis; intermediate	16.5	9.16	.46	47	44	140 to 110	Negative.
11	Mo.	Feb. 8, 1916	45	M	Glomerulonephritis; intermediate	31.2	21.42	.39	83	103	185 to 135	Neuroretinitis.
12	Ch.	Mar. 9, 1916	36	M	Glomerulonephritis; intermediate	15.35	15.75	.37	67	61	176 to 110	Sclerosis.
13	Dav.	Apr. 17, 1916	21	F	Chronic degenerative nephritis	13.07	13.35	.37	58	59	160 to 130	Negative.
14	Wh.	Feb. 2, 1916	24	F	Glomerulonephritis; intermediate	19.18	2.93	.31	49	62	198 to 175	Early retinitis.

ritis; but in which there is more or less vascular disease or cardiac decompensation or both; tabulated in Chart III.

CHART III.—CASES WITH VASCULAR DISEASE.

No.	Name.	Date.	Age.	Sex.	Diagnosis.	Urine urea per 24 hours.	Urine urea per liter.	Blood urea per liter.	Weight (kilo).	Index.	Blood-pressure.
1	Bo.	Nov. 22, 1915	50	F	Sclerotic hypertension; cardiac decompensation	32.76	11.7	.34	56	15	200–110
2	Ro.	Feb. 29, 1916	57	M	Arteriosclerosis	9.2	7.1	.31	60	38	142– 85
3	Hur.	Nov. 8, 1915	63	M	Myocardial weakness	16.45	2.94	.30	84	33	122– 83
		Nov. 18, 1915				11.25	4.50	.24	85	43	145– 98
4	Da.	Feb. 27, 1916	59	M	Sclerotic hypertension	17.41	6.00	.37	54	47	170– 12
5	Ki.	Nov. 2, 1915	36	M	Chronic myocarditis	22.07	4.32	.28	79	64	121– 72
		Nov. 16, 1915				10.72	22.33	.38	79	36	
		Nov. 16, 1915				26.55	14.62	.30	..	176	

CHART IV.

Name.	Diagnosis.	Date.	Urine urea per liter.	D. Urine urea per 24 hours.	Ur. Blood urea per liter.	$\frac{Ur}{\sqrt{D}}$
Van S.	Endothelioma; lymph nodes	Feb. 1, 1916	12.00	20.90	.21	.045
		Feb. 5, 1916	12.61	28.27	.24	.045
Ma.	Angina pectoris (very mild)	Feb. 1, 1916	7.68	17.18	.21	.051
		Feb. 8, 1916	6.71	29.46	.27	.050
		Feb. 15, 1916	8.16	16.78	.24	.058
McC.	Advanced nephritis	Dec. 17, 1915	13.85	33.00	.98	.171
		Dec. 20, 1915	13.45	15.25	1.17	.300
St.	Advanced nephritis	Nov. 4, 1915	4.00	18.02	.45	.106
		Nov. 15, 1915	4.02	18.01	.42	.099
		Dec. 3, 1915	5.11	23.70	.30	.062

			Blood urea per liter.	D. Urine urea per 24 hours.	C. Urine urea per liter.	$D \times \sqrt{C}$.
Ke.	Perihepatitis	Jan. 17, 1916	.23	17.90	9.23	54
		Jan. 21, 1916	.22	20.08	5.22	46

Inspection of the cases tabulated in Chart I, cases in which presumably the kidneys are normal, shows the wide variation that is observed in the index in the same individual on different occasions and in different individuals. We may profitably examine certain of these cases from another point of view. If those instances of repeated examinations on the same individual where the urea concentration in the urine was approximately constant be chosen we find five examinations (see Chart IV) on two normal individuals. If to the data of these examinations Ambard's first law be applied it will be found to be confirmed in the first case, the ratio of $\frac{Ur}{\sqrt{D}}$

being constant in the two examinations on this case and also in the first two examinations of the second case. Among the nephritic cases five examinations from two cases exhibit approximately constant urinary urea concentration, but Ambard's first law does not hold in these cases. This is, perhaps, due to progression of the renal impairment in the first case and amelioration in the second.

If we select from our data those non-nephritic cases with repeated examinations showing constant blood urea concentration for the purpose of verifying Ambard's second law we can find only one case (see Chart IV). In this case Ambard's second law is but poorly supported. This finding is quite in harmony with the results previously quoted from Pepper and Austin; it is when there is variation in the urinary urea concentration that Ambard's formula most frequently gives unsatisfactory results. However, since variability in the concentration of urea in the urine is the rule and not the exception, this defect may seriously impair the value of Ambard's formula as a gauge of renal function under ordinary clinical conditions. The variability which we have found in the constant at repeated examinations of the same non-nephritic case or in comparing with each other one group of non-nephritic cases leads us to conclude that Ambard's formula is not a mathematically accurate expression of the behavior of renal function as regards urea excretion, but that it is merely a crude diagrammatic indication of certain relations between blood urea and urea excretion and urinary urea concentration.

The question must arise, however, whether Ambard's quotient or its modification, in spite of this objection that may be urged against it, is of value clinically as an index of renal function. If we can demonstrate that the quotient gives with reasonable constancy, information evidently more consistent with the clinical condition and subsequent course of the patient than does the careful inspection of those factors separately that go to make the quotient, we might well employ the quotient for diagnostic and prognostic purposes. On the other hand the determination of the index, because of the necessity for great accuracy in collection of the urine, is a much more difficult procedure to carry out than is the simple estimation of the blood urea, and more important still there are possibilities of undetected errors occurring through the loss of a small quantity of urine. Hence, unless some definite advantage can be urged in favor of the quotient as compared with the simple blood urea considered with due regard to the character of the urinary excretion, the latter would appear to be the safer criterion in clinical diagnosis and prognosis.

Inspection of our results would lead us to conclude that if we are to accept McLean's normal blood urea figures, 0.20 to 0.50 gms. per liter as correct, then the index is a more delicate measure of renal impairment than is the blood urea.

In our nephritic group, 10 out of 23 examinations show a blood urea within McLean's normal figures and 21 out of 23 give figures for the index below 80, which, according to McLean, indicates impairment of renal function. The clinical picture would lead us to suspect impairment of renal function in those cases. If, however, we accept Tileston's and Comfort's[9] figures for blood urea, 0.35 gms. per liter, or Folin and Denis's[10] urea figures, 0.23 and 0.28 gm. per liter as normal, then in out studies the index no longer possesses any advantages over the blood urea.

Inspection of Chart I will show that we have never found a blood urea in a normal or non-nephritic individual above 0.35 gm. per liter under ordinary conditions, and we have, accordingly, accepted this figure as the upper normal limit, which is in accord with the views of Tileston and Comfort. Accepting 0.35 gm. per liter as the upper limit of normal, then it is readily seen that in our nephritic cases blood urea alone gave as satisfactory evidence of impairment of function, as did the index except in the third examination of St. and in the case of Wh.

In the case of Wh. and the third examination of St. the index gave information which the blood urea alone would have failed to show.

Inspection of Chart III shows that in cases in which in all probability the only impairment in renal function is the result of arteriosclerotic changes or of renal passive congestion, the index may show pronounced depression while the blood urea remains within normal limits. There is evidence to suggest, therefore, that the index is at least in certain cases a more delicate gauge of renal impairment than is the blood urea. It is at least possible, however, and indeed highly probable, that for clinical purposes of diagnosis and prognosis these cases present rather an argument against the use of the index and for the blood urea than the reverse. It is in part because the estiamtion of the blood urea often helps to distinguish true nephritis from arterioselerotic lesions, or from congestion of the kidney, that it is of value in these cases. Clearly for this purpose the index would be of less value, being too sensitive and too readily reduced by arteriosclerotic condition and by passive congestion as well as by nephritis.

On examination of Chart III, which consists of frank cases of nephritis, one fails to see the marked variability in the index in individuals in whom more than one examination has been made that was noted in the non-nephritic cases. This tendency to constancy of the index in cases of nephritis with impaired function has also been emphasized by McLean.

The patient St. shows marked rise of the index on the fourth

[9] The Total Non-protein Nitrogen and the Urea of the Blood in Health and Disease as Estimated by Folin's Method, Arch. Int. Med., 1914, xiv, 620.

[10] On Uric Acid, Urea, and Total Non-protein Nitrogen in Human Blood, Jour. Biol. Chem., 1913, xiv, 29.

examination, due to the elimination of a urine of higher urea concentration and to a higher urea output which have most probably been caused by large doses of infusion of digitalis which he received between the time of the second and third examination.

Finally, it seems possible that in conditions in which there is either an extremely high nitrogenous intake or in which there is very rapid tissue catabolism, the blood urea may be raised above 0.35 gm. per liter, although renal function is quite normal, and that this may be accompanied by an increased rate of urea elimination in the urine with a normal index. In such cases the index would be a better gauge of the state of renal efficiency than would the blood urea alone. McLean has induced just such a condition by the administration of urea to normal individuals. Under ordinary circumstances, however, such conditions do not obtain, and our conclusion would be that, as a rule, the blood urea alone is a better guide in ordinary clinical diagnosis and prognosis than is the index.

Conclusions. 1. The Ambard formula in its original form or as modified by McLean does not express precisely the law of renal function with respect to the elimination of urea, and this is particularly true as regards the effect of urinary urea concentration.

2. The upper limit of blood urea in non-nephritic and normal individuals under ordinary conditions of diet and life is about 0.35 gm. urea per liter of blood. Figures higher than this are, under ordinary conditions of diet, to be considered evidence of impaired renal function.

3. Using McLean's modification of Ambard's formula, it was found that in the great majority of nephritic cases a lowering of the index was accompanied by an elevation of the blood urea above normal limits, 0.35 gm. per liter, and that the index afforded no information of diagnostic or prognostic value that could not be as readily deduced from the blood urea alone.

4. In certain cases the index was found to be lowered when the blood urea was within normal limits. This was especially true in arteriosclerotic cases and in cases with cardiac decompensation, which probably detracts from the clinical value of the index as compared with that of the blood urea rather than the reverse, since it is of importance to distinguish between cases of vascular and renal character.

5. In the determination of the index there is a possibility of error arising from undetected incomplete collection of the urine, which cannot occur in the simple blood urea estimation.

6. The urea index estimated repeatedly in the same individual exhibits wider variations in the normal or non-nephritic individual than in the nephritic.

7. For purposes of ordinary clinical diagnosis and prognosis the estimation of blood urea is a more reliable and more useful guide than is the urea index or the Ambard quotient.

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CLINICAL STUDIES OF ACIDOSIS.

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During the past few years much progress has been made in the study of the regulation of the body fluids as regards acidity and alkalinity and in the development of our conception of the condition known as acidosis.

Acidosis may well be compared with disturbance of the body temperature. It is recognized that life can continue only within a limited range of temperature variation and that health is compatible only with a still more restricted range. We are, more or less, familiar with the mechanisms which regulate the body temperature and maintain its constancy. Similarly, we have learned that the reaction of the body fluids as regards acidity and alkalinity must possess a degree of constancy even greater than must their temperature, if a normal condition or, indeed, life itself is to continue. Largely through the work of Lawrence Henderson,[1] and his associates we have become familiar with at least some of the mechanisms by which this reaction is kept constant. The normal reaction in the body is maintained chiefly by three means: The first of these is the presence in the blood and lymph of the salts, chiefly the sodium salts, of two very weak acids, carbon dioxide and phosphoric acid. Of these two acids the former is the more abundant and important. In the blood and lymph, sodium carbonate (alkaline) and carbon dioxid (acid) are both present in such proportion as to give a nearly neutral reaction. Similarly

[1] Clinical Studies on Acid Base Equilibrium and the Nature of Acidosis, Arch. Int. Med., 1913, xii, 153. Sellards, A. W., the Determination of Equilibrium in the Human Body between Acids and Bases, with Especial References to Acidosis and Nephropathies, Bull. Johns Hopkins Hosp., 1912, xxiii, 289.

disodium hydrogen phosphate (alkaline) and monosodium phosphate (acid) are both present in the proportions to give the same nearly neutral reaction. If to this almost neutral fluid a stronger acid, such as oxybutyric or lactic or hydrochloric acid be added, there occurs an interchange which diagrammatically may be expressed as follows: Each unit of strong acid combines with sodium of the salts of these weak acids, thereby liberating one unit of weak acid for each unit of strong acid introduced.

Sodium phosphate + hydrochloric acid = sodium chloride + phosphoric acid.

Sodium carbonate + hydrochloric acid = sodium chloride + carbon dioxid.

Thus for every unit of strong acid introduced into the blood there is liberated a unit of weak acid which possesses much less power of altering the reaction of the blood. The capacity of a free acid in a given concentration to alter the reaction of a fluid is dependent upon its ionization. For example, in a decinormal solution of hydrochloric acid, 91 per cent. of the hydrogen is dissociated in aqueous solution at ordinary temperature. Thus: $1000\ HCl = 910\ \overset{+}{H} + 910\ \overset{-}{Cl} + 90\ HCl$; on the other hand, in a decinormal solution of acetic acid only 1.3 per cent. of the reacting hydrogen is dissociated under similar conditions. Thus: $1000\ H(C_2H_3O_2) = 13\ \overset{+}{H} + 13\ \overset{-}{C_2H_3O_2} + 987\ H(C_2H_3O_2)$. Hence, a decinormal solution of hydrochloric acid although containing the same amount of reacting hydrogen as a decinormal solution of acetic acid is seventy times as strong an acid as the acetic acid, because the hydrogen ion concentration of the hydrochloric acid is seventy times as great as that of the acetic acid. The reaction, the color of an indicator added to a solution and in general the properties which we group under the term acidity are dependent upon the hydrogen ion concentration of the solution.

For the purpose of denoting the hydrogen ion concentration or reaction of a solution the notation now generally used in medical work is the logarythmic notation. In this notation the hydrogen ion concentration of a neutral solution is about 7; increasing degrees of acidity are indicated by decreasing figures and increasing degrees of alkalinity by increasing figures. By this notation the reaction of urine ranges, according to Henderson and Palmer,[2] depending upon the character of the diet and other factors between the acid urine 4.7 and the alkaline urine 8.7. As a rule, it lies between 5 and 7.5. The blood possesses a very constant reaction at 7.4.

The first factor, therefore, in maintaining the constancy of reaction of the blood is the presence of considerable amounts of sodium carbonate with carbon dioxid and of sodium phosphate in the

[2] Extreme Variations of the Concentration of Ionized Hydrogen in Human Urine, Jour. Biol. Chem., 1913, xiv, 81.

blood. When acids are added to the blood they combine with this sodium and liberate carbonic and phosphoric acids, both having very low coefficients of dissociation, hence producing a minimal change in the hydrogen ion concentration of the blood. This factor alone is extremely potent in diminishing the changes in the reaction of the blood and body fluids when subjected to the addition of acids or alkalies. The second and third factors are the rapid elimination from the body of these weak acids when so liberated, the phosphoric acid being eliminated by the kidney and the carbonic acid by the lungs. To these weak salts constituted with the weak acids, the sodium phosphate and sodium carbonate, Henderson has given the name "buffer substances," because of their effect in limiting the changes in reaction that would follow the introduction of acids or alkalies into the blood. As a result of their presence in the body fluids and of the rapid elimination by the lungs and kidneys of the carbonic acid and phosphoric acid liberated from them the blood and body fluids always preserve that constancy of reaction essential to life. It must be clear, however, that the continued introduction of acids into the blood will tend to reduce the amount of these buffer substances available, and must invariably do so if their depletion exceeds the body's capacity for replacing them. When such a reduction in the buffer substances of the blood has occurred the condition is that known as "acidosis." Acidosis may be defined as any condition in which the buffer substances of the blood and body fluids are reduced below the normal. The primary effect of such a reduction is a diminution in the capacity of the blood to transport acids or alkalies. The acid most abundantly produced in the body is carbonic acid, and when there is a reduction of the buffer substances of the blood there is a reduction in the blood's capacity for carrying carbonic acid. If this be marked enough there occurs an accumulation of carbonic acid in the tissues, and among other tissues in the respiratory center. As is well known, any increase of the acidity in the respiratory center, such as will be induced by an accumulation of CO_2, serves as stimulant to this center. A more thorough ventilation of the lungs results. A more thorough ventilation of the blood follows, and this favoring the removal of the CO_2 from the tissues, limits its further accumulation. Hence, an equilibrium is established as a result of the response of the respiratory center to the carbon dioxid stimulation, and there is maintained an increased respiratory activity with more thorough ventilation of the lungs. This adjustment gives us one clinical symptom of acidosis, namely, hyperpnea, and one of our laboratory methods for detecting acidosis, namely, the reduction in the carbon dioxid concentration of the alveolar air; the latter, of course, being used as a measure of the thoroughness of pulmonary ventilation. Since the carbon dioxid tension of the alveolar air is, at least under normal

conditions of the lungs, equal to the carbon dioxid tension of the arterial blood leaving the lungs, this increased ventilation of the lungs with lowering of the carbon dioxid tension of the alveolar air leads also to a lowering of the carbon dioxid tension of the arterial blood. It is well to emphasize the distinction between carbon dioxid tension and carbon dioxid content of a fluid or atmosphere. Like any other gas, carbon dioxid, whether in the air or dissolved in fluid, is present at some definite pressure or tension. In air the relation between the percentage of carbon dioxid and its pressure or tension is a simple one. Its pressure bears the same proportion to the total pressure of the air that its volume does to the total volume of the air. Thus, if carbon dioxid constitutes 6 per cent. of a sample of air, and that air is at atmospheric pressure (barometric pressure), which at the time is, let us suppose, 765 mm. of Hg., then the carbon dioxid tension in the sample of air will be 6 per cent. of 765, or 45.9 mm. Hg. The carbon dioxid of the alveolar air may be expressed either in per cent. or in mm. of Hg. tension, usually the latter. The carbon dioxid tension of a fluid is the pressure which the carbon dioxid exerts at the surface of the fluid. It is measured by determining the carbon dioxid which must be maintained in an atmosphere in contact with the fluid in order to keep the carbon dioxid content of the fluid unchanged. If the carbon dioxid tension of the atmosphere contiguous to a fluid is greater than that of the fluid the fluid absorbs carbon dioxid until the tension in each is equal, and *vice versa*. The amount or percentage of carbon dioxid, however, which a fluid must contain to possess a certain carbon dioxid tension varies greatly, depending upon the nature and temperature of the fluid. This is illustrated in the following table, giving the approximate carbon dioxid content and carbon dioxid tension of distilled water, a sample of blood and of serum at 38°. If acid be added to any fluid the carbon dioxid content of the fluid will be decreased for any given carbon dioxid tension. Reduction of the temperature increases the carbon dioxid content for any given carbon dioxid tension.

It is, of course, the carbon dioxid tension, not content, of alveolar air and of arterial blood which are equal. In some of the discussions of this subject it has been stated that in acidosis the carbon dioxid tension of the blood is decreased. This statement is open to criticism. In acidosis the total carbon dioxid content of the blood (carbonate plus free carbon dioxid) is decreased; the amount of CO_2 that the blood can carry at a given CO_2 tension is decreased. As a result of stimulation of the respiratory center the pulmonary ventilation is greater, hence the CO_2 tension of the alveolar air and hence of the arterial blood is lower than normal; but, however much the total carbon dioxid content of the blood, arterial and venous, is reduced following the introduction of acids into the blood, and however much the carbon dioxid tension of

the arterial blood is decreased by the increased respiratory activity, there is no proof that the carbon dioxid tension of the venous blood is decreased; indeed, it is possible that it may even be increased. Adequate studies upon this point are difficult, and are not yet available. Until the last few months only the very indirect method of gauging the existence of acidosis, namely, the determination of the thoroughness of pulmonary ventilation by estimating the carbon dioxid content or tension of the alveolar air, was our best laboratory method for the recognition of acidosis. The technic of this method has been greatly simplified recently by Marriott,[3] so that it may be carried out at the bedside in about five minutes' time and without any complicated apparatus. The value of this method has always been limited, however, first, by the fact that as a guage of the state of the blood it is dependent upon a normal irritability of the respiratory center. A hyperirritability of the respiratory center will maintain an increased pulmonary ventilation and a lowered alveolar CO_2 content without there being any acidosis, and conversely a diminished irritability of the respiratory center may diminish the evidence by this method of an existing acidosis. In the second place an alteration in the pulmonary ventilation may readily occur from unintentional alteration by the patient of his manner or rate of breathing during the time of examination. Moreover, in certain conditions, such as Cheyne-Stokes breathing, the pulmonary ventilation is variable and in pulmonary disease, such as pneumonia, we do not yet know what effect the local disease has upon the relation between the pulmonary ventilation and the state of the blood.

AMOUNT OF CO_2 IN VOLUMES PER CENT. HELD IN DISTILLED WATER, IN A SAMPLE OF WHOLE BLOOD (BOHR) AND IN A SAMPLE OF SERUM (JACQUET) AT THE SAME TEMPERATURE, 38°, AND THE INDICATED CO_2 TENSIONS.

CO_2 tension in mm. Hg.	Volumes per cent. of CO_2 in Water, per cent.	Whole blood, per cent.	Serum, per cent.
15 mm.	1.1	30.5	47.2
30 mm.	2.2	38.9	62.1
50 mm.	3.7	45.3	64.6

Fortunately, for the progress of our knowledge of acidosis, two methods have been given us during the past year which enable us to study directly from the blood itself the "buffer value" of the blood. The first devised by Van Slyke, Stillman, and Cullen[4] is a method for measuring directly by means of a special gas buret the amount of the sodium carbonate buffer in the blood. The

[3] The Determination of Alveolar Carbon Dioxid Tension by a Simple Method, Jour. Am. Med. Assn., 1916, lxvi, 1594.

[4] Nature and Detection of Diabetic Acidosis, Proc. Soc. Exper. Biol. and Med., 1915, xii, p. 165.

second, devised by Levy, Rowntree, and Marriott,[5] is a method for gauging the same factor indirectly by determining the hydrogen ion concentration of a sample of the blood under certain conditions.

The first of these methods has been used by us in a series of clinical cases, and the results of these studies are here presented, together with some considerations concerning certain features of the technic.

The principle of this method is to obtain blood from the patient, oxalte it, separate the plasma and subject the latter to an atmosphere of definite carbon dioxid content or tension, about 6 per cent. (45 mm. tension). When the plasma has been brought into equilibrium with this atmosphere 1 c.c. of the plasma is transferred to a special gas buret, and by means of acid and a vacuum all the CO_2 held as carbonate is liberated and the total CO_2 drawn from the plasma into the vacuum and measured. The total CO_2 content after reduction to 0°, 760 mm. pressure, and correction for vapor tension, may be expressed as volumes per cent. of the original plasma. Thus, 1 c.c. of normal human plasma so treated will yield about 0.70 c.c. of CO_2, or seventy volumes per cent.

Technic. Upon first using this method we employed oxalated plasma obtained in the usual way by centrifuging the oxalated blood in open centrifuge tubes. The results secured were frequently surprising, however, and especially the remarkable variations often observed in repeated examinations of the blood of the same individual at different times. Investigation of the various steps in our procedure showed that these irregularities were due to the fact that the carbon dioxid binding capacity of a plasma is greatly influenced by the carbon dioxid tension present in the whole blood at the moment the cells and plasma are separated. If the carbon dioxid tension of the whole blood be high at the time of the separation into cells and plasma the plasma will have a higher binding capacity for carbon dioxid at any given tension than if the carbon dioxid tension of the whole blood be low at the time of the separation. This relation first pointed out by Zuntz[6] has been subsequently more thoroughly investigated by Guerber[7] and Petry.[8]

By these observers it was shown that when CO_2 escapes from the plasma of whole blood and leaves behind the base chiefly sodium, with which it has been combined, thus increasing the alkalinity of the plasma, a diffusion of hydrochloric acid occurs from the

[5] A Simple Method for Determining Variations in the Hydrogen ion Concentration of the Blood, Arch. Int. Med., 1915, xvi, 389.

[6] Bohr, C., Blutgase und respiratorischer Gaswechsel. In Nagel, W.: Handb. der Physiol. des Menschen, 1905, i, 116. Zuntz: Beiträge zur Physiologie des Blutes, Inaug, Diss., Bonn, 1868.

[7] Ueber den Einfluss der Kohlensäure auf die Verteilung von Basen und Säuren zwischen Serum und Blutkörperchen, Sitzbericht. d. phys. med. Gesellschaft z. Wurzburg, 1895–96, p. 28.

[8] Ueber die Verteilung der Kohlensäure im Blute, Beiträge z. chem. Phys. und Path., Hofmeister, 1902–3, iii, 247.

cells into the plasma to combine with at least a part of this free base. If this plasma is now separated from the cells its combining power for CO_2 is obviously less than that of the original plasma, since a portion of its base originally capable of holding CO_2 is now combined with hydrochloric acid diffused from the cells. When the whole oxalated blood is drawn and centrifuged in the ordinary way in open tubes a variable escape of CO_2 occurs during the process, and consequently from the same portion of blood, plasmas of very different binding capacities may be secured. It is in our experience highly important that either the escape of CO_2 from the blood be prevented from the time the blood is drawn until the removal of the plasma from the cells, or else that the blood be brought to some standard carbon dioxid tension at the time of centrifuging and kept at this tension until the plasma is separated. We are indebted to Dr. Van Slyke[9] for the suggestion of a simple method for securing the former of these two conditions. This consists of drawing the blood directly from the patient's vein through tubing which passes to the bottom of a centrifuge tube containing a few oxalate crystals and which ends beneath a layer of paraffin oil. The oil floating above the blood effectually prevents the escape of CO_2 until the centrifuging is complete and the plasma pipetted off. This paraffin oil method has been our standard method in these studies. In some cases, however, we have also saturated the whole oxalated blood at a tension of 6 per cent. CO_2 and kept it at this tension by stoppering or by covering with paraffin oil during the centrifuging.

To show the importance of this step we obtained in a series of seventeen cases the oxalated plasma from the whole blood in three ways: (1) blood drawn from the vein directly into a centrifuge tube beneath paraffin oil and hence protected from loss of CO_2 until removal of the plasma; (2) blood drawn and oxalated, exposed to the air, but subsequently saturated at 6 per cent. CO_2 tension and kept at this tension during centrifuging; (3) blood drawn, oxalated, and centrifuged in the ordinary way exposed throughout to the air. The plasmas obtained in these three ways were all saturated at 6 per cent. CO_2 tension and analyzed for their CO_2 content by Van Slyke's method. The results are shown in Table I, A. It will be seen that the paraffin oil and the 6 per cent. saturation of the whole blood give closely parallel results, the latter, as a rule, yielding a plasma that holds from 3 to 6 more volumes per cent. of CO_2. On the other hand the exposed blood invariably yields a plasma of lower binding capacity, but in different individuals or in the same individual at different times the resulting plasma may be only one volume per cent. lower, or as much as 24 volumes per cent. lower.

[9] Personal communication.

TABLE I.—PART A.—THE EFFECT OF THE CO_2 TENSION OF THE WHOLE BLOOD UPON THE CO_2 CAPACITY OF THE PLASMA DERIVED FROM IT.

	CO_2 content of plasma saturated at 45 mm. CO_2 tension, the whole blood having been				
Case No.	Kept under paraffin oil.	Saturated at 45 mm. CO_2 at 20° C.		Exposed to air.	
	P.	S.	S–P.	E.	P–E.
1	73	77	4	50	23
2	73	72	–1	51	22
3	70	66	–4	68	2
3	66	68	2	49	17
4	66	70	4	49	17
5	65	68	3	49	16
6	64	73	9	48	16
7	64	68	4	57	7
8	64	68	4	43	21
8	62	68	6	50	12
9	62	68	6	59	3
10	60	64	4	55	5
11	59	62	3	38	21
12	53	59	6	43	10
13	52	58	6	44	8
14	51	49	–2	38	13
14	46	48	2	45	1

If the oxalated blood before centrifuging be thoroughly aerated by being poured from beaker to beaker for five minutes the resulting plasma exhibits a still lower CO_2 capacity, as shown in Table I, B.

PART B.

Case No.	Kept under paraffin oil. P.	Aerated 5 minutes A.	P–A.
15	70	48	22
16	70	59	11
17	66	47	19
18	65	46	19
19	64	49	15
20	61	44	17
21	58	51	7

At first sight it might seem surprising that saturation of the whole blood at 6 per cent. (45 mm.) CO_2 tension should increase the CO_2 capacity of the plasma as compared with the blood as drawn from the vein under paraffin oil. That this occurs is due to the fact that the saturation of the whole blood at 45 mm. tension was carried out at room temperature (18° to 20° C.), and the blood will, as a rule, hold more CO_2 at 18° C. at 45 mm. tension than at the same or higher tension that exists in the veins at 37° C.

Effects of Cyanosis. In considering the relative merits of the paraffin oil method and the 6 per cent. saturation of the whole blood in the study of acidosis it will be recognized that most factors will alter the plasma in the same direction and about equally whichever of these methods be used. One conspicuous exception to this relation exists, however, namely, any alteration in the CO_2 tension

of the venous blood as drawn, such, for example, as occurs in asphyxia or cyanosis. This may be seen in the experiments shown in Table II.

TABLE II.—EFFECT OF INJECTION OF ACID, ALKALI, AND OF ASPHYXIA ON THE CO_2 CAPACITY OF THE PLASMA.

Dog No.	Blood drawn after:	CO_2 content of plasma saturated at 45 mm. CO_2 tension, the whole blood having been Kept under paraffin oil. P.	Saturated at 45 mm. CO_2 at 20° C. S.	S– P.
1	Control period . . .	54	56	2
	KH_2PO_4	47	52	5
	Asphyxia	48	46	–2
	Na_2CO_3	67	70	
2	Control period . . .	52	62	10
	Asphyxia	55	58	3
	KH_2PO_4	48	58	10

These experiments were performed to show the effect upon the CO_2 binding capacity of the plasma of injection of alkalies, of injection of acids, and of increase in the CO_2 tension of the venous blood from asphyxia.

Experiment I. A normal dog, weighing 10 kilos, was etherized; 10 c.c. of blood drawn and oxalated under paraffin oil from the right jugular vein and immediately a second portion of 10 c.c. drawn and oxalated exposed to the air for subsequent saturation as whole blood at 6 per cent. CO_2 (control blood). There was then injected in the course of fifteen minutes 150 c.c. of KH_2PO_4 solution (13.6 gms. per liter) into the left femoral vein. Two portions of blood were immediately taken from the right jugular vein as before (KH_2PO_4 blood). The trachea was then compressed until the tongue was deeply cyanosed and two more portions of blood taken as before from the right jugular vein (asphyxia blood). After a few minutes' interval 150 c.c. of 3 per cent. Na_2CO_3 solution was injected in fifteen minutes into the left femoral vein and two portions of blood taken as before from the right jugular vein (Na_2CO_3 blood). The injection of acid phosphate reduced the CO_2 capacity of the plasma obtained both by the paraffin oil and by the 6 per cent. saturation method. Asphyxia still further reduced the CO_2 capacity of the plasma by the 6 per cent. saturation method, but slightly increased that of the plasma from the paraffin oil method. Thus a slight acidosis of asphyxia was wholly obscured by the increased CO_2 tension of the venous blood when the plasma was obtained by the paraffin oil method.

Experiment II. This experiment is identical, except that the asphyxia was performed before the injection of the acid phosphate. The results are similar, but perhaps even more striking. Whether this effect of asphyxia upon the plasma obtained by the paraffin oil method would ever be of clinical importance is not certain, but in a very cyanotic patient it is possible.

Van Slyke Method in Clinical Cases. A series of clinical cases chosen more or less at random have been studied by the Van Slyke method, using the paraffin oil method for obtaining the plasma. The results are shown in Table III. Throughout this study all figures are the volumes of CO_2 per cent. reduced to 0° C., 760 mm. and corrected for vapor tension. It has seemed to us that the normal limits by this method may be considered as lying between 65 and 80 volumes per cent. Between 55 and 65 volumes per cent. the patients have been, as a rule, mildly nephritic, mildly diabetic, or markedly arteriosclerotic, and might, therefore, be expected to exhibit the slightest grade of acidosis. Below 55 the patients have been for the most part advanced nephritics, except for one moderately severe diabetic and one quite septic case.

Comparison of Three Methods. In a series of cases we have compared the carbon dioxid capacity of the plasma obtained under paraffin oil, the alveolar air, using the Plesch-Higgins method[10] and the hydrogen ion concentration of the serum by the dialysis method of Levy, Rowntree, and Marriott after blowing off the free CO_2 from the dialysate as recently suggested by Marriott. The results are shown in Table IV. In general the results agree, but the method of Van Slyke is distinctly the most sensitive of the three and gives much more perfect duplicates than does the method of alveolar air.

Van Slyke Method Following Anesthesia. The Van Slyke method has been applied to the study of a few cases following nitrous oxide-ether anesthesia. The results are shown in Table V. It will be seen that after from thirty to one hundred and fifteen minutes, ether anesthesia, a lowering of the CO_2 capacity of the plasma, was constantly observed. The degree to which it was lowered was, in general, proportional to the duration of the anesthesia. In a thirty minutes' anesthesia the lowering was only two to four volumes per cent. while in an anesthesia of one hundred and five minutes it was reduced to forty-nine volumes per cent., about fifteen below the normal. The reduction is apparently at or near its maximum at the close of the anesthesia and exhibits no marked changes in either direction for the next four or five hours, perhaps for twenty-four hours. The time required for return to normal has not been determined.

In eight of ten cases in which the urine was studied, acetone was studied by the sodium nitroprusside test in the first or second twenty-four hours after operation. The ferric chloride test was positive once. Even when the acetone test was strongly positive, however, the total ketone bodies were never present in more than very small amounts, the largest amount excreted in twenty-four hours being 0.83 mgm. expressed as acetone (Shaffer's method). The total acid output by Henderson's and Palmer's method was normal.

TABLE III.—CLINICAL CASES. CO_2 CONTENT OF PLASMA (BLOOD TAKEN UNDER PARAFFIN OIL).

No.	Diagnosis.	Age.	Date.	Plasma CO_2.	Alveolar CO_2.	Blood-pressure.	Blood urea, nitrogen.	Ketonuria gms. per 24 hours.
	Controls:							
1	Headache	.....	.....	78				
2	Carcinoma of lip	.....	.....	78				
3	Sprain	.....	.....	71				
4	Angioneurotic edema	.....	.....	71				
5	Sarcoma of leg	.....	.....	71				
6	Papilloma of bladder	.....	.....	70				
7	Pneumonia	.....	.....	69				
8	Varicocele	.....	.....	65				
9	Hemorrhoids	.....	.....	65				
10	Fracture of arm	.....	.....	65				
11	Myoma uteri	.....	.....	65				
12	Gastric neurosis	.....	Feb. 22	70				
	Gastric neurosis	.....	Feb. 26	64				
	Gastric neurosis	.....	Feb. 28	66				
13	Endothelioma	.....	Feb. 5	63				
	Endothelioma	.....	Feb. 8	65				
	Arteriosclerotics:							
14	Arteriosclerosis	57 years	.....	68	..	142–85	14	
15	Arteriosclerosis	60 years	.....	66	..	150–85		
16	Arteriosclerosis	49 years	.....	66				
17	Arteriosclerosis	59 years	Feb. 27	65	..	170–120	17	
			Mar. 9	71	..	170–120	29	
18	Arteriosclerosis	63 years	Mar. 16	61	..	230–130		
			Mar. 29	63	..	230–130		
19	Arteriosclerosis	56 years	.....	62	..	220–135	14	
20	Cerebral hemorrhage	.....	.....	60				
21	Atrophic cirrhosis	56 years	.....	57	..	160–120	13	
	Nephritics:							
22	Early nephritis	36 years	.....	72	..	176–110	17	
23	Chronic nephritis	42 years	.....	64	..	.....	33	
24	Early nephritis	24 years	.....	62	..	198–150	14	
25	Parenchymatous nephritis	21 years	Apr. 17	61	..	150–110	17	
			Apr. 19	63	..	150–110		
			May 6	64	..	150–110	16	
			May 15	69	48	135–90		
26	Chronic nephritis	15 years	.....	59	..	165–115	23	
27	Chronic nephritis	45 years	.....	59	..	185–135	18	
28	Acute nephritis	30 years	Apr. 12	53	30	115–65	35	
			Apr. 27	77	55	115–65	13	
29	Chronic nephritis	26 years	.....	53	..	140–100	21	
30	Advanced nephritis	44 years	Feb. 25	51	..	180–140	67	
			Mar. 4	46	..	180–140	49	
			Mar. 8	46	..	180–140	51	
31	Advanced nephritis	39 years	Apr. 17	48				
			Apr. 27	55				
			May 6	48	..	.....	116	
			May 12	46	35	.....	119	
32	Advanced nephritis	55 years	.....	37	33	190–100	142	
33	Advanced nephritis	56 years	.....	35	25	158–80	122	
34	Advanced nephritis	34 years	Mar. 27	40	..	178–115	195	
			Mar. 29	33	..	178–115		
	Mercurial nephritis:							
35	Bichloride poisoning (severe)	21 years	June 6	70	..	120–75	16	
			June 7	70	..	120–75	20	
			June 15	71	..	120–60	17	
36	Bichloride poisoning (mild)	24 years	June 12	70	..	115–70	14	
			June 15	70	..	120–75	13	
	Eclampsia:							
37	Eclampsia	.....	.....	57	..	.....	21	
38	Eclampsia	.....	.....	52	..	.....	29	
39	Eclampsia	.....	.....	52	35	.....	20	
40	Eclampsia	.....	.....	48	..	.....	13	
41	Eclampsia	.....	Mar. 28	46				
			Mar. 30	57	..	.....	14	
	Septic:							
42	Pelvic inflammation	32 years	Feb. 29	52	..	195–120	12	
			Mar. 27	63				
	Diabetes:							
43	Mild	48 years	.....	71	..	.....	...	2.0
44	Mild	25 years	.....	64	..	.....	...	0.4
45	Mild	51 years	.....	60	..	.....	...	2.0
46	Mild	35 years	Mar. 8	52	..	.....	...	4.6
			Mar. 29	64	..	.....	...	0.4

The blood urea nitrogen estimations in the table were performed by the urease method (Van Slyke and Cullen); the urinary ketones by Schaffer's method.

TABLE IV.—COMPARISON OF CO_2 CONTENT OF PLASMA, CO_2 TENSION OF ALVEOLAR AIR AND HYDROGEN ION CONCENTRATION OF SERUM AFTER AËRATION OF DIALYSATE.

Case No.	CO_2 content plasma.	CO_2 tension alveolar.	Hydrogen ion of serum.
1	77	44	8.1
2	72	50	8.0
3	69	48	8.0
4	67	43	8.0–
5	65	50	7.9
6	64	39	8.0
7	63	41	8.0
8	62	38	8.1
9	58	44	8.1
10	52	40	8.0
11	52	35	7.9
12	46	35	7.9
13	37	33	7.7

TABLE V.—EFFECT OF ETHER ANESTHESIA ON CO_2 CONTENT OF PLASMA.

Case No.	Operation.	Age.	Duration of anesthesia, minutes.	CO_2 content: Before.	After.
1	Carcinoma of lip	72	30	73	69
2	Amputation of leg	17	30	70	66
3	Amputation of leg	30	30	65	63
4	Herniorrhapy	20	35	..	64
5	Hemorrhoids	19	35	65	59
6	Perineal, plastic	51	40	..	56
7	Nephrotomy	30	50	..	60
8	Ectopic pregnancy	35	50	..	53[10]
9	Nephrotomy	48	70	..	59
10	Perineal, plastic	46	85	..	47[10]
11	Suprapubic cystotomy	24	90	70	60
12	Pan hysterectomy	56	100	..	53[10]
13	Pan hysterectomy	58	105	..	51
14	Pan hysterectomy	19	105	..	49[10]
15	Pan hysterectomy	35	110	65	57
16	Gastrojejunostomy	39	115	..	56

Four of the patients received just at the close of the anesthesia two pints each of 5 per cent. glucose solution per rectum. These cases showed quite as marked a reduction of the carbonate content of the plasma as did comparable cases not receiving the glucose.

The cause of acidosis is different in different conditions. In diabetes it is due largely to the presence in the blood of the ketone acids. In nephritis and severe diarrhea it is probably, according to Howland and Marriott,[11] due to an impaired capacity on the part of the kidneys to excrete phosphoric acid. For other types the cause of the acidosis is not known. That the clinical symptomatology varies somewhat with the type and cause of the acidosis is apparently true. Whether the treatment of acidosis, *per se*,

[10] Received glucose per rectum at close of anesthesia.

[11] Acidosis Occurring with Diarrhea, Am. Jour. Dis. of Child., 1916, xi, 309.

apart from its cause will be of importance remains to be determined. Henderson has urged that in any conditions associated with reduction in the buffer value of the blood, sodium bicarbonate be given by mouth to the point of rendering the urine less acid, but not distinctly alkaline. Using this simple criterion one may endeavor to replenish the supply of buffer substances in the blood and yet avoid overtaxing the system with excessive alkali. In this connection attention may be called to the suggestion of Magnus-Levy[12] that in giving sodium carbonate solutions intravenously for the treatment of severe acidosis the injection of a highly alkaline solution may well be a severe insult to the system. He suggested that a safer plan is to pass CO_2 gas through the sterile sodium carbonate solution, to which a drop of phenolphthalein has been added, until the solution is colorless, when it becomes more closely analogous to the normal sodium carbonate-carbon dioxid buffer of the blood —the reaction of which is nearly neutral—the substance which one is aiming by such injections to replace.

Conclusions. 1. In the new methods for studying acidosis directly from the blood we have a means of investigation that constitutes a distinct advance upon our previous methods.

2. As criteria of the supply of "buffer substance" in the blood the carbon dioxid capacity of the plasma (Van Slyke, Stillman, and Cullen method) the hydrogen ion concentration of the serum (Levy, Rowntree, and Marriott method) and the alveolar air (Plesch-Higgins method) give results that are in general parallel. The first of these is the most sensitive of the three and gives much more satisfactory duplicates than does the alveolar air determination. It affords a simple and quick method of determining the presence and degree of acidosis.

3. In using the method for the CO_2 capacity of the plasma, and presumably in any method intended to measure directly or indirectly the alkalinity of the plasma, the CO_2 concentration of the whole blood must be kept unchanged or brought to a standard tension while centrifuging and separating the plasma from the cells.

4. Asphyxia or any condition of high CO_2 tension in the blood *in vivo* raises the CO_2 capacity of the plasma if the latter is separated by the paraffin oil method, and may interfere with the recognition of a slight acidosis. This may be overcome by saturating the whole blood at a standard CO_2 tension before centrifuging and maintaining this tension until the plasma is pipetted off.

5. By the Van Slyke method the normal CO_2 capacity of the plasma reduced to 0° 760 mm. pressure and correcting for vapor tension appears to be about sixty-five to eighty volumes per cent. This is slightly reduced in arteriosclerotic conditions and moderately

[12] Ueber subkutane Infusionen von Mononatriumkarbonat, Therap. Monatsch., 1913, xxvii, 838.

to markedly reduced in diabetes and nephritis, especially in the advanced stage.

6. After ether anesthesia there is a depression of the CO_2 capacity of the plasma of from two to twenty volumes per cent. This depression is proportional to the duration of the anesthesia. The lowest figure observed was 47. This reduction is present and probably maximal at the close of the anesthesia, and apparently remains little altered for at least five hours. A single injection of two pints of a 5 per cent. glucose solution per rectum at the close of the anesthesia does not lessen the reduction in the CO_2 capacity during the next five hours.

BLOOD CHANGES IN ALBINO RATS FOLLOWING REMOVAL OF THE SPLEEN *

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INTRODUCTORY

About a year ago, during the course of studies in the albino rat (*Mus norvegicus-albinus*) carried on under the direction of Dr. Alfred Stengel, on the relation of the spleen to the other glands of internal secretion, it was thought worth while to note incidentally any blood changes that might occur after splenectomy, since no references to such observations on the rat could be found in the literature. Among the first observations a few unusual and interesting phenomena were noted, which seemed to make a somewhat systematic study worth while.

A fairly complete bibliography of the literature up to 1914 of the blood changes after splenectomy may be found among the references given by Meyer.[1] The most important work since that time is that of Pearce and his collaborators.[2]

Observations on man, dogs, rabbits, goats, sheep and other animals have shown that removal of the spleen is usually followed by certain alterations in the blood. The time of onset, the extent of the changes and the period of recovery are highly variable. This variability applies not only to the results of different investigators, but also to the data obtained from single series, in which the experimental conditions may be considered fairly comparable. The most important of the blood changes may be summed up as follows:

1. Slight to moderate anemia, variable in time of onset, but usually within the first three or four weeks after splenectomy. Recovery from this anemia is usually complete within a period varying from a few weeks to a few months.

2. Marked rise in leukocyte count followed by a gradual decline. A slight leukocytosis with excess of lymphocytes and eosinophils tends to persist for a long time.

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1. Meyer, A.: Centralbl. f. d. Grenzgeb. d. Med. u. Chir., 1914, **18**, 41.

2. Pearce and Collaborators: The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice, numerous papers, Jour. Exper. Med., 1912-1916.

3. Increased resistance of erythrocytes to hemolytic agents, such as hypotonic salt solution and hemolytic immune serums.

None of the theories offered in the attempt to explain the cause of the anemia of splenectomy have proved adequate. Krumbhaar, Musser and Pearce,[3] as a result of their studies on blood regeneration following bleeding and the administration of hemolytic agents to splenectomized dogs, conclude that the anemia of splenectomy is caused by some factor, as yet unknown, operating in the absence of the spleen.

MATERIAL AND METHODS

All litters of rats used in this series were carefully selected from the colony kept at the Wistar Institute of Anatomy and Biology. The animals were kept in the animal house of the Wistar Institute during the course of the work. For these privileges I am indebted to Prof. H. H. Donaldson. I am further indebted to Professor Donaldson for many helpful suggestions during the course of the work.

The ages of the animals at the time of operation varied between 30 and 70 days, averaging about 45 days. In every experiment parallel observations were made on the splenectomized animal and a control of the same sex and from the same litter. Animals operated on and controls were always kept in the same cage. Operations were performed under ether anesthesia. Splenectomy in the rat requires only three to four minutes for its performance. There is practically no loss of blood. Recovery takes place promptly; within an hour the rat will run about the cage and by the next morning is apparently quite as active as the control. No wound infection, peritonitis or evidences of internal hemorrhage were discovered in the series.

The blood for cell counts, hemoglobin estimations and study of reticulated cells was obtained by snipping the end of the tail with a pair of sharp scissors. A fairly free oozing of blood is required to obtain accurate cell counts and hemoglobin estimations. The Fleischl hemometer was used throughout. For the study of reticulated cells brilliant cresyl blue stain was employed, according to the usual technic.

BLOOD CHANGES FOLLOWING REMOVAL OF NORMAL SPLEENS

The results of observations on red blood cell count, hemoglobin and reticulated cells are given in Table 1. The results are somewhat variable. Most splenectomized animals show a transient drop in cell count and hemoglobin, reaching its maximum in from one to three weeks. Nearly all appear to have recovered completely by the end of the fourth week after operation. Three out of sixteen show no evidence of anemia, but it is possible that more frequent blood counts

3. Krumbhaar, E. B.; Musser, J. H., Jr., and Pearce, R. M.: Jour. Exper. Med., 1913, **18,** 665.

TABLE 1.—Studies of Red Blood Cell Count, Percentage of Hemoglobin and Percentage of Reticulated Cells in Rats with Normal Spleens

Rat No.	Days After Splenectomy	Red Blood Cell Count in Millions		Hemoglobin Percentage		Percentage of Reticulated Red Cells	
		Operated	Control	Operated	Control	Operated	Control
54 and control.....	Preliminary	7.6	7.3	85	78	2.8	7.2
	3	7.1	7.3	80	82	4.6	10.4
	8	5.7	7.2	69	85	2.8	1.0
	16	6.5	8.1	71	82	4.0	8.0
	29	8.2	8.2	80	82	3.4	4.0
	45	7.2	8.7	78	94	8.6	5.2
55 control same as for 54	Preliminary	7.3	....	82	...	3.4	....
	3	7.0	....	81	...	2.4	....
	8	6.0	....	67	...	1.4	....
	16	7.6	....	77	...	16.0	....
	29	8.1	....	84	...	0.6	....
	45	7.4	....	82	...	3.2	....
57 and control.....	Preliminary	9.2	9.2	92	86	2.0	4.2
	14	7.9	8.0	88	87	....	....
	28	7.6	8.3	72	84	1.0	3.2
	44	9.9	8.3	87	84	0.8	1.6
49 and control.....	Preliminary	8.5	9.2	88	91	0.1	0.1
	3	8.2	....	73	...	2.0	2.0
	6	7.7	8.6	79	84	3.4	1.2
	20	8.0	7.6	90	86	0.2	1.8
	34	8.5	7.9	90	84	1.4	1.6
	50	9.4	9.7	96	90	0.2	1.4
	141	10.3	10.7	84	92	0.8	0.6
	254	9.1	8.8	99	104	0.6	0.5
60 and control.....	Preliminary	8.9	8.4	86	90	0.	0.2
	5	9.1	8.8	88	93	1.2	1.2
	13	8.9	8.4	85	87	1.2	2.0
	28	8.0	9.2	84	86	1.4	1.0
	231	9.2	9.4	91	82	1.8	2.2
64 and control same as for 60	Preliminary	8.9	....	90	...	0.2	....
	5	8.4	....	74	...	0.5	....
	13	8.2	....	83	...	3.4	....
	28	8.1	....	82	...	0.4	....
	231	9.7	....	95	...	1.4	....
7 and control.....	Preliminary	8.8	9.0	94	93	....	....
	7	7.6	7.9	76	84	....	....
	14	7.8	9.2	69	86	....	....
	21	7.3	7.8	64	86	....	....
	30	8.7	7.6	88	86	....	....

TABLE 1.—Studies of Red Blood Cell Count, Percentage of Hemoglobin and Percentage of Reticulated Cells in Rats with Normal Spleens—(*Continued*)

Rat No.	Days After Splenectomy	Red Blood Cell Count in Millions		Hemoglobin Percentage		Percentage of Reticulated Red Cells	
		Operated	Control	Operated	Control	Operated	Control
10 and control.....	Preliminary	8.5	8.8	94	94	....	....
	6	7.4	7.3	81	88	....	....
	13	7.6	8.2	68	86	....	....
	20	7.7	7.6	75	86	....	....
	29	7.4	8.7	73	88	....	....
19 and control.....	Preliminary	8.6	8.8	86	96	....	....
	7	6.9	7.8	66	82	....	....
	20	6.9	7.5	75	86	....	....
	30	7.2	8.6	70	94	....	....
22 and control.....	Preliminary	9.4	9.7	94	92	....	....
	7	7.4	7.8	78	82	....	....
	20	6.9	7.5	69	80	....	....
	30	6.9	7.5	72	94	....	....
30 and control.....	Preliminary	8.2	....	88	...	....	....
	3	8.5	8.1	78	80	....	....
	13	8.4	8.8	85	95	....	....
	47	9.5	9.9	85	95	....	....
32 and control.....	Preliminary	8.3	....	90	...	....	....
	3	8.4	8.7	90	96	....	....
	13	7.8	8.1	82	90	....	....
	25	8.1	8.2	82	84	....	....
	32	8.9	8.2	84	87	....	....
40 and control.....	Preliminary	7.8	8.0	84	90	....	....
	7	7.3	7.3	84	81	....	....
	14	7.9	7.6	80	79	....	....
	22	7.9	7.9	75	92	....	....
	31	8.2	7 4	88	81	....	....
41 same control as for 40	Preliminary	7.7	....	90	...	....	....
	7	7.9	....	85	...	....	....
	14	7.9	....	86	...	....	....
	22	7.6	....	81	...	....	....
	31	8.9	....	86	...	....	....
70 and control.....	Preliminary	8.1	8.3	85	84	0.8	1.0
	3	7.0	9.8	74	92	8.0	....
	13	7.9	8.1	64	82	2.8	0.8
	25	8.7	8.6	91	90	....	....
	230	9.4	9.3	96	97	1.2	0.8
71 and control.....	Preliminary	7.8	8.6	94	94	1.6	0.8
	3	9.7	9.8	89	90	....	....
	13	7.9	7.7	75	82	2.6	1.4
	25	9 1	8.2	85	93	....	....
	230	8.8	9.1	110	102	3.0	1.0

might have disclosed a slight degree. The drop in red cells is slight, but the hemoglobin percentage shows a distinct change. The average of fifty-eight examinations in the first five weeks after splenectomy is 7,850,000 red cells and 79.2 per cent. hemoglobin; the average of forty-five readings in controls is 8,160,000 red cells and 86.6 per cent. hemoglobin. The average of the lowest readings in sixteen animals operated on is 7,350,000 red cells and 72.9 per cent. hemoglobin, the average of the lowest readings in thirteen controls is 7,740,000 red cells and 82.1 per cent. hemoglobin. The tendency to slightly more marked decrease in hemoglobin than red cells was found in dogs by Musser and Krumbhaar.[4] Five rats examined about eight months after splenectomy showed normal cell counts and hemoglobin.

The leukocyte counts in the albino rat show such variations that conclusions regarding possible changes produced by splenectomy are difficult to draw. There seems to be a tendency toward development of slight leukocytosis.

Examinations of stained specimens of blood from the young rat frequently show a few nucleated red cells. During the period of anemia following splenectomy they are sometimes found in fairly large numbers, as many as five normoblasts to one leukocyte.

The normal variation in percentage of reticulated cells in young rats was found to be large; some showed as high as 16 per cent. In eight adult animals 3 per cent. was the highest number found. No changes that could be attributed to the splenectomy were discovered.

RESISTANCE OF ERYTHROCYTES TO HYPOTONIC SALT SOLUTION

Because of the extremely rapid clotting time of rat blood, a slight modification of technic was deemed advisable.

Sodium citrate in 1.5 per cent. solution, just sufficient to prevent coagulation, was added to the blood immediately after its withdrawal. Two small drops of the citrated blood were added to each of seventeen tubes containing graduated concentrations of salt solution, beginning with 0.2 per cent. and increasing by 0.025 per cent. up to 0.6 per cent. The tubes were incubated for two hours, then placed in the ice-box for about sixteen hours, after which the readings were made. The first tinge of red visible throughout the entire solution was considered beginning hemolysis; the first tube showing no distinct red sediment was considered as showing complete hemolysis.

The blood of nine splenectomized animals tested four to five weeks after operation (Table 2) showed in every case markedly greater resistance to hypotonic salt solution than that of controls. Tests made

4. Musser, J. H., Jr., and Krumbhaar, E. B.: Jour. Exper. Med., 1913, **18,** 487.

about eight months after operation yielded somewhat different results. In one case resistance in animals operated on and control was identical. In four other animals operated on the increase of resistance over that of the control animals was distinctly less marked than that observed in the earlier period. These few studies seem to indicate a tendency to eventual loss of the increased resistance.

TABLE 2.—Resistance of Red Cells to Hypotonic Salt Solutions

Rat No.	Days After Splenectomy	Hemolysis Begins		Hemolysis Complete	
		Operated	Control	Operated	Control
1	30	0.400	.....	0.250	.....
3	..	.....	0.450	.....	0.350
4	30	0.400	.....	0.250	.....
5	..	.....	0.475	.....	0.300
7	30	0.400	.....	0.250	.....
8	..	.....	0.475	.....	0.300
19	29	0.425	.....	0.275	.....
20	..	.....	0.500	.....	0.375
32	34	0.425	.....	0.250	.....
33	..	.....	0.475	.....	0.300
36	34	0.450	.....	0.300	.....
37	..	.....	0.475	.....	0.350
38	35	0.425	.....	0.275	.....
39	..	.....	0.475	.....	0.325
40	35	0.350	.....	0.250	.....
41	35	0.400	.....	0.275	.....
42	..	.....	0.450	.....	0.325
49	254	0.450	.....	0.225	.....
50	..	.....	0.475	.....	0.275
60	231	0.450	.....	0.275	.....
64	231	0.425	.....	0.275	.....
62	..	.....	0.500	.....	0.300
70	230	0.500	.....	0.325	.....
71	230	0.500	.....	0.350	.....
72	..	.....	0.500	.....	0.375
73	..	.....	0.500	.....	0.375

BLOOD CHANGES FOLLOWING REMOVAL OF ENLARGED SPLEENS

Many investigators have observed the occurrence of enlarged spleen in albino rats. The cause of this enlargement, according to Hatai,[5] has never been determined. Hatai found also that a heavy

5. Hatai, S.: Am. Jour. Anat., 1913, **15,** 87.

liver was likely to be associated with the heavy spleen. Professor Donaldson[6] has observed that otherwise apparently healthy rats kept out in the cold during the winter showed enlarged spleens.

During the course of this investigation eight rats with enlarged spleens were operated on and the spleens removed. These eight rats all came from three litters. The general appearance, nutrition, weight and preliminary blood count in each case were within normal limits and corresponded with the data obtained from the other animals in the same litters. In two litters all the rats were slightly above the average weight for their age. In the third, all, including those with normal as well as those with enlarged spleens, were slightly below the average. No means were found of distinguishing the rat with enlarged spleen from the normal animal until the spleen itself was exposed to view.

The gross appearance of the organ is characteristic. It is large, soft, with somewhat rounded edges. The color is dark, reddish blue. Microscopically there is found engorgement with blood and moderate hyperplasia of the lymphoid and endothelial cells. No evidences of inflammation or excessive hemolysis were observed.

In view of these observations it was thought possible that the enlargement might be of the nature of a functional hypertrophy, either in response to a demand for increased splenic function to compensate for lessened or increased activity on the part of functionally correlated organs or tissues, or as part of a protective mechanism invoked by the body against infection or the products of infection.

The data obtained in seven of these rats by examination of the blood is given in Table 3. In six of the seven the onset of an extremely rapid and severe anemia occurred in from three to five days. In Rat 25 the onset of the anemia was less rapid. It was not observed until the twentieth day after operation. Rat 47 died two days after splenectomy, before any study of the blood had been made. Examination, however, showed intense pallor and distinct icteroid tinge of tissues.

Of the seven rats studied, all showed percentage of hemoglobin under 25 and red blood cell counts under 2,540,000. Six showed red blood cell counts under 2,000,000. These findings were in striking contrast to what was observed in sixteen rats from which presumably normal spleens were removed, in which the lowest hemoglobin estimation was 65 per cent. and the lowest red blood cell count 5,700,000. The leukocyte counts also showed differences. As stated before, while the removal of the normal spleen is followed by a tendency to

6. Donaldson: Personal communication to the author.

TABLE 3.—Studies of Red and White Cell Counts, Hemoglobin, Reticulated and Nucleated Red Cells of Rats with Enlarged Spleens

Rat No.	Days After Splenectomy	Red Blood Cell Count in Millions		Percentage of Hemoglobin		Leukocyte Count in Thousands		Per Cent. Reticulated Red Cells		Nucleated Red Cells	
		Operated	Control	Operated	Control	Operated	Control	Operated	Control	Operated	Control
45 and control...	Preliminary	7.7	7.6	87	83	14.6	13.6	....	...	....*	..
	1	7.8	7.5	91	86	10.8	9.4	....	...	....	..
	2	8.2	7.1	91	88	28.6	20.2	....	...	....	..
	3	7.3	7.6	77	90	23.6	11.2	....	...	....	..
	4	1.8	7.4	24	85	20.4	9.6	....	...	Mod. No.	..
	5	1.9	8.2	25	84	36.8	15.2	....	...	Mod. No.	..
	12	3.9	7.4	53	81	44.6	12.2	....	...	Mod. No.	..
	20	5.0	8.1	77	86	15.4	12.0	....	...	Mod. No.	..
	28	1.8	8.0	29	90	36.2	16.2	65	1.4	Mod. No.	..
	37	7.9	8.3	94	85	34.4	23.0	15	8.0	Mod. No.	..
	43	Died	...	..	..	....	....	....	...	....	..
43 and control, same as for 45	Preliminary	7.6	...	83	..	15.0	....	....	...	....	..
	1	7.3	...	76	..	15.0	....	....	...	....	..
	2	6.8	...	76	..	29.8	....	....	...	....	..
	3	1.9	...	29	..	52.8	....	....	...	3	..
	4	Died	...	..	..	....	....	....	...	....	..
44 control, same as 45	Preliminary	7.4	...	86	..	14.0	....	....	...	....	..
	1	7.6	...	88	..	11.6	....	....	...	....	..
	2	6.6	...	76	..	22.0	....	....	...	....	..
	3	6.2	...	72	..	23.0	....	....	...	....	..
	4	1.6	...	21	..	28.0	....	....	...	....	..
	5	2.2	...	23	..	24.6	....	....	...	....	..
	12	6.2	...	72	..	13.8	....	....	...	....	..
	18	Died	...	..	..	....	....	....	...	....	..
53.....	Preliminary	8.4	...	82	..	16.0	....	0.6	...	....	..
	3	4.7	...	46	..	47.6	....	92	...	Few	..
	4	1.3	...	19	..	....	....	89	...	....	..
	4	Died	...	..	..	....	....	....	...	....	..
52.....	Preliminary	8.5	...	100	..	26.2	....	4.5	...	....	..
	3	2.5	...	26	..	49.6	....	84	...	Mod.	..
	4	Died	...	..	..	....	....	....	...	....	..

TABLE 3.—Studies of Red and White Cell Counts, Hemoglobin, Reticulated and Nucleated Red Cells of Rats with Enlarged Spleens—(*Continued*)

Rat No.	Days After Splenectomy	Red Blood Cell Count in Millions		Percentage of Hemoglobin		Leukocyte Count in Thousands		Per Cent. Reticulated Red Cells		Nucleated Red Cells	
		Operated	Control	Operated	Control	Operated	Control	Operated	Control	Operated	Control
25 and control...	Preliminary	8.9	7.9	86	88	8.6	12.4	....	...	....	..
	6	6.7	7.3	68	84	15.2	19.0	....	...	....	..
	13	7.8	7.7	77	88	21.4	14.4	....	...	....	..
	20	1.9	8.2	22	83	22.2	10.2	....	...	Many	..
	27	2.0	9.7	38	90	28.4	9.0	....	...	Many	..
	34	2.9	8.2	52	87	34.8	11.6	....	...	Many	..
	41	2.1	8.2	23	88	17.8	17.0	....	...	Many	..
	48	3.5	9.0	60	87	29.8	15.0	....	...	....	..
	55	3.3	8.5	47	92	22.4	12.0	....	...	Mod. No.	..
	62	4.6	8.8	55	90	11.2	15.0	....	...	....	..
	70	3.7	9.6	52	92	64.6	10.8	52.0	3.2	....	..
	71	Died	...	..	..	....	....	....	...	....	..
28 and control...	Preliminary	8.6	9.5	94	92	10.0	9.8	....	...	....	..
	5	1.9	8.0	24	82	46.4	12.6	....	...	Few	2
	12	2.1	7.4	25	88	12.2	12.4	....	...	Mod.	..
	19	3.3	8.1	52	85	36.2	12.8	....	...	Many	..
	26	1.8	7.7	35	81	33.2	13.2	....	...	Many	..
	33	2.8	7.6	51	89	19.8	12.8	....	...	Few	..
	40	3.6	8.5	49	89	20.8	13.4	....	...	....	..
	47	8.7	8.2	75	89	31.6	10.2	....	...	....	..
	54	3.9	8.5	55	84	19.8	14.0	....	...	Few	..
	61	5.6	8.2	70	88	17.0	18.4	....	...	....	..
	79	6.0	8.0	75	84	10.6	10.4	38.0	1.4	....	..
	78	8.1	9.2	95	102	21.4	10.6	4.3	1.4	....	..
	84	7.3	8.1	85	94	....	11.8	6.2	5.0	....	..
	111	5.6	9.9	74	86	....	....	41.0	4.0	....	..
	125	8.4	8.9	86	88	....	....	3.0	2.8	....	..
	216	9.3	9.2	83	87	20.2	16.1	0.8	5.0	....	..
	Killed	...	...	..	..	....	....	....	...	....	..

* Where no mention is made of nucleated red cells, it signifies that none were seen during a differential count of 300 leukocytes.

leukocytosis, the changes are not very marked. In this series there is a marked rise in the leukocyte count so that counts of 40,000 to 50,000 are not unusual.

Nucleated red cells were observed frequently, and occasionally in large numbers during the periods of anemia. They tended to disappear as the blood count returned to normal. A few studies of reticulated red cells showed extraordinary rises during the period of anemia. This is shown in striking manner in Rat 53, in which a preliminary count just before splenectomy showed 0.6 per cent. of skeined cells. A count made three days later showed 92 per cent. The following day, only a few hours before death, 89 per cent. of the red cells were reticulated. All observations made on anemic blood showed high percentages of reticulated cells. None of the preliminary counts or those made after the blood had been regenerated gave abnormally high percentages.

Seven of the eight rats succumbed. Four died within four days after operation. Rat 44 had one period of partial regeneration of the blood, but died eighteen days after operation. Rat 25 also had two remissions, in the latter of which the blood count returned completely to normal. This animal died forty-three days after operation. Rat 28, the only animal that recovered, showed three periods of anemia before complete recovery. Examination of the blood 216 days after splenectomy showed it to be normal except for a slight leukocytosis.

In all the five animals examined post mortem, the most striking thing observed was the marked pallor of the tissues and evident extreme anemia. Two showed distinct icteroid coloration of the tissues. No evidences of hemorrhage or infection could be found anywhere in the body. The absence of the lung disease, so prevalent in older rats, was noted. The mortality of 87.5 per cent. in this series of rats with enlarged spleen following splenectomy is in striking contrast to the mortality observed among twenty-five other splenectomized rats, of which one died about four weeks after operation. None of the other twenty-four died during periods of observation ranging from twenty-nine days to four months.

COMMENT

It seems probable that there is no important function peculiar to the spleen. The slight transient alterations following splenectomy, together with the new lymphoid tissue, make it seem likely that this lymphoid type of tissue normally shares with the spleen certain of its duties, and in the absence of that organ is capable of assuming a large part of the burden.

If a diseased spleen were removed we should expect the results of splenectomy to be less in degree than usual, because compensation for splenic function had already partially occurred.

Musser has observed that in some chronic conditions the spleen may have been diseased so long and so extensively that a vicarious compensation of its function by other organs may have occurred, thus obscuring the effect of removal. The same idea is expressed by Meyer.[1] The latter would exclude observations after removal of the spleen for leukemia, pseudoleukemia and malaria enlargement, Banti's disease, tuberculosis, echinococcus cyst and purulent affections, from the data of pure experiment, because compensation may be expected to have occurred.

When a normal spleen is removed the alterations which result depend on the capability of related tissues to carry on in entirety the particular functions which had been in part performed by the spleen. The extent of the alterations probably depends in part on the amount and functional capability of the substituting tissues, the duration on the rapidity with which these tissues undergo functional hypertrophy.

If, however, conditions of some sort were present in the body demanding increased function of the type carried on by the spleen, in response to which that organ had hypertrophied, we should not expect to find after splenectomy the large factor of safety which is present in the normal animal. We should expect to find an exaggeration of the phenomena that usually occur after splenectomy.

As far as known with certainty at the present time the only untoward result of splenectomy is anemia. This anemia is variable as to degree and duration, probably depending in an inverse relation on the functional capability of the tissue ready to take the place of the spleen. Therefore, if a truly hyperfunctioning spleen were removed we should expect a severe anemia to develop. Such a result has occurred in all our rats with enlarged spleens.

The cause of the anemia cannot be explained at the present time, but certain phenomena in connection with it stand out prominently and are suggestive in their relation to the rôle of the spleen in the mechanism of blood destruction and regeneration.

The hematogenic function seems not only unimpaired, but capable of tremendous activity in the absence of the enlarged spleen. This is shown during the periods of severe anemia, when at times nearly every cell in the circulating blood is a young form. Thus we are forced to explain the anemia on the ground of increased hemolysis. The rapidity of development of the anemia, the jaundice, the overwhelming preponderance of young red cells, in some cases almost to the exclusion of other types, plainly tell the story of hemolysis.

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7. Musser, J. H., Jr.: The Archives Int. Med., 1912, **9**, 592.

SUMMARY

1. Results of splenectomy were studied in sixteen rats whose spleens were presumably normal; also in eight rats with enlarged spleens.

2. Rats after excision of a normal spleen showed a slight transient anemia, slight tendency to leukocytosis, well-marked increase in resistance of erythrocytes, no change in percentages of reticulated red cells. There was an inconstant increase in the number of nucleated red cells during the periods of anemia.

3. Removal of enlarged spleens was followed by rapid and usually fatal anemia, hyperleukocytosis, marked increase in the number of nucleated and reticulated red cells and, in two cases, by distinct jaundice.

CONCLUSIONS

1. The variability of results following splenectomy is due to several factors, including the functional activity of the spleen and the functional activity and ability to compensate on the part of the tissues with function similar to that of the spleen.

2. The associated phenomena make it appear almost certain that the anemia which develops after the removal of an enlarged spleen is of hemolytic type; thus more evidence is brought forward that the anemia of splenectomy is of hemolytic origin.

3. The type of function exerted by the spleen in the mechanism of blood destruction and regeneration is necessary to life. Usually after the removal of the spleen there are left in the body other tissues capable of carrying on the function successfully. Under circumstances in which the function cannot be successfully assumed by other tissues, removal of the spleen is attended with disastrous results.

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A STUDY OF THE ACIDOSIS, BLOOD UREA, AND PLASMA CHLORIDES IN URANIUM NEPHRITIS IN THE DOG, AND OF THE PROTECTIVE ACTION OF SODIUM BICARBONATE.

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PLATES 57 AND 58.

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HISTORICAL.

Numerous investigations have been made concerning the relation between nephritis and the alkalinity of the blood. The earliest studies were made by titration of the blood serum or of a dialysate of the serum with a weak acid, usually phosphoric or tartaric acid with litmus paper as an indicator. By such a method von Jaksch (1) noted a decrease in the alkalinity of the blood in uremia and concluded that the diminished alkalinity of the blood is a factor in inducing some of the phenomena of uremic intoxication. Brandenburg (2, 3), using a similar method, confirmed von Jaksch's finding. The methods employed by these investigators are, however, admittedly inadequate. In 1912 Straub and Schlayer (4), using Haldane's method, found a diminution in the carbon dioxide content of the alveolar air in uremia and thus established on a secure basis the association of acidosis with uremia. Von Noorden (5), however, considered that although a slight grade of acidosis may be present in uremia, it represents merely an associated phenomenon (perhaps the result of inanition) and not the underlying cause of the intoxication. Sellards (6) observed that a larger quantity of sodium bicarbonate must be ingested by certain nephritics, in order to render their urine alkaline, than is the case with normal individuals. This phenomenon he called "increased tolerance to sodium bicarbonate" and concluded that it indicates a condition of acidosis. In 1913 Palmer and Henderson (7), applying the principle of Sellards, but with a more highly developed method for determining the acidity of the urine, found evidence of some degree of acidosis in many pathological conditions. Among these were certain acute infections, severe anemias, the cachexias of malignant neoplasms, and certain types of nephritis.

There are also numerous investigations concerning the effect of alkali and acid upon the alkalinity of the blood and of the urine. Miquel (8) noted a diminution of the alkalinity of the blood of normal animals following the administration

of acids. Gaethgens (9), on the other hand, observed a rapid excretion of the acid introduced without detecting any depletion of the bases of the blood. Lassar (10) using the tartaric acid titration method with litmus paper as an indicator reported a slight, but unquestionable decrease in the alkalinity of the blood of a dog to which acid had been administered. A more reliable method was that employed by Walter (11) in 1877, who made use of the fact that the carbon dioxide content of the blood is proportional to the content of available base in the blood. He demonstrated that when an animal is treated by injecting an acid into the blood stream, the blood undergoes a diminution in its content of base as shown by its diminished carbon dioxide content. He also showed that under such conditions there is an increased ammonia excretion in the urine and eventually a respiratory death, which may be delayed by the administration of soda. Von Hösslin (12) found an intimate relation between the acidity of the urine as determined by the method of Moritz and the amount of albumin and casts in the urine. He observed that following a lowered acidity of the urine after administration of soda, the albumin in the urine as well as casts diminishes, and, furthermore, that such lowered acidity is accompanied by an improvement in the renal functional capacity as indicated by a better excretion of sodium chloride. Von Hösslin (13), therefore, advocated the administration of sodium bicarbonate in order to reduce the acidity of the urine in nephritis. He pointed out that the initial acidity of the urine is no gauge of the amount of soda that must be given to render the urine alkaline. Henderson and Palmer (14) have investigated the effect of acid ingestion upon the hydrogen ion concentration in urine and found a constant increase of acidity in the urine after the ingestion of considerable amounts of acid, but they were not able to produce a urine of an acidity as great as that common in many pathological conditions. Scheltema (15) also obtained favorable effects from the administration of alkali to nephritic individuals. Henderson and Palmer (16), in a study of the factors of acid excretion in nephritis, found "first, that the urinary concentration of ionized hydrogen is, in a statistical sense, increased in the various forms of nephritis; and secondly that such pathological states are frequently marked by a condition of acidosis." They found (17) a renal retention of alkali in those cases of nephritis in which excretion of ammonia is diminished in the urine.

EXPERIMENTAL.

The present investigation was undertaken to study the development of acidosis in nephritis produced by uranium nitrate and the relation of this acidosis to the changes in urea and chlorides of the blood, and also to study the effect of administration of sodium bicarbonate upon all these factors. In these experiments the following determinations were made: (1) the carbon dioxide content of

the plasma and the hydrogen ion concentration of the serum, (2) the urea nitrogen of the blood, (3) the chlorides of the plasma, and (4) the reaction of the urine and its content of albumin and casts. For the carbon dioxide content of the plasma, the Van Slyke-Stillman-Cullen (18) method was used; for the hydrogen ion concentration of the serum, Marriott's modification of the Levy-Rowntree-Marriott method (19, 20); for urea determinations, the Van Slyke-Cullen method (21, 22); and for the plasma chlorides, the method of McLean and Van Slyke (23). In every instance duplicate determinations were done.

The blood for these determinations, except for the hydrogen ion concentration of the serum, was obtained by drawing the blood from the external jugular vein through a tube passing to the bottom of a centrifuge tube containing either sodium oxalate or potassium oxalate crystals and a layer of paraffin oil which, floating on the surface of the blood, excluded contact with the air. The amount of oxalate employed was about 1 per cent by weight of the amount of blood. A portion of the whole blood was removed for urea determination and the remainder was centrifuged. Of the plasma so obtained, 1 cc. was transferred directly from beneath the paraffin oil to the Van Slyke burette for determination of its carbon dioxide content. The results of these determinations are given in the tables in the columns headed "direct." The remainder of the plasma was removed from the cells and used for chloride determination and for the carbon dioxide content after saturation in an atmosphere of 5.5 per cent carbon dioxide at room temperature. This saturation was performed by introducing about 3 cc. of the plasma into a 250 cc. separatory funnel and filling this with normal alveolar air by exhaling deeply five times through the funnel. The funnel was then closed and the plasma shaken in this atmosphere for 1 minute. 1 cc. portions were then transferred to the burette for analysis. The results of these determinations are given in the columns headed "funnel." These readings were always completed within an hour of the drawing of the blood. For the hydrogen ion concentration of the serum, blood was drawn directly from the vein into a small test-tube and the serum separated by centrifuging. In addition, plasma was examined by Marriott's method. The columns headed

"direct" represent the reading obtained at the close of dialysis; the columns headed "A. B." are the readings after the removal of carbon dioxide from the dialysate by aeration.

Plasma Carbon Dioxide Content, Blood Urea, and Plasma Chlorides in Dogs with Uranium Nephritis.

Since it has been noted by MacNider (24) that the age of a dog has an important bearing on the degree of nephritis caused by uranium nitrate, dogs of the same age have been used as far as possible. The importance of diet in determining the toxicity of uranium nitrate has been shown by Opie (25). Animals have been shown to be more susceptible to this poison when kept upon a diet rich in meat than when upon a diet rich in carbohydrates. For this reason, the animals employed have received a constant diet of milk and dog biscuit with no meat. The animals were fed daily at 5 p.m. In regard to the method of injection, the crystalline uranium nitrate has been dissolved in distilled water and given to the dogs, at the first injection in the proportion of 0.015 gm. per 10 kilos of body weight. After an interval of 20 days another injection of the same dose was given, followed by two subsequent injections at 10 day intervals. The amount of uranium given was increased in the third and fourth injections; in the third injection 0.0185 gm. and in the fourth injection 0.045 gm. per 10 kilos of body weight. The object of the increased doses was to produce severe nephritis, in which the carbon dioxide content, urea, and chlorides of the blood might undergo a more marked alteration. Finally, at the end of the experiment, the animals were killed and the kidneys examined. Four dogs were used in the experiments. They were bled at 9 a.m. on the days when the blood was studied. Three preliminary examinations were made on each dog before administering either uranium or soda. The results of these examinations are shown in Table I. The results following the administration of uranium to Dogs 1 and 2 are shown in Tables II and III and Text-figs. 1, 2, and 3.

In Dog 1, after the first injection of uranium, the carbon dioxide content of the plasma underwent a considerable diminution in the course of a week, and, at the same time, the urea and chlorides of

TABLE I.

Normal Dogs.

	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
			Serum.		Plasma.						
	Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts.
	per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
Dog 1	55	58	7.5	8.0	7.4	7.9	11	5.6	Acid.	—	—
	63	66	7.9	8.1	7.5	7.8	13	5.9	"	—	—
	62	65	7.8	8.0	7.4	7.9	12	6.0	"	—	—
Average.....	60	63	7.7	8.0	7.4	7.9	12	5.8			
Dog 2	56	61	7.4	7.9	7.3	7.7	14	5.4	Acid.	—	—
	60	65	7.8	8.0	7.4	7.7	13	5.9	"	—	—
	61	65	7.6	8.0	7.4	7.8	12	5.6	"	—	—
Average.....	59	64	7.6	8.0	7.4	7.7	13	5.6			
Dog 3	55	62	7.8	7.9	7.6	7.9	13	5.8	Acid.	—	—
	56	62	7.9	8.0	7.6	8.0	13	5.9	"	—	—
	57	64	7.9	8.0	7.4	8.0	13	5.8	"	—	—
Average.....	56	63	7.9	8.0	7.6	8.0	13	5.8			
Dog 4	55	60	7.8	7.9	7.3	7.9	13	5.5	Acid.	—	—
	58	65	7.8	7.9	7.4	7.7	15	6.0	"	—	—
	60	66	7.9	8.0	7.4	7.8	14	5.8	"	—	—
Average.....	58	64	7.8	7.9	7.4	7.8	14	5.8			

Extent of Variability.

Van Slyke method.		Marriott method.				Urea nitrogen.	Chlorides.
		Serum.		Plasma.			
Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.		
per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*
55–63	58–66	7.4–7.9	7.9–8.1	7.3–7.6	7.7–8.0	11–15	5.4–6.0

TABLE II.

Dog 1. Weight 8 kilos.
First injection, 2 p.m., July 3, 1916.

Day.	Date.	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
				Serum.		Plasma.						
		Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts.
		per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
Average		60	63	7.7	8.0	7.4	7.9	12	5.8	Acid.	–	–
1	*1916* July 3	12 mg. of uranium nitrate at 2 p.m.										
2	July 4											
3	" 5	57	63	7.8	7.9	7.1	7.6	21	6.3	Acid.	Tr.	–
4	" 6									"	"	–
5	" 7	53	56	7.7	7.8	7.3	7.7	28	6.7	"	"	+
6	" 8	48	55	7.1	7.8	7.0	7.8	32	6.7	"	++	+
7	" 9									"	+	+
8	" 10	45	50	7.1	7.7	6.9	7.9	36	6.8	"	+	+
9	" 11	40	46	7.0	7.8	6.9	7.8	23	7.0	"	+	+
10	" 12									"	+	+
11	" 13	55	60	7.2	7.9	7.1	7.8	21	6.0	"	+	–
12	" 14									"	+	–
13	" 15	60	65	7.5	7.7	7.3	7.6	19	6.3	"	+	–
14	" 16									"	+	–
15	" 17									"	Tr.	–
16	" 18	58	60	7.7	8.0	7.3	7.8	21	5.8	"	"	–
17	" 19									"	"	–
18	" 20	60	65	7.8	7.9	7.4	7.7	16	5.8	"	"	–
19	" 21									"	"	–
20	" 22	63	65	7.9	8.0	7.4	7.9	16	6.2	"	"	–
21	" 23											
22	July 24	12 mg. of uranium nitrate at 9 a.m.								Acid.	Tr.	–
23	July 25									Acid.	Tr.	–
24	" 26			7.7	7.8	7.3	7.4	23	6.6	"	+	–
25	" 27									"	+	+
26	" 28			7.6	7.8	7.3	7.8	26	6.6	"	++	+
27	" 29									"	++	+
28	" 30									"	++	+
29	" 31			7.5	7.8	7.3	7.8	20	6.3	"	++	+
30	Aug. 1									"	+	+
31	" 2			7.6	7.8	7.4	7.7	21	6.2	"	+	–
32	" 3									"	Tr.	–
33	" 4	60	65	7.7	7.8	7.4	7.6	21	6.1	"	"	–

TABLE II—*Concluded.*

Day.	Date.	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
				Serum.		Plasma.						
		Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts
33	Aug. 4	15 mg. of uranium nitrate at 9 a.m., after bleeding.								Acid.	Tr.	—
		per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
34	Aug. 5									Acid.	Tr.	—
35	" 6									"	"	—
36	" 7									"	+	—
37	" 8	48	52	7.8	7.8	7.4	7.8	21	6.5	"	+	—
38	" 9									"	+	—
39	" 10	46	50	7.6	7.8	7.2	7.6	22	6.9	"	+	—
40	" 11									"	+	+
41	" 12	49	55	7.9	8.0	7.4	7.8	22	6.7	"	++	+
42	" 13									"	+	—
43	" 14									"	+	—
44	Aug. 15	36 mg. of uranium nitrate at 9 a.m.								Acid.	+	—
45	Aug. 16	49	53	7.6	7.9	7.4	7.8	29	7.0	Acid.	+	+
46	" 17	45	47	7.4	7.8	7.2	7.7	29	7.1	"	++	+
47	" 18	42	46	7.2	7.8	7.1	7.7	26	7.3	"	++	+
48	" 19	40	47	7.2	7.8	7.1	7.6	25	7.3	"	++	+
49	" 20	45	49	7.3	7.8	7.1	7.6	26	7.4	"	++	+
50	" 21									"	+	+
51	" 22	52	56	7.7	7.8	7.2	7.6	25	7.1	"	+	+

the blood exhibited an increase associated with the appearance of albumin and casts in the urine. It will be noted that the minimum of the plasma carbon dioxide content coincides approximately with the maximum of the blood urea and plasma chlorides in time of occurrence. At about this period casts also appeared in the urine. During the 3rd week after the first injection, the plasma carbon dioxide content returned to its normal level, and on the 20th day, the values for plasma chlorides and blood urea nitrogen were 6.2 and 16 respectively. The chlorides, however, had three times been within normal limits between the 11th and 20th days. Urine at

TABLE III.

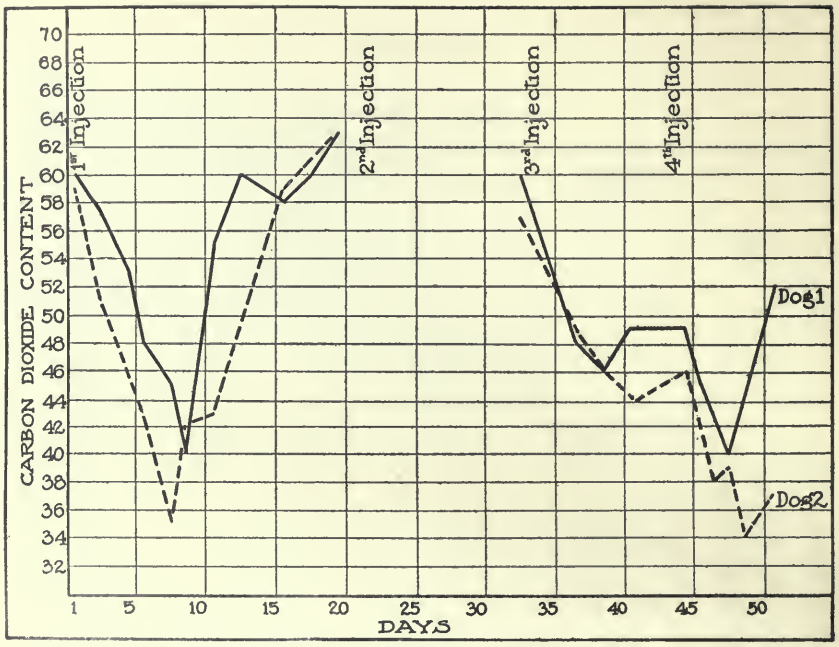
Dog 2. Weight 27 kilos.
First injection, 2 p.m., July 3, 1916.

Day.	Date.	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
				Serum.		Plasma.						
		Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts.
		per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
Average.....		59	64	7.6	8.0	7.4	7.7	13	5.6	Acid.	–	–
1	*1916* July 3	40 mg. of uranium nitrate at 2 p.m.										
2	July 4									Acid.		
3	" 5	51	58	7.8	8.0	7.3	7.8	21	6.4	"	Tr.	–
4	" 6									"	"	–
5	" 7	46	48	7.6	7.8	7.4	7.6	23	6.6	"	+	+
6	" 8	43	47	6.9	7.7	6.8	7.6	25	7.2	"	++	+
7	" 9									"	++	+
8	" 10	35	39	6.9	7.4	6.8	7.3	42	7.3	"	++	+
9	" 11	42	45	7.1	7.8	7.0	7.5	41	6.9	"	++	+
10	" 12									"	++	+
11	" 13	43	46	7.1	7.8	7.0	7.5	42	6.9	"	+	+
12	" 14									"	+	+
13	" 15	49	52	7.5	7.7	7.3	7.5	41	6.6	"	+	+
14	" 16									"	+	–
15	" 17									"	Tr.	–
16	" 18	59	62	7.7	7.9	7.4	7.9	36	6.1	"	"	–
17	" 19									"	"	–
18	" 20	61	63	7.9	8.1	7.3	7.6	26	6.0	"	"	–
19	" 21									"	"	–
20	" 22	63	65	7.8	8.0	7.4	7.8	16	5.9	"	"	–
21	" 23											
22	July 24	40 mg. of uranium nitrate at 9 a.m.								Acid.	Tr.	–
23	July 25									Acid.	Tr.	–
24	" 26			7.8	7.9	7.4	7.8	22	5.9	"	+	–
25	" 27									"	+	+
26	" 28			7.6	7.8	7.3	7.7	26	6.3	"	++	+
27	" 29									"	++	+
28	" 30									"	++	+
29	" 31			7.4	7.7	7.3	7.7	26	6.5	"	++	+
30	Aug. 1									"	+	+
31	" 2			7.5	7.7	7.3	7.6	20	6.1	"	+	?
32	" 3									"	+	?
33	" 4	57	60	7.6	7.7	7.3	7.5	20	6.1	"	+	+

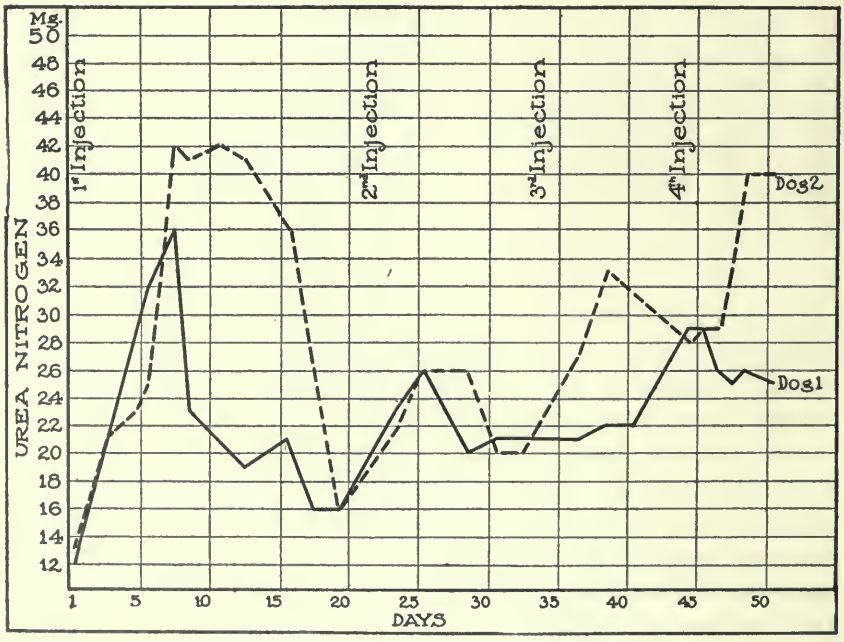
TABLE III—*Concluded.*

Day.	Date.	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
				Serum.		Plasma.						
		Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts.
33	Aug. 4	50 gm. of uranium nitrate at 9 a.m., after bleeding.										
		per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
34	Aug. 5									Acid.	+	+
35	" 6									"	+	+
36	" 7									"	+	+
37	" 8	49	53	7.7	7.7	7.4	7.7	27	6.7	"	++	+
38	" 9									"	++	+
39	" 10	46	48	7.6	7.8	7.3	7.7	33	7.0	"	++	+
40	" 11									"	++	?
41	" 12	44	46	7.7	7.8	7.3	7.8	31	6.9	"	+	+
42	" 13									"	+	+
43	" 14									"	+	+
44	Aug. 15	120 mg. of uranium nitrate at 9 a.m.								Acid.	+	+
45	Aug. 16	46	49	7.6	7.7	7.3	7.6	28	7.0	Acid.	+	+
46	" 17	42	45	7.3	7.7	7.2	7.6	29	7.3	"	+	+
47	" 18	38	40	7.1	7.7	7.0	7.5	29	7.2	"	++	+
48	" 19	39	45	7.1	7.6	7.0	7.5	34	7.3	"	++	+
49	" 20	34	40	7.1	7.7	7.0	7.6	40	7.3	"	++	+
50	" 21									"	++	+
51	" 22	37	43	7.1	7.6	7.1	7.5	40	7.1	"	++	+

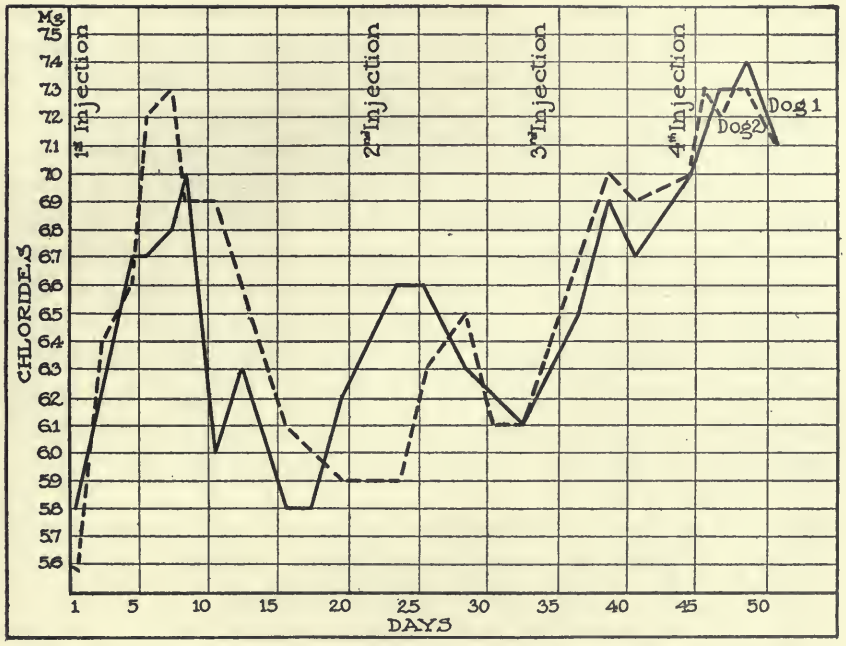
this time still showed the presence of a trace of albumin, although casts were absent. After the second injection of uranium similar changes occurred in the blood urea and plasma chlorides, but less pronounced in degree; the carbon dioxide content could not be studied at this time as the only burette at hand was broken. After the third and larger injection, a marked reduction of the carbon dioxide content occurred and a synchronous marked rise of plasma chlorides; no significant change was noted in the blood urea. It may be noted that casts in the urine appeared rather late After the fourth injection, which was given before the blood had returned to a normal



TEXT-FIG. 1. Curves showing the carbon dioxide content of the plasma of Dogs 1 and 2 after injections of uranium nitrate. No soda administered.



TEXT-FIG. 2. Curves showing the urea nitrogen in 100 cc. of blood in Dogs 1 and 2 after injections of uranium nitrate. No soda administered.



TEXT-FIG. 3. Curves showing the chlorides in 1 cc. of the plasma in Dogs 1 and 2 after injections of uranium nitrate. No soda administered.

condition, the minimum of plasma carbon dioxide content and the maximum of plasma chloride coincided approximately in their occurrence about the 4th day after injection. During this last period the chlorides in the blood were much higher and the albumin in the urine was much greater in quantity than after the previous injections. The increase in the quantity of blood urea, however, was less than after the first injection.

In the second dog nearly identical relations in the carbon dioxide content, urea, and chlorides of the blood and albumin and casts in the urine were observed as in the first dog. We may, however, note the following slight differences. After their reappearance 3 days after the second injection, the urinary casts persisted throughout the experiment. After each injection the carbon dioxide content, the chloride content, and the blood urea showed greater disturbances than in the first dog. This is prob-

ably to be attributed to the much larger absolute dose of uranium, although the same dose was used per kilo. We may conclude from these experiments (Dogs 1 and 2) that the plasma carbon dioxide content undergoes a considerable diminution in uranium nephritis, while the plasma chlorides and blood urea increase, and, furthermore, that the occurrence of the minimum content of carbon dioxide coincides approximately with the occurrence of the maximum content of chlorides and of urea in the blood. Both the carbon dioxide content and plasma chlorides returned nearly to their normal condition 2 to 3 weeks after the first injection. Urea, on the other hand, did not return quite to its normal level at any period, although 3 weeks after the first injection, the change from the normal value was slight. Albumin after appearing did not disappear entirely from the urine, although casts occasionally did.

Influence of Sodium Bicarbonate on the Plasma Carbon Dioxide Content, Blood Urea, and Plasma Chlorides in Dogs with Uranium Nephritis.

For this experiment Dogs 3 and 4 were used. Each dog received through a stomach tube 1 gm. of sodium bicarbonate dissolved in 10 cc. of water per kilo of body weight at 9 a.m. throughout the entire period of the experiment. On the 3rd day uranium nitrate was injected. The amounts of the uranium per kilo and the intervals between succeeding injections were exactly the same as in the experiment on Dogs 1 and 2. The blood was taken at 9 a.m., before administering the soda. The results are shown in Tables IV and V and Text-figs. 4, 5, and 6. While the dogs were receiving sodium bicarbonate alone, that is, before the giving of uranium, a considerable increase of the plasma carbon dioxide content was observed. Following the first uranium injection this carbon dioxide content showed a decrease and at the same time there occurred an increase in the plasma chlorides and blood urea, as in Dogs 1 and 2.

The relative decrease in the carbon dioxide content following uranium in the dogs receiving soda is comparable with that in the dogs receiving no soda, but the absolute level reached was not so low, because of the higher level already existing when the uranium

TABLE IV.

Dog 3. Weight 13.5 kilos.
First injection, 9 a.m., July 5, 1916.
July 3. 12 a.m. 13.5 gm. of sodium bicarbonate + 135 cc. of water.
From July 4 sodium bicarbonate + water was given every day, after bleeding.

Day.	Date.	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
				Serum.		Plasma.						
		Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts.
		per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
Average		56	63	7.9	8.0	7.6	8.0	13	5.8	Acid.	—	—
	1916											
1	July 3, 2 p.m.	69	71	7.8	8.0	7.5	7.9	13	5.6			
2	" 4									Alkaline.	—	—
3	July 5	20 mg. of uranium nitrate at 9 a.m.								Alkaline.	—	—
4	July 6	66	68	7.8	8.0	7.4	7.9	14	6.1	Alkaline.	Tr.	—
5	" 7	56	60	7.7	8.0	7.3	7.9	15	6.2	"	"	—
6	" 8	55	60	7.4	7.9	6.9	7.8	19	6.2	"	"	—
7	" 9									"	"	—
8	" 10	47	52	7.3	7.7	6.9	7.8	26	6.8	"	"	+
9	" 11									"	"	+
10	" 12	48	50	7.2	7.8	6.9	7.6	29	6.7	"	+	+
11	" 13									"	+	+
12	" 14	55	58	7.4	7.8	7.0	7.6	30	6.0	"	+	+
13	" 15									"	+	—
14	" 16									"	+	—
15	" 17	61	64	7.7	7.9	7.4	7.9	29	6.4	"	Tr.	—
16	" 18									"	"	—
17	" 19	65	68	7.9	8.1	7.4	7.9	32	6.0	"	"	—
18	" 20									"	"	—
19	" 21	70	75	8.0	8.1	7.5	7.9	16	6.0	"	"	—
20	" 22									"	"	—
21	" 23									"	"	—
22	" 24	70	73	7.9	8.1	7.5	7.9	22	5.6	"	"	—
23	" 25											
24	" 26											
25	" 27			8.0	8.1	7.4	7.8	25	5.9	Alkaline.	Tr.	—

TABLE IV—*Concluded.*

Day.	Date.	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
				Serum.		Plasma.						
		Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts.
25	July 27	20 mg. of uranium nitrate at 9 a.m., after bleeding.										
		per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
26	July 28									Alkaline.	+	−
27	" 29			7.8	8.0	7.4	7.9	26	6.5	"	+	−
28	" 30									"	+	−
29	" 31									"	+	−
30	Aug. 1			7.5	7.8	7.3	7.5	17	6.0	"	+	−
31	" 2									"	+	−
32	" 3			7.6	7.8	7.2	7.5	15	5.8	"	+	−
33	" 4									"	+	−
34	" 5	62	55	7.6	7.8	7.3	7.6	15	6.0	"	Tr.	−
35	" 6									"	"	−
36	Aug. 7	25 mg. of uranium nitrate at 9 a.m.								Alkaline.	Tr.	−
37	Aug. 8									Alkaline.	Tr.	−
38	" 9									"	+	−
39	" 10	52	55	7.5	7.8	7.3	7.6	28	6.3	"	+	−
40	" 11									"	+	−
41	" 12	49	52	7.3	7.8	7.2	7.6	23	6.2	"	+	−
42	" 13									"	+	−
43	" 14	50	55	7.5	7.9	7.3	7.5	26	6.4	"	+	−
44	" 15									"	+	−
45	" 16									"	+	−
46	Aug. 17	60 mg. of uranium nitrate at 9 a.m.										
47	Aug. 18	62	67	7.7	7.8	7.4	7.8	28	6.4	Alkaline.	+	−
48	" 19	56	59	7.6	7.8	7.4	7.8	20	6.3	"	+	+
49	" 20									"	+	+
50	" 21	54	61	7.5	7.8	7.3	7.7	19	6.6	"	+	+
51	" 22									"	+	+
52	" 23	58	62	7.5	7.8	7.3	7.6	18	6.3	"	+	+
53	" 24	62	65	7.7	7.9	7.4	7.8	15	6.6	"	+	+

TABLE V.

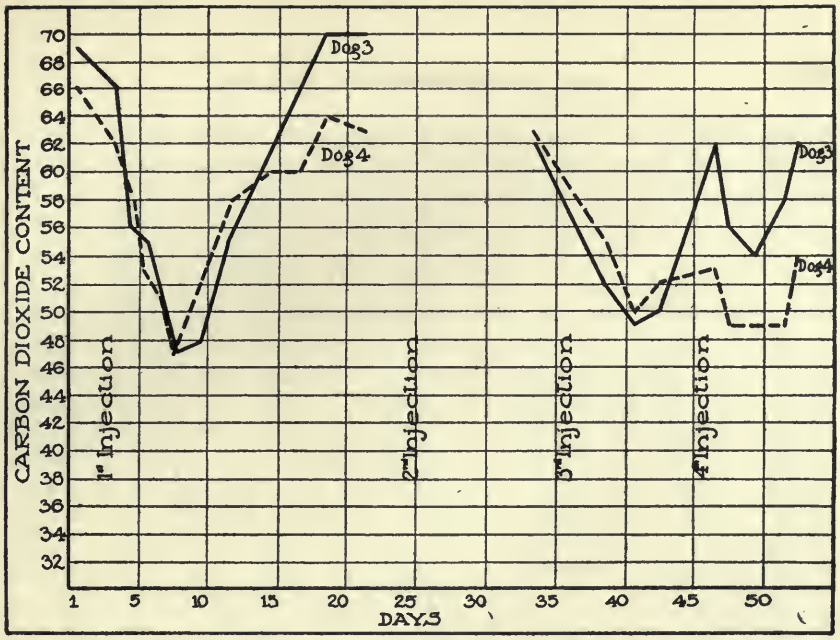
Dog 4. Weight 19 kilos.
First injection, 9 a.m., July 5, 1916.
July 3. 12 a.m. 19 gm. of sodium bicarbonate + 190 cc. of water.
From July 4 sodium bicarbonate + water was given every day, after bleeding.

Day.	Date.	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
				Serum.		Plasma.						
		Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts.
		per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
Average.....		58	64	7.8	7.9	7.4	7.8	14	5.8	Acid.	−	−
	1916											
1	July 3, 2 p.m.	66	70	7.8	8.0	7.5	8.0	13	6.0			
2	" 4									Alkaline.	−	−
3	July 5	29 mg. of uranium nitrate at 9 a.m.								Alkaline.	−	−
4	July 6	62	66	7.9	8.0	7.4	7.8	16	5.6	Alkaline.	Tr.	−
5	" 7	58	60	7.7	7.9	7.3	7.7	18	6.0	"	"	−
6	" 8	53	56	7.4	7.8	7.2	7.7	29	6.3	"	"	−
7	" 9									"	"	−
8	" 10	47	52	7.2	7.6	7.0	7.4	32	6.8	"	+	−
9	" 11									"	+	+
10	" 12	52	55	7.3	7.8	7.2	7.7	32	6.7	"	+	+
11	" 13									"	+	+
12	" 14	58	61	7.4	7.8	7.2	7.6	38	6.3	"	+	+
13	" 15									"	+	−
14	" 16									"	Tr.	−
15	" 17	60	65	7.8	8.0	7.5	7.9	28	6.3	"	"	−
16	" 18									"	"	−
17	" 19	60	66	7.8	7.9	7.4	7.9	36	6.1	"	"	−
18	" 20									"	"	−
19	" 21	64	68	7.9	8.0	7.4	7.8	26	5.8	"	"	−
20	" 22									"	"	−
21	" 23									"	"	−
22	" 24	63	66	7.8	8.0	7.4	7.8	25	5.8	"	"	−
23	" 25									"	"	−
24	" 26									"	"	−
25	" 27			8.0	8.1	7.4	7.7	20	6.0	"	"	−
25	July 27	29 mg. of uranium nitrate at 9 a.m., after bleeding.										
26	July 28									Alkaline.	+	−
27	" 29			7.9	8.0	7.5	7.9	28	6.1	"	+	−
28	" 30									"	+	−
29	" 31									"	+	+

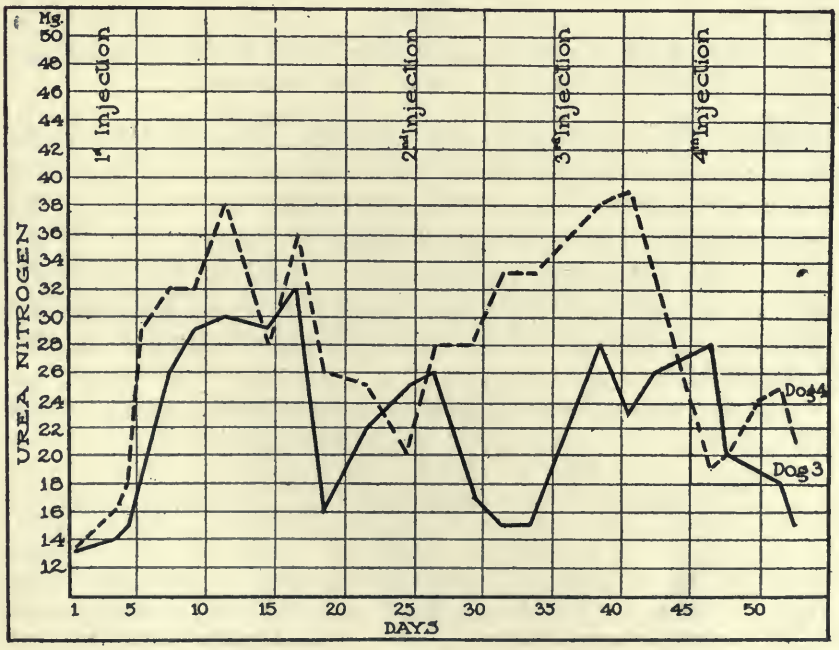
TABLE V—*Concluded.*

Day.	Date.	Van Slyke method.		Marriott method.				Urea nitrogen in 100 cc. of blood.	Chlorides in 1 cc. of plasma.	Urine.		
				Serum.		Plasma.						
		Direct.	Funnel.	Direct.	A. B.	Direct.	A. B.			Reaction.	Albumin.	Casts.
		per cent	*per cent*	*log.*	*log.*	*log.*	*log.*	*mg.*	*mg.*			
30	Aug. 1			7.7	7.9	7.3	7.7	28	5.8	Alkaline.	+	+
31	" 2									"	+	−
32	" 3			7.8	7.9	7.4	7.8	33	6.3	"	+	−
33	" 4									"	+	−
34	" 5	63	65	7.7	7.8	7.4	7.7	33	6.1	"	Tr.	−
35	" 6									"	"	−
36	Aug. 7	36 mg. of uranium nitrate at 9 a.m.								Alkaline.	Tr.	−
37	Aug. 8									Alkaline.	Tr.	−
38	" 9									"	+	−
39	" 10	55	58	7.5	7.6	7.2	7.5	38	5.8	"	+	−
40	" 11									"	+	−
41	" 12	50	52	7.5	7.6	7.3	7.6	39	6.0	"	+	−
42	" 13									"	+	−
43	" 14	52	54	7.6	7.7	7.3	7.5	33	6.4	"	+	−
44	" 15									"	+	−
45	" 16									"	+	−
46	Aug. 17	87 mg. of uranium nitrate at 9 a.m.								Alkaline.	+	−
47	Aug. 18	53	55	7.7	7.8	7.4	7.8	19	6.6	Alkaline.	+	−
48	" 19	49	52	7.6	7.9	7.3	7.7	20	7.0	"	+	+
49	" 20									"	+	+
50	" 21	49	53	7.6	7.7	7.3	7.7	24	6.9	"	+	+
51	" 22									"	+	+
52	" 23	49	52	7.6	7.7	7.3	7.7	25	7.0	"	+	+
53	" 24	54	58	7.7	7.8	7.4	7.7	21	7.0	"	+	+

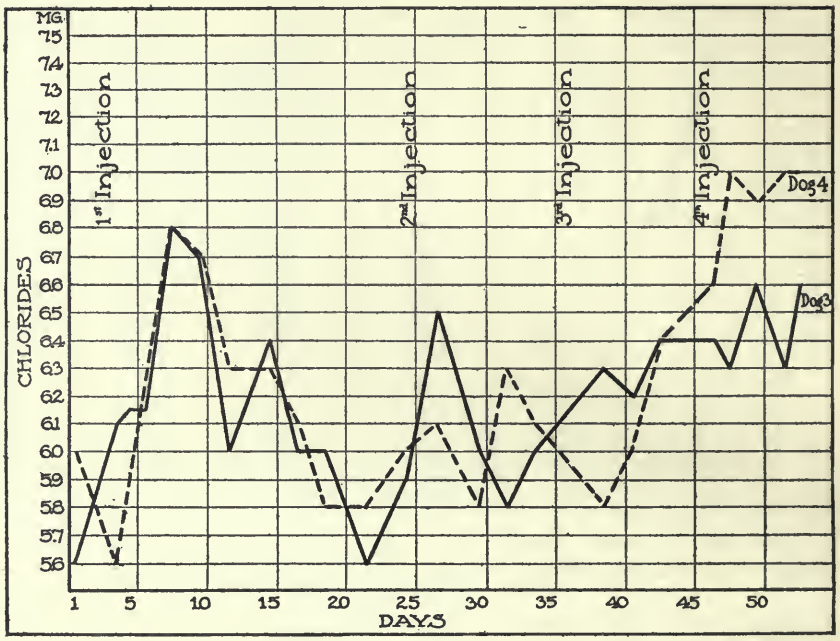
administration was commenced. The increase in the blood urea in the dogs receiving soda is comparable with that in the dogs receiving no soda except following the large fourth injection, when the dogs receiving soda showed a less pronounced rise. The increase in the plasma chlorides was consistently lower throughout the experiments in the dogs receiving the soda. This was especially marked following the fourth injection of uranium. The soda administered was sufficient to keep the urine in these dogs persistently



TEXT-FIG. 4. Curves showing the carbon dioxide content of the plasma of Dogs 3 and 4 after injections of uranium nitrate. Soda administered.



TEXT-FIG. 5. Curves showing the urea nitrogen in 1 cc. of blood in Dogs 3 and 4 after injections of uranium nitrate. Soda administered.



TEXT-FIG. 6. Curves showing the chlorides in 1 cc. of the plasma in Dogs 3 and 4 after injections of uranium nitrate. Soda administered.

alkaline. The albuminuria was persistent from the time of the first appearance, but was definitely less marked than in the animals receiving no soda. Casts were likewise less abundant in these animals and disappeared entirely for longer intervals of time. In general, following the fourth injection, the carbonate dogs showed a considerable difference from the control dogs as to the diminution of carbon dioxide content and as to increase of chlorides and urea in the blood.

The protocols of the histological examination of the kidneys follow.

Dog 1. Weight 8 Kilos. No Soda.

Day of experiment.	Uranium nitrate. Absolute dose.	Relative dose per kilo.
	gm.	*mg.*
1	0.012	1.5
22	0.012	1.5
33	0.015	1.85
44	0.036	4.5
52	Killed.	

Interval before fixation of tissues: a few minutes.

Under the low power the glomeruli appear normal. The epithelium of the proximal convoluted tubules appears swollen, especially in the tubules in the region of the corticomedullary junction. The epithelium of the ascending loops of Henle appear swollen. The medulla appears normal. The medullary rays stand out conspicuously.

Under high power some of the glomeruli exhibit moderate congestion, others appear normal. The epithelium of the proximal convoluted tubules is greatly swollen, but the nuclei are well preserved and only rarely is there any evidence of necrosis. The distal convoluted tubules are almost all normal, except for the presence of serum in the lumina of some. The medulla appears normal.

Diagnosis.—Marked cloudy swelling of the proximal convoluted tubules, especially those near the corticomedullary junction (Fig. 1).

Dog 2. Weight 27 Kilos. No Soda.

	Uranium nitrate.	
Day of experiment.	Absolute dose.	Relative dose per kilo.
	gm.	*mg.*
1	0.040	1.5
22	0.040	1.5
33	0.050	1.85
44	0.120	4.5
52	Killed.	

Interval before fixation of tissues: a few minutes.

Under low power this kidney appears highly abnormal with moderate to marked congestion of the glomerular tufts and with patches of marked epithelial swelling in the proximal convoluted tubules, of interstitial edema, and of serum in the lumina in the cortex, especially in the corticomedullary region. The medulla exhibits marked congestion and edema with some swelling of epithelium and with serum or casts in the lumina.

Under high power congestion of the glomeruli is confirmed. The proximal convoluted tubules show intense epithelial swelling and in many places the nuclei are pale or necrotic. In many places the epithelium has entirely desquamated from these tubules, leaving simply the basement membrane. The distal convoluted tubules and ascending limbs of Henle are in many places encroached upon and compressed by interstitial edema and exhibit degeneration of epithelium and serum or casts in many of the lumina. In some of the epithelial cells of the distal convoluted tubules there is a yellowish pigment resembling hemosiderin.

Diagnosis.—Intense cloudy swelling and necrosis of the epithelium of the proximal convoluted tubules. Moderate cloudy swelling of the epithelium of the ascending limbs of Henle and the distal convoluted tubules. Interstitial edema of the cortex and medulla. Serum and casts in tubule lumina. Congestion of glomerular tufts (Fig. 2).

Dog 3. Weight 13.5 Kilos. Soda.

Day of experiment.	Uranium nitrate. Absolute dose.	Relative dose per kilo.
	gm.	*mg.*
1	0.020	1.5
23	0.020	1.5
34	0.025	1.85
44	0.060	4.5
52	Killed.	

Interval before fixation of tissues: a few minutes.

Under low power the picture is practically normal.

Under high power the glomeruli appear normal. The epithelium of all the tubules is in fair condition with little swelling, although this can be observed in occasional cells in the proximal convoluted tubules. The medullary cells are normal. The lumina are almost entirely free from serum.

Diagnosis.—Approximately normal.

Dog 4. Weight 19 Kilos. Soda.

Day of experiment.	Uranium nitrate. Absolute dose.	Relative dose per kilo.
	gm.	*mg.*
1	0.029	1.5
23	0.029	1.5
34	0.036	1.9
44	0.087	4.6
52	Killed.	

Interval before fixation of tissues: a few minutes.

Under low power the picture is normal except for serum or casts in the lumina of the medullary tubes.

Under high power the glomeruli seem normal. The proximal convoluted tubules show little cloudy swelling. The medulla is normal except for serum or casts in the lumina in a considerable number of the tubules.

Diagnosis.—Slight cloudy swelling. Serum or casts in the medullary tubules (Fig. 3).

It is a well established fact that uranium nitrate produces primarily and most conspicuously degeneration and necrosis of the epithelium of the renal tubules, and especially of the proximal convoluted tubules. The physiological studies of Schlayer and Hedinger (26), Pearce, Hill, and Eisenbrey (27), and others have shown that the reaction of the vascular apparatus of the kidney may be impaired or not by uranium nitrate, depending upon the dosage employed. Christian and O'Hare (28) have found that uranium nitrate causes also a lesion of the glomeruli characterized by the presence of hyaline droplets in the capillary loops and by other changes.

MacNider (29) reported a difference in the degree of pathological change in the kidney following uranium in dogs given sodium carbonate intravenously as compared with control dogs receiving no soda. The most marked difference was seen in the degree of involvement of the epithelium of the convoluted tubules.

It is evident from a study of the histology of the kidneys that the kidneys from both dogs receiving no soda exhibited more pronounced nephritis than those of either of the dogs receiving soda. The most severe nephritis occurred in Dog 2, which received the largest absolute dose of uranium; Dog 1, which received the smallest absolute dose of uranium, but no soda, showed also definitely more marked lesions than did Dogs 3 and 4, which received soda.

From the facts given both in the functional studies and in the pathological examination, it is clear that the nephritis in the dogs receiving sodium bicarbonate is less severe than that in the control dogs.

The toxic effect of uranium for the kidney is usually ascribed to the action of the metal as such. According to the experimental results of MacNider the toxicity of uranium runs parallel with its ability to lead to the formation of various acid bodies, and if the appearance of these substances in the urine is delayed and their amount in the body diminished by the administration of alkali, there is less evidence of the toxic action of the metal. In order to exclude the possibility of the toxic salt of uranium having been itself rendered inert by the direct action of sodium carbonate, he injected two animals with uranium nitrate in which the solvent for the uranium was a 3 per cent solution of sodium carbonate in 0.9 per cent sodium chloride. The toxic effect of uranium was in no way diminished when employed in a 3 per cent solution of the carbonate. He ascribes the protective action of sodium carbonate in uranium nephritis to the neutralization of acid bodies produced by the uranium in the animal economy.

In the study of the hydrogen ion concentration of the blood, the most consistent results were obtained by Marriott's modification of the technique, the figure obtained after aeration of the dialysate from the serum and given in the tables in the column "serum A. B." Determination upon the plasma by the same method gave, on the whole, parallel but probably less consistent results. A comparison of the methods of Marriott and of Van Slyke shows a greater delicacy in the Van Slyke method, so that while the evidences as to the acid

base equilibrium of the blood afforded by the two methods agree, Marriott's method is hardly delicate enough to permit of as satisfactory conclusions in such experiments as the present ones.

Action of Alkali and of Acid upon the Carbon Dioxide Content in Plasma.

Acid was administered by stomach tube to two dogs and alkali to two other dogs. For the acid, hydrochloric acid was chosen, and for the alkali, sodium bicarbonate. 1 cc. of 0.5 per cent hydrochloric acid per kilo of body weight was introduced into the stomach through the stomach tube. When acid was thus administered a diminution of the plasma carbon dioxide content developed, but since in this experiment a severe nephritis resulted, it might be questioned whether the decrease of the carbon dioxide content was produced wholly by the acid administered or in part also by the acidosis of the nephritis.

The microscopic examination gave clear evidence of nephritis in these kidneys.

When alkali (10 per cent sodium bicarbonate) was administered, the plasma carbon dioxide content of the blood showed a constant increase (Tables VI to XI.)

TABLE VI.

Dog 5. Weight 20.5 kilos.
Bled every day at 9 a.m.

Date.	Van Slyke method.		Urine.			Remarks.
	Direct.	Funnel.	Reaction.	Albumin.	Casts.	
1916	*per cent*	*per cent*				
Aug. 5	57	59	Acid.	—	—	
" 6	56	59	"	—	—	Acid at 10 a.m., after bleeding.
" 7	49	52	"	++	++	Died at 11 a.m.

TABLE VII.

Dog 6. Weight 18 kilos.
Bled every day at 9 a.m.

Date.	Van Slyke method.		Urine.			Remarks.
	Direct.	Funnel.	Reaction.	Albumin.	Casts.	
1916	*per cent*	*per cent*				
Aug. 5	58	60	Acid.	—	—	
" 6	55	58	"	—	—	Acid at 10 a.m., after bleeding.
" 7	48	53	"	++	++	

TABLE VIII.

Dog 7. Weight 10.5 kilos.
Bled every day at 9 a.m.

Date.	Van Slyke method.		Urine.			Remarks.
	Direct.	Funnel.	Reaction.	Albumin.	Casts.	
1916	*per cent*	*per cent*				
Aug. 5	56	58	Acid.	—	—	
" 6	55	58	"	—	—	After bleeding, 200 cc. of 10 per cent sodium bicarbonate were given.
" 7	60	63	Alkaline.	—	—	

TABLE IX.

Dog 8. Weight 14 kilos.
Bled every day at 9 a.m.

Date.	Van Slyke method.		Urine.			Remarks.
	Direct.	Funnel.	Reaction.	Albumin.	Casts.	
1916	*per cent*	*per cent*				
Aug. 5	56	58	Acid.	—	—	
" 6	55	58	"	—	—	After bleeding, 280 cc. of 10 per cent sodium bicarbonate were given.
" 7	60	63	Alkaline.	—	—	

TABLE X.

Dog 3. Weight 13.5 kilos.

	Van Slyke method.		Urine.			Remarks.
	Direct.	Funnel.	Reaction.	Albumin.	Casts.	
	per cent	*per cent*				
Average.....	56	63	Acid.	—	—	
	69	71	Alkaline.	—	—	2 hrs. after 135 cc. of 10 per cent sodium bicarbonate were given.

TABLE XI.

Dog 4. Weight 19 kilos.

	Van Slyke method.		Urine.			Remarks.
	Direct.	Funnel.	Reaction.	Albumin.	Casts.	
	per cent	*per cent*				
Average.....	58	64	Acid.	—	—	
	66	70	Alkaline.	—	—	2 hrs. after 190 cc. of 10 per cent sodium bicarbonate were given.

DISCUSSION.

These investigations show that the nephritis produced by means of uranium nitrate presents a diminution of the plasma carbon dioxide content, associated with an increase of blood urea and plasma chlorides and the appearance of albumin and casts in the urine. These changes indicate the presence of an acidosis in the nephritis produced by uranium nitrate.

Moreover, both the nephritis thus produced and the acidosis which accompanies it can be diminished by means of sodium bicarbonate. In dogs receiving sodium bicarbonate and given uranium nephritis, there is maintained a higher plasma carbon dioxide content, a less pronounced increase of chlorides in the blood, as well as

a diminution of albumin and casts in the urine as compared with animals given uranium nephritis and receiving no soda. In severe nephritis the amount of urea is also diminished in the carbonate dogs as compared with the controls. The nephritis of the carbonate dogs is less severe as regards the histological picture than that of the controls.

CONCLUSIONS.

1. The presence of an acidosis in dogs with experimental uranium nephritis is demonstrable by the Van Slyke-Stillman-Cullen method and that of Marriott. It is detected more readily by the former method.
2. This acidosis is associated with increase in the blood urea and plasma chlorides and with the appearance of albumin and casts in the urine.
3. The oral administration of sodium bicarbonate diminishes the acidosis, the increase in plasma chlorides, the amount of albumin and casts in the urine, and, to a lesser degree, the increase in the blood urea following the administration of uranium. It also diminishes the severity of the changes produced by uranium in the kidneys.
4. The oral administration of sodium bicarbonate to normal dogs raises the carbon dioxide content of the plasma as determined by the Van Slyke-Stillman-Cullen method.

I wish to thank Dr. J. Harold Austin for his constant suggestions and interest throughout the course of this investigation, and Dr. Herbert Fox, Director of the William Pepper Clinical Laboratory, for extending to me the privileges of the laboratory.

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EXPLANATION OF PLATES.

PLATE 57.

FIG. 1. Dog 1. Section of the kidney of a dog with uranium nephritis, to which no soda was given.

FIG. 2. Dog 2. Section of the kidney of a dog with uranium nephritis, to which no soda was given.

PLATE 58.

FIG. 3. Dog 4. Section of the kidney of a dog with uranium nephritis, to which soda was given.

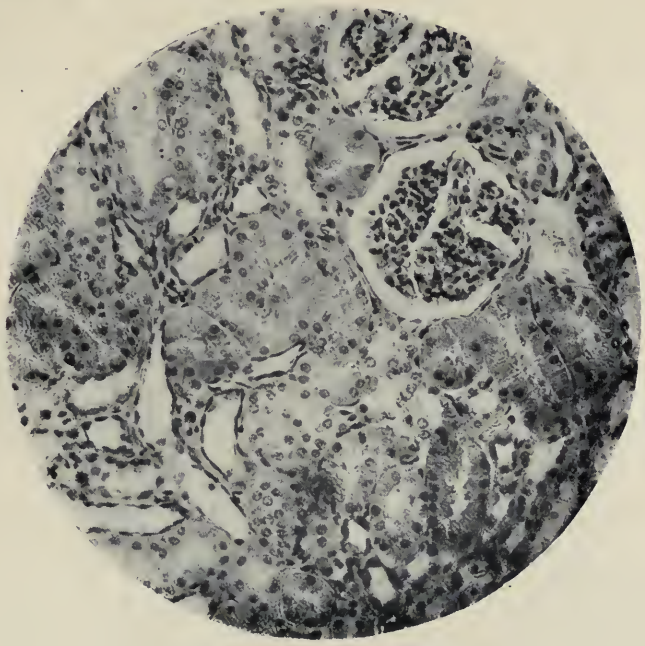

FIG. 1.

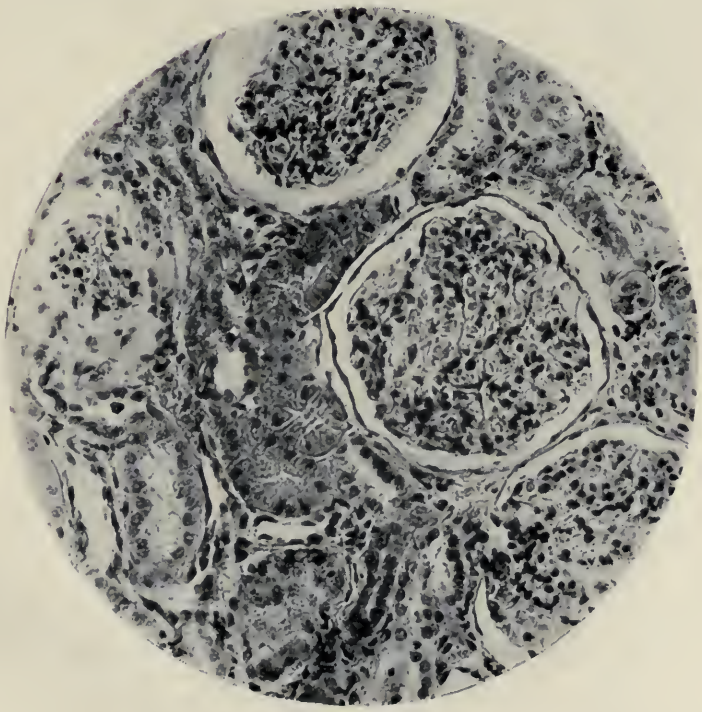

FIG. 2.

(Goto: Uranium Nephritis in the Dog.)

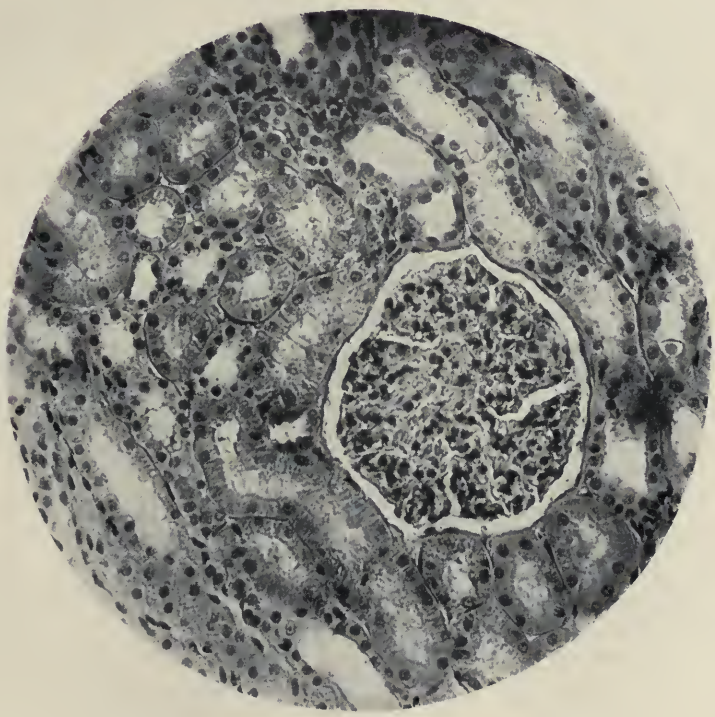

FIG. 3.

(Goto: Uranium Nephritis in the Dog.)

Transient Heart Block—Electrocardiographic Studies

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TRANSIENT HEART BLOCK — ELECTROCARDIOGRAPHIC STUDIES *

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The study of the heart beat by means of modern instruments of precision, such as the polygraph and the string galvanometer, has revealed the fact that the graver forms of cardiac arrhythmia, as heart block and auricular fibrillation, are much more common than was previously suspected. It has also been recognized that such arrhythmias as paroxysmal tachycardia, flutter and premature contractions (extrasystoles), are notoriously transient in most cases, but until very recently heart block (especially complete block) and auricular fibrillation were looked on as being nearly always permanent conditions due to extensive organic changes. In 1910, however, I[1] was able to show that complete heart block may exist for years without demonstrable lesion of the bundle of His at autopsy, and similar cases have occasionally been reported since that time (see Ref. 8). In the case of auricular fibrillation, I have recently shown[2] that transient attacks are not only fairly common, but may be divided into three well-defined groups, whereas transient heart block occurring during an acute infection (Naish and Kennedy[3]), or as the result of digitalis medication (Hewlett[4]), is now an even better recognized condition. It is to such cases that Hart[5] has applied the term "functional heart block," "not because we believe organic changes are absent, but because such changes are of such a moderate degree or are of such a nature that by the administration of drugs the evidence of functional abnormalities can be considerably modified." With these reservations the term is a serviceable one, and yet it would be unfortunate to distract attention too much from the myocardial damage, whether acute or chronic, slight though it may be, which undoubtedly underlies the majority of such cases. The following cases illustrate different types of this rather complex condition.

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1. Krumbhaar, E. B.: Adams-Stokes Syndrome, with Complete Heart Block without Destruction of the Bundle of His, THE ARCHIVES INT. MED., 1910, **5,** 583.

2. Krumbhaar, E. B.: Transient Auricular Fibrillation, THE ARCHIVES INT. MED., 1916, **18,** 263.

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5. Hart, T. S.: Functional Heart Block, Am. Jour. Med. Sc., 1915, **149,** 62.

I. Transient Partial A-V Block in Acute Rheumatic Carditis.—The case selected as an example of this type of block was observed during a recurrence of acute articular rheumatism. It illustrates the temporary nature of the block, a parallelism between the block and the degree of rheumatism, the type of ventricular arrhythmia produced, and the influence of vagus stimulation and inhibition on the disturbed mechanism.

Case 1.—M. C., single, white, actress, aged 34, was admitted to the medical service of the University Hospital, Feb. 19, 1915, complaining of "an acute attack of rheumatism" of two weeks' duration, following tonsillitis and exposure. Severe pains occurring simultaneously in several joints and in most of the muscles of the body, were partially relieved by salicylates, but still persisted in the back, sides and arms. The wrist and elbow of the left arm were painful, tender, red and swollen.

When 16 years old the patient had had a severe attack of rheumatic fever, with cardiac complications, and since then she had had several recurrent attacks, and had experienced more or less palpitation and dyspnea. Her profession exposed her to inclement weather and she was addicted to excess in

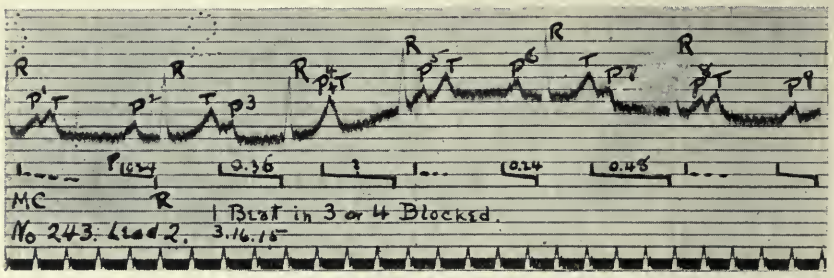

Fig. 1.—Case 1. Electrocardiogram (Lead 2) of M. C., showing partial heart block. It will be noticed that the first, fifth and eighth P waves fail to be followed by a ventricular complex. After each such "dropped" beat, the succeeding P-R interval is almost within normal limits, but the P-R interval of each succeeding cycle is lengthened until another "dropped" beat occurs. In this, as in other electrocardiograms of this series, platinum wire strings were used with resistances varying between 3,000 and 4,000 ohms. The tension of the string was so standardized that a change in potential of 1 millivolt caused a deflection of 1 cm. As the string could not be standardized with the patient in circuit, 1,400 ohms were added as an arbitrary equivalent of the patient's resistance. Time intervals are expressed by a Jaquet time marker in fifths of seconds, and occasionally by vertical lines indicating $\frac{1}{5}$ and $\frac{1}{25}$ second.

eating and late suppers, with moderate indulgence in beer, whisky and coffee. Several other members of the family suffered with "rheumatism." At no time during the present illness had the patient received any digitalis or allied drugs.

Physical Examination and Course of Illness.—The condition of the muscles and joints corresponded to the description given by the patient. Dental caries, pyorrhea alveolaris, general nervousness and the state of the heart were the other points requiring consideration. The heart dulness began above at the third rib, the right border was 3 cm. to the right of the midsternal line, and the left border 12 cm. to the left of the midsternal line and 2 cm. outside the midclavicular line. There was a well-marked apex beat in the fifth space, 2 cm. inside the limit of dulness. At the apex was heard a loud, harsh, blowing systolic mur-

mur, largely replacing the muscle sound and transmitted to the axilla. The second pulmonic sound was slightly accentuated. The radial pulses were equal, synchronous, regular, of moderate quickness, volume and tension, and not sclerotic. The patient refused to have the routine electrocardiogram taken at this time. Blood pressure: 110 systolic, 70 diastolic; hemoglobin, 70 per cent.; red blood count, 4,260,000; leukocytes, 13,200. The urine contained a trace of albumin, leukocytes, mucus, epithelial cells and no casts.

In short, the case was that of an ordinary recurrent attack of acute articular rheumatism in a patient suffering with chronic mitral endocarditis (regurgitation). During the first week of her stay at the hospital an extension to other joints and an increase in the temperature indicated that the administration of salicylates should be increased. Following 1.5 gm. of sodium salicylate every hour the temperature was quickly reduced so that the dosage was reduced to 0.3 gm. every. fourth hour. Four days later, full doses were resumed in

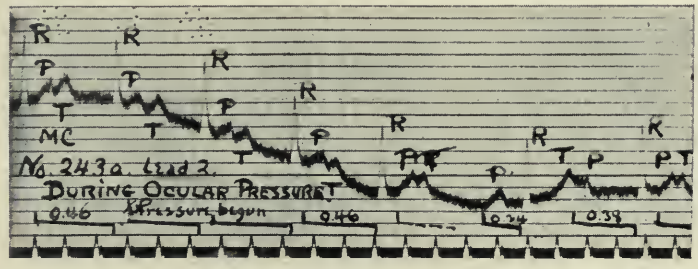

Fig. 2.—Case 1. Electrocardiogram (Lead 2) of M. C., showing the effect of vagus stimulation, by ocular pressure, on partial heart block. Note in the first four beats that although the P-R interval is greatly prolonged, it is equal in all, and no beat fails to provoke a ventricular response. After the fifth P wave is blocked, there is a return to gradual prolongation of the P-R interval.

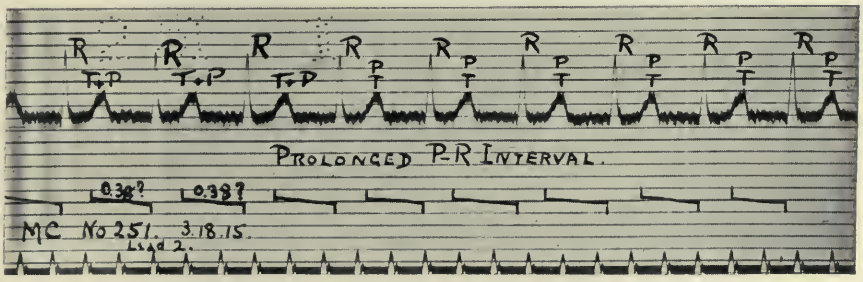

Fig. 3.—Case 1. Electrocardiogram (Lead 2) of M. C., after the administration of 1 mg. atropin. No beats are blocked, and the P-R intervals are equal. They are so long, however, that the P wave is superimposed on the preceding T.

response to higher fever and a new involvement of the right wrist. The patient again responded to treatment after four days, but the medication was continued for another week, at the end of which time the heart rate dropped suddenly from 90 to 44, the sounds became of poorer quality and the second pulmonic sound was occasionally reduplicated. The cardiac dulness was found to be 1 cm. wider, both to right and left, than on previous examination. Although no note of cardiac arrhythmia was made until one week later, on which date the record of Figure 1 was taken, it is most probable that the arrhythmia began at the time the first drop in rate was noted. The electrocardiogram shows a varying degree of partial block. In Lead 2 there is a gradual increase

of the P-R interval, until, after about every fourth auricular impulse, the ventricle fails to respond. Following such a "dropped" beat, the P-R interval is shortened and a similar cycle recommended. In Leads 1 and 3 the degree of block is different, there being a 2:1 rhythm in which every other auricular contraction fails to be followed by a ventricular contraction. These findings confirmed polygraphic tracings taken on the same day. Contrary to expectation, during vagus stimulation by ocular pressure the degree of block was slightly less. The "dropped beats" occurred as before, but with lesser frequency, and the preceding P-R intervals, though prolonged for a short time to more than 0.4 second, were all equal for several beats. Electrocardiograms taken on the next two days revealed the same state of affairs. At this time, 1 mg. of atropin administered hypodermically proved sufficient temporarily to prevent the "dropping" of beats. The P-R interval, however, remained prolonged (0.38 second) and there was no appreciable rise in the auricular rate. After four days

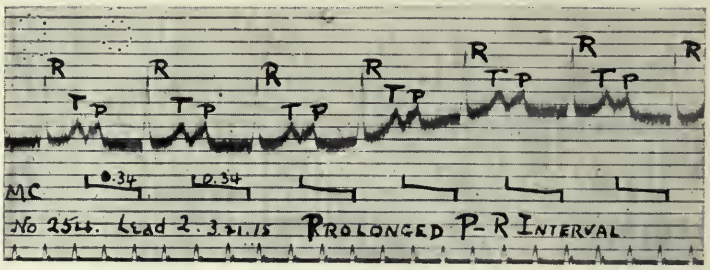

Fig. 4.—Case 1. Electrocardiogram (Lead 2) of M. C., three days later after the administration of 1 mg. of atropin. The slightly slower rate (longer diastole) and shorter P-R interval allow the P wave to be distinguished from the preceding T.

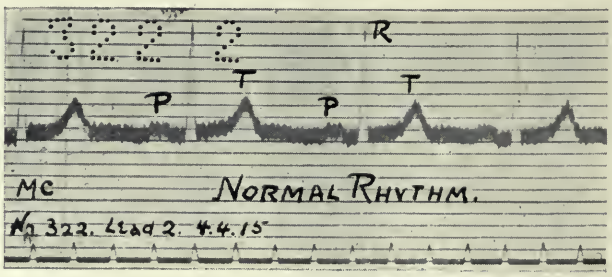

Fig. 5.—Case 1. Electrocardiogram (Lead 2) of M. C., showing normal rhythm and normal P-R interval.

of atropin medication (0.3 mg. hypodermically three times a day), the rhythm remained regular for several days; the P.R interval was not as much prolonged as before, and the P wave no longer coincided with the preceding T. In other words, although the block was not due to medication, and from the nature of the disease process was presumably due to an acute myocarditis involving His' bundle, nevertheless the administration of atropin was sufficient to prevent any beats being "dropped." Does this indicate that the vagus as well as the diseased bundle was a factor in the block, or does it indicate that atropin exerts a direct dromotropic effect on His' bundle? Two days after this tracing was taken, the acute arthritis recurred in one finger, together with fever and episcleritis of the right eye. Electrocardiograms at this period showed the same condition of partial block as before described. Atropin medication was again

begun, together with atophan. In four days the temperature had returned almost to normal, the patient was without pain, and the cardiac rhythm was regular. From that time until the patient's discharge from the hospital, electrocardiograms showed not only a regular rhythm, but a normal P-R interval (0.19). A slight recurrence of pain, swelling and redness in two knuckles of the left hand, with a rise of 1 degree in temperature, did not have any effect on the cardiac rhythm, and the patient progressed to an uninterrupted recovery from the acute rheumatism. It was later ascertained, however, that the patient died, within two months of the time of her discharge, of acute yellow atrophy of the liver. In the terminal illness the heart continued regular at the rate of 100 beats per minute. Necropsy was refused.

DISCUSSION

During an attack of recurrent acute articular rheumatism in a patient suffering with chronic mitral endocarditis, cardiac hypertrophy and probably some chronic myocarditis, there was presumably superimposed an acute myocardial involvement of His' bundle. This was extensive enough to lower the conductivity of the bundle sufficiently to cause partial heart block. Vagus stimulation failed to change the stage of heart block, but did slightly change its character. After the administration of atropin, impulse conduction was delayed but no longer blocked. Coincident with improvement in the other rheumatic symptoms, the block disappeared, to reappear again with a recrudescence of symptoms. At this time, during the administration of atropin, the dropped beats disappeared and the P-R interval became normal. A third minor recurrence of rheumatic symptoms, however, failed to affect the now normal heart rhythm, which continued normal as long as the patient was under observation. The administration of salicylates cannot be considered to have had any connection with the production of the block; not only because this class of drugs is not considered to have any effect on conductivity, but also because the degree of block in this case usually varied inversely with the degree of salicylic medication.

It is of interest that although the block was presumably of myocardial origin, it was nevertheless influenced by factors affecting the vagus, and thus indirectly affecting the damaged conductive system.

II. Transient Complete A-V Block Due to Digitalis.—Transient digitalis block of lesser degrees is not an uncommon condition, but a transient complete block, especially when following relatively small doses of digitalis, must be considered as very unusual.

CASE 2.—M. F., married, Irish, hospital orderly, aged 65, had been suffering for over a year with attacks of giddiness, weakness, loss of vision, and on two or more occasions apoplectiform attacks, which lasted some hours, but were not followed by any hemiplegia. On one of these occasions he was admitted to this hospital for one week, but no bradycardia or arrhythmia was observed, although tincture digitalis (0.32 c.c. three times a day) was given for six days. His past and family histories are unimportant, except for a life of hard work and exposure, with moderate use of alcohol, tobacco, tea and coffee.

Physical Examination and Course.—On the present admission, Jan. 11, 1916, the following pertinent signs were observed: blood pressure, systolic, 130, diastolic, 70; marked pyorrhea; emphysematous chest; cardiac dulness: right border 4 cm. from midline, left border 14.5 cm. from midline, outside of left midclavicular line; apex beat neither visible nor palpable; heart sounds weak, no murmurs; blood and urine normal.

Tincture of digitalis (0.65 c.c. three times a day) was given for two days, when it was noticed that the patient's pulse had become slow (38 beats per minute) and irregular. It was afterward ascertained that previous to admission, the patient had taken 0.3 c.c. of the tincture of digitalis four times a day for four days, thus making a total dosage of only 8.7 c.c. of the tincture spread over six days. An electrocardiogram taken at this time showed a complete block with

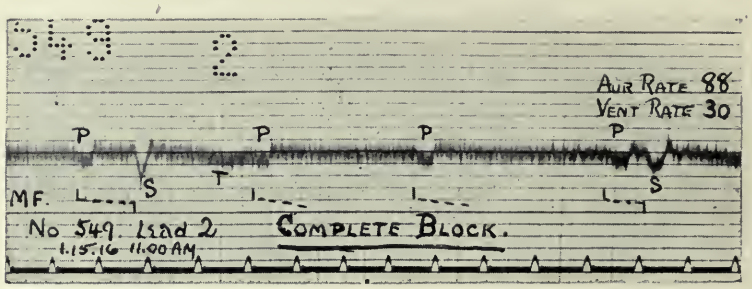

Fig. 6.—Case 2. Electrocardiogram (Lead 2) of M. F., showing complete heart block. Note that there is no constant relation between the occurrence of P and S (ventricular complex); although P twice falls before S, the P-R interval is different in both cases. The complete dissociation is even more obvious when the whole length of film is consulted. Note that the P waves are inverted and the ventricular complex of peculiar shape.

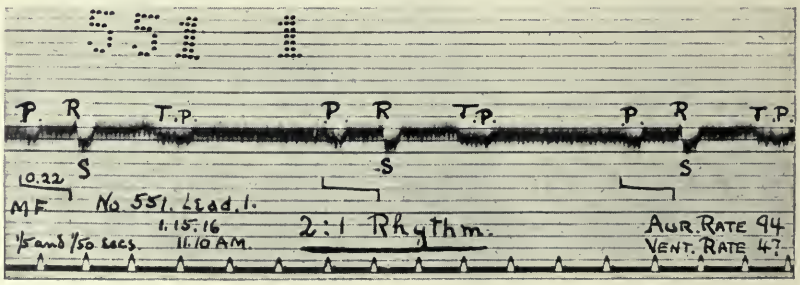

Fig. 7.—Case 2. Electrocardiogram (Lead 1) of M. F., showing partial heart block (2:1 rhythm) after the administration of 2 mg. of atropin.

a varying form of ventricular complex (auricular rate, 88; ventricular rate, 30). Not only were the ventricular deflections very small, but the heart sounds were quite inaudible. Immediately after this record was made, 2 mg. atropin were given hypodermically. The pulse rate rose in ten minutes to 50, and an electrocardiogram showed that the complete block had been replaced by a 2:1 rhythm.[6] The P-R interval was longer than normal (0.22 second). One-half hour later,

6. This might be interpreted as a normal rhythm, the second P wave being considered as part of a long diphasic T; but such an interpretation is highly improbable, as it would presuppose not only a very unusual form of T wave, but also the very slow auricular rate of 47.

complete block was reestablished (auricular rate, 100; ventricular, 46). During all this period the patient was quite comfortable, resting quietly in bed. Digitalis medication was stopped and thirty-six hours later the pulse rate had risen to 70, about which point it stayed during the rest of the patient's stay in the hospital. The P-R interval, however, remained prolonged (0.20 second), and was not changed by increasing the heart rate with atropin. When seen two months later, the patient had had no more "attacks," though he was still somewhat "shaky" in his legs. Electrocardiogram showed a normal rhythm, but the P-R interval still remained long.

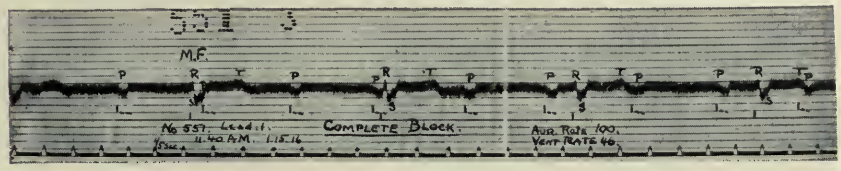

Fig. 8.—Case 2. Electrocardiogram (Lead 1) of M. F., showing the return to complete heart block, forty minutes after the administration of atropin. Note that P occurs at regular intervals but without relation to, and occasionally superimposed on, the ventricular complex (R S).

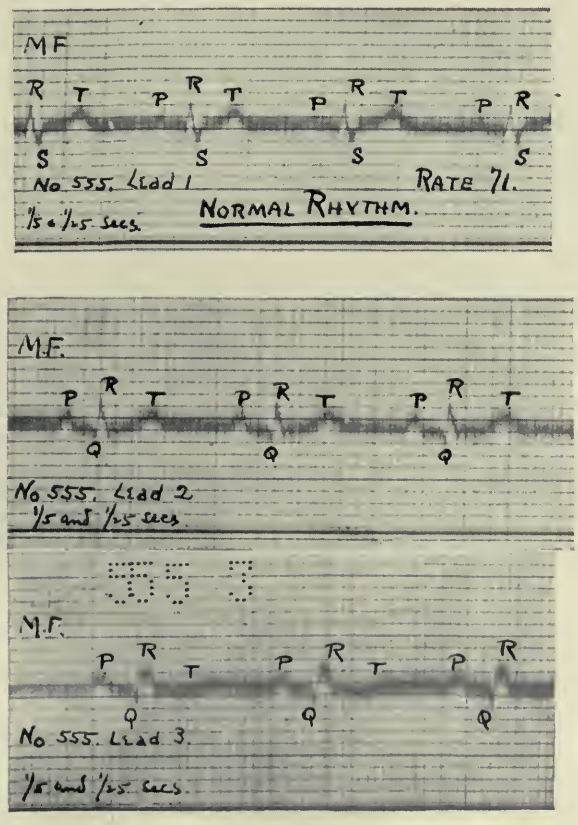

Fig. 9.—Case 2. Electrocardiogram of M. F. (three leads). Normal rhythm. Note also that the P waves are no longer inverted and that the ventricular complexes are of normal shape.

DISCUSSION

In an old man suffering from chronic myocardial degeneration and arteriosclerosis, and subject to spells of weakness and unconsciousness, moderate digitalis dosage for less than a week, brought on a transient, complete A-V heart block, with varying types of ventricular complexes. This was temporarily relieved by atropin, and disappeared spontaneously thirty-six hours after the digitalis was stopped. Although digitalis was obviously the determining factor in the causation of the block, it is but fair to assume that degenerative changes in the fibers of His' bundle contributed to the easier production of the digitalis effect. The temporary cessation of the block after the administration of atropin offers an interesting comparison with the first case. If in that case, as seems probable, atropin exerted a direct dromotropic effect on His' bundle, one might assume that here also the atropin effect may have been obtained partly through direct action on the bundle, although on account of the obvious importance of digitalis effects, the paralyzing action on the vagus must, of course, also be taken into account. The varying forms of ventricular complexes during the digitalis period are of the same kind as previously described by Cohn,[7] Oppenheimer and Williams[8] and Christian.[9]

III. Development of Defective Conductivity of Right Branch of His' Bundle.—The subject of defective conductivity in one or other branch of His' bundle has been thoroughly presented by Carter[10] and Matthewson[11] from the clinical, and by Eppinger and Rothberger[12] from the experimental, side. At least one case is on record also in which transient block occurred in the right branch of His' bundle during a febrile attack (Lewis[13]), and Carter cites instances in which, as in the present case, the bundle branch block is complicated by extrasystoles. The following case, however, is unique in one particular,

7. Cohn, A. E.: A Case of Transient Complete Auriculo-Ventricular Dissociation, Showing Constantly Varying Ventricular Complexes, Heart, 1913, **5,** 5.

8. Oppenheimer, B. S., and Williams, H. B.: Prolonged Complete Heart Block without Lesion of the Bundle of His and with Frequent Changes in the Idioventricular Electrical Complexes, Proc. Soc. Exper. Biol. and Med., 1913, **10,** 86.

9. Christian, H. A.: Transient Auriculoventricular Dissociation with Varying Ventricular Complexes Caused by Digitalis, The Archives Int. Med., 1915, **16,** 341.

10. Carter, E. P.: Clinical Observations on Defective Conduction in the Branches of the Ventricular Bundle, The Archives Int. Med., 1914, **13,** 803.

11. Matthewson, G. D.: Lesions of the Branches of the A-V Bundle, Heart, 1913, **4,** 385.

12. Eppinger, H., and Rothberger: Ueber die Folgen der Durschneidung der Tawaraschen Schenkel des Reizleitungssystem, Ztschr. f. klin. Med., 1910, **70,** 1.

13. Lewis, T.: Certain Physical Signs of Myocardial Involvement, Brit. Med. Jour., 1913, **1,** 484.

that the earliest record was taken at the time when the branch block was apparently in the process of formation, and opportunity was offered to follow the case until the block was permanently established.[14]

Case 3.—F. L., man, married, retired, aged 76, had been under observation for ten years for symptoms suggesting arteriosclerosis and myocardial weakness (precordial pains, especially after meals, dyspnea on exertion, enlarged liver, occasional cough). After exercise the precordial oppression increased and the pain occasionally radiated down either arm. The pulse had always been slow (55 to 65), the blood pressure was but moderately increased (average examples are: in 1912, systolic, 130; diastolic, 80; in 1913, 135 and 90; 1914, 155 and 80; 1915, 155 and 80; 1916, 155 and 70). A systolic murmur and an occasional slight arrhythmia had been noticed in the previous two years. The heart was enlarged to the left, the supracardiac dulness increased and the lungs emphysematous. The blood and urine were negative.

Except for an attack of biliary obstruction twenty-five years previously, and a tendency to constipation and flatulence, the past medical history is negative. For years the patient had smoked six to twelve cigars a day and indulged moderately in whisky. No venereal disease. Family history negative.

One month after the last electrocardiogram was taken, the patient died suddenly of acute cardiac failure. No necropsy was had.

Examination and Course.—In the first electrocardiogram, taken in January, 1915, although most of the complexes are of the type indicating left ventricular preponderance, occasionally one appears with the characteristic form of defective branch conduction, that is, notching and prolongation of the Q-R-S interval to more than 0.1 second. These occur as premature contractions. (The electrocardiogram is further complicated by the appearance of occasional auricular extrasystoles.) One month later almost all complexes were of this form and present typical examples of defective conductivity of the right branch of His' bundle. They are no longer premature. The auricular extrasystoles were still present. The P-R interval also had been prolonged from 0.20 second to 0.25 second. As the patient had been taking moderate doses of digitalis during this month, it was thought that a digitalis block might have been present, but 2 mg. of atropin hypodermically only raised the rate from 63 to 105 without altering the block. It became impossible to make further studies at this time, as the patient was living in another city. One year later, however, he returned, having been given digitalis for one month previously, and again the branch defect was found, this time in every complex. In fact, the electrocardiogram taken at this time was almost the counterpart of the one taken the year before, as far as the blocked complexes were concerned. All digitalis medication was stopped, but electrocardiograms taken on several occasions after this, on one occasion after one week's administration of atropin ($\frac{1}{150}$ grain by mouth four times a day) showed the same condition of defective conductivity to be constantly present. The patient felt badly when digitalis was given and was subjectively improved during the administration of atropin.

DISCUSSION

A man 76 years of age, suffering from arteriosclerosis, cardiac hypertrophy and chronic myocarditis, and mild anginoid attacks, was

14. Oppenheimer, B. S., and Rothschild, M. H.: Abnormalities in the Q R S Groups of the Electrocardiogram Associated with Myocardial Involvement. Soc. Exper. Biol. and Med., Dec. 20, 1916. These authors have pointed out that electrocardiograms can be produced by lesions involving terminal fibers of a branch of His' bundle, and claim a different picture for a block of the main stem of a branch.

examined electrocardiographically to determine the form of arrhythmia that was present. This was found to be due to occasional auricular extrasystoles, and anomalous ventricular beats, due to deficient conductivity in the right branch of His' bundle. One month later, almost all complexes were of this type, but no longer premature. The P-R interval was prolonged to 0.25 second, and the condition was not altered

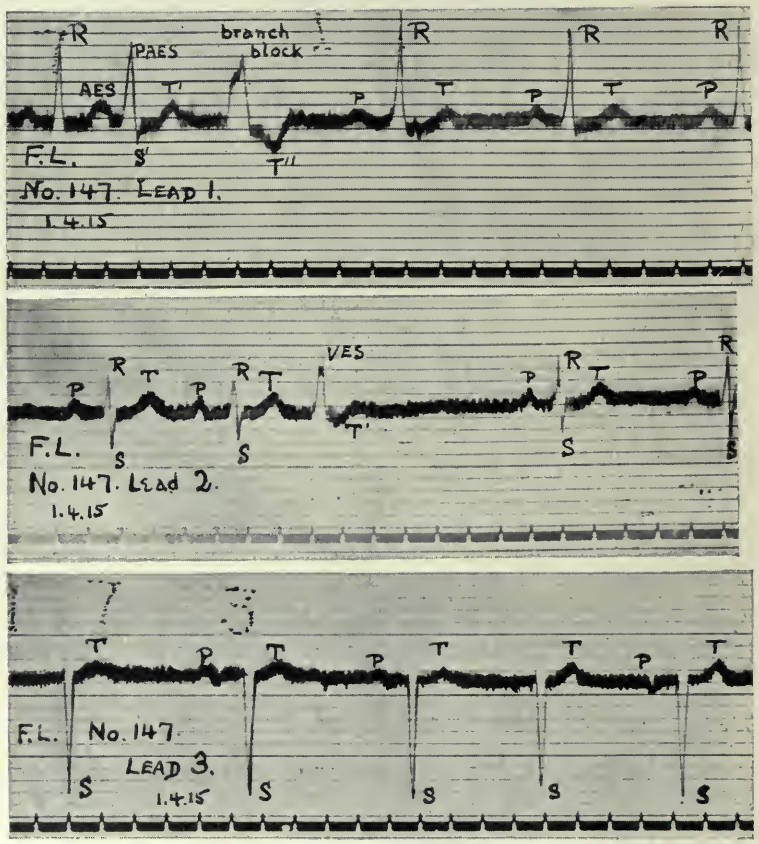

Fig. 10.—Case 3: Electrocardiogram of F. L. (three leads). Note that although most of the complexes are of the type indicating preponderance of the left ventricle, one in Lead 1 occurring prematurely is notched and broader (that is, slower). In the light of future records this is undoubtedly due to the impulse being blocked in the right branch of His' bundle. It is preceded by an auricular extrasystole and in Lead 2, an isolated ventricular extrasystole (probably of the branch block type) occurs. For purposes of reproduction, this print and the Q R S group of a few others have been retouched.

by atropin. One year later, in spite of long continued abstinence from digitalis, the same condition of defective conductivity was found to be present. It is, therefore, safe to assume that the defective conductivity (probably caused by the chronic myocarditis) was developing when

the patient was first seen and later became permanent, and it is important to note that the block developed without clinical symptoms or signs other than those revealed by the string galvanometer.

IV. Transient Prolongation of P-R Interval (of Unexplained Origin), Associated with Paroxysmal Tachycardia.—Prolongation of the P-R interval as a result of digitalis medication is too common a condition to require further comment. In the present instance, however, in a healthy young adult who also had attacks of paroxysmal tachycardia, the condition had occurred on at least two occasions in the absence of digitalis medication, or in fact of any other adequate cause.

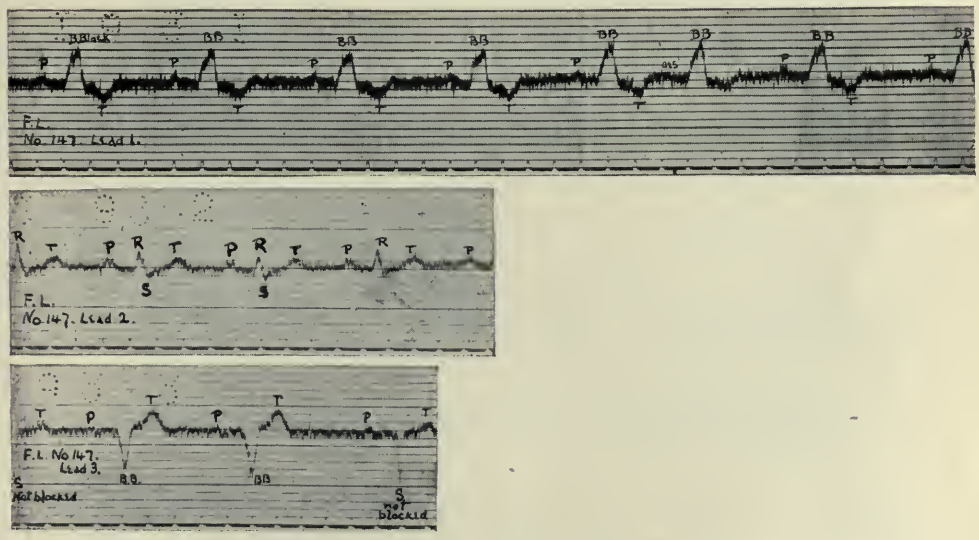

Fig. 11.—Case 3. Electrocardiogram of F. L. (three leads), showing defective conductivity (block in right branch of His' bundle) in almost all leads. Note that in Lead 3, two beats (S) are of the original type; all the others are notched and slow. An auricular extrasystole in Lead 1 is followed by the same kind of complex.

Case 4.—H. W. B., medical student, aged 23, was admitted to the student's ward of the University Hospital, Feb. 2, 1915, suffering from an attack of acute tonsillitis, vague joint pains and very rapid heart rate (over 150). These conditions subsided, after one day's rest in bed, with such suddenness that a diagnosis of paroxysmal tachycardia was made. After the paroxysm the pulse varied between 72 and 100.

The past history showed that the patient had had a slight attack of rheumatic fever when 10 years old, had had frequent attacks of tonsillitis since that time, and had a mild, chronic, atrophic nasopharyngitis. He smoked four or five cigarets and drank two cups of coffee a day, but took no alcohol. He denied any history of venereal disease. He was distinctly neurotic, with vasomotor instability, and at the time of admission had been studying unusually hard and worrying over examinations. His health was otherwise excellent

and he could undertake violent exercise without any cardiac distress, further than that he "notices that he has a heart."

Examination and Course.—The first electrocardiographic examination taken the day after admission, when the patient was feeling well, revealed a regular slow rhythm, normal in every respect except for the greatly prolonged P-R interval (0.36 second). On account of the recent history of tachycardia, however, another record was taken immediately after exercise. After climbing two flights of stairs, a task sufficient to raise a normal heart rate from 72 to 96, the patient's heart rate was raised from 78 to 108. An electrocardiogram

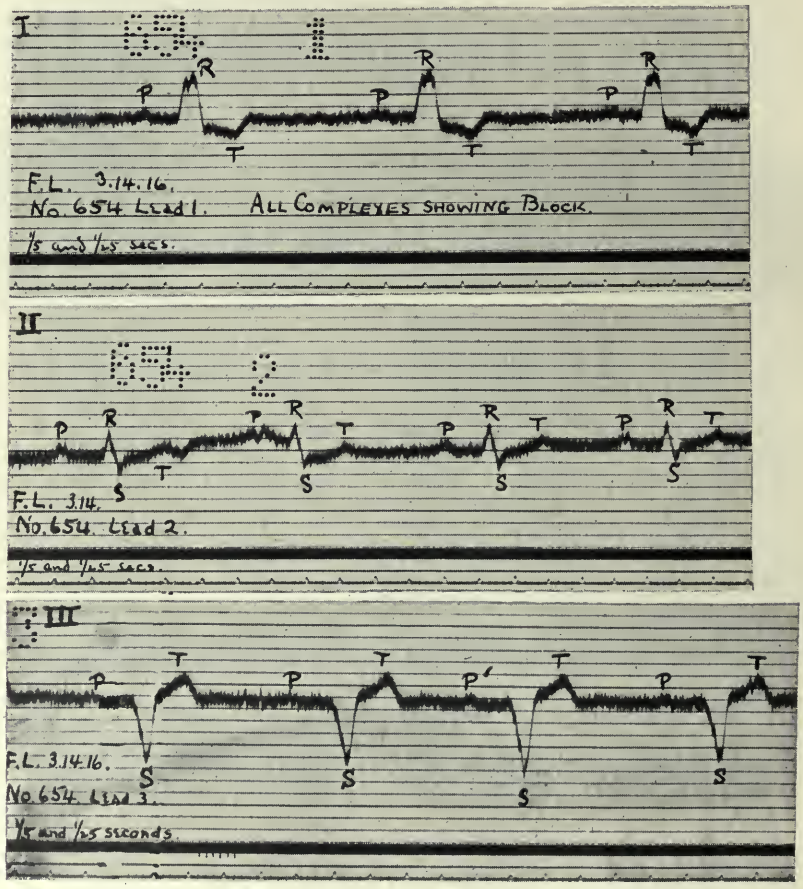

Fig. 12.—Case 3. Electrocardiogram of F. L. (three leads), showing defective conductivity (block of right branch of His' bundle) constant in all complexes.

taken at this time showed disappearance of the P wave (fused with the preceding T) during the rapid period (chiefly due to the prolonged P-R interval), and fortunately also caught the sudden cessation of the paroxysm, with reappearance of P. (Change in rate from 100 to 67.) (This record (Fig. 13) is almost a duplicate of figure 62 in Lewis' Clinical Electrocardiography). A record was then taken during forced respiration, which produced an arrhythmia, due both to sinus arrhythmia and to changes in the P-R interval. Atropin

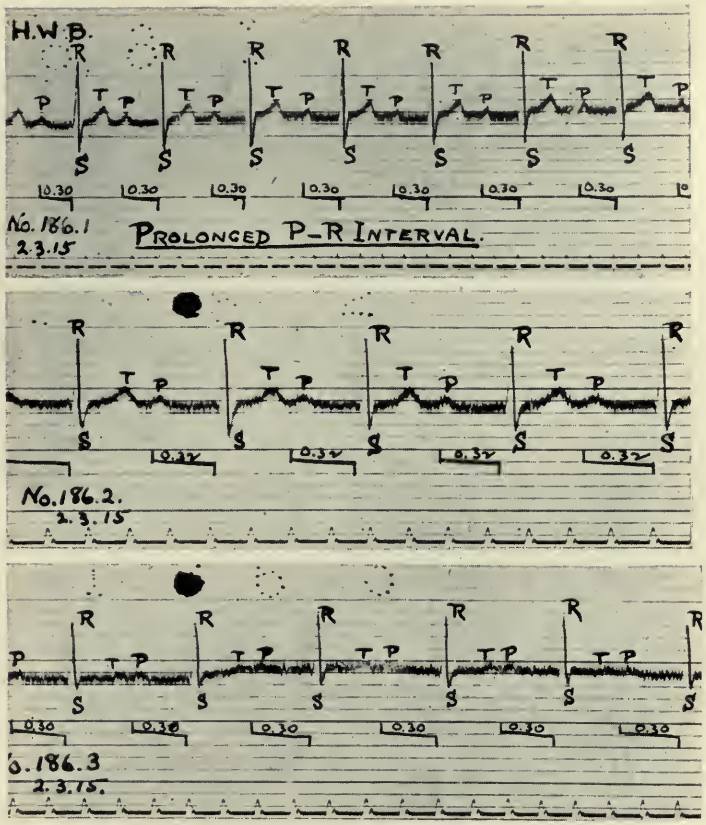

Fig. 13.—Case 4. Electrocardiogram of H. W. B. (three leads), showing prolongation of the P-R interval, earliest stage of heart block. Note that except for the greatly prolonged P-R interval (0.30 second) the record is normal.

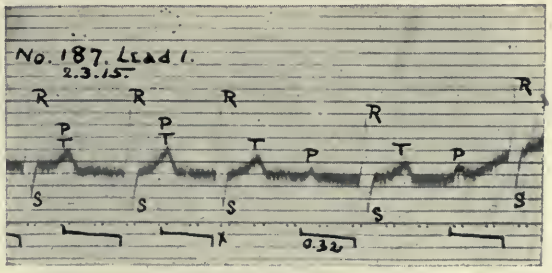

Fig. 14.—Case 4. Electrocardiogram of H. W. B. (Lead 1), showing termination of an attack of paroxysmal tachycardia. The P wave during the paroxysm is superimposed on the preceding T, so that it is apparent that the P-R interval remains prolonged during the paroxysmal period. The fact that this combined wave is higher than the single T wave of the normal period is an indication that the P waves of the paroxysm were upright, and therefore arose at or near the sinus.

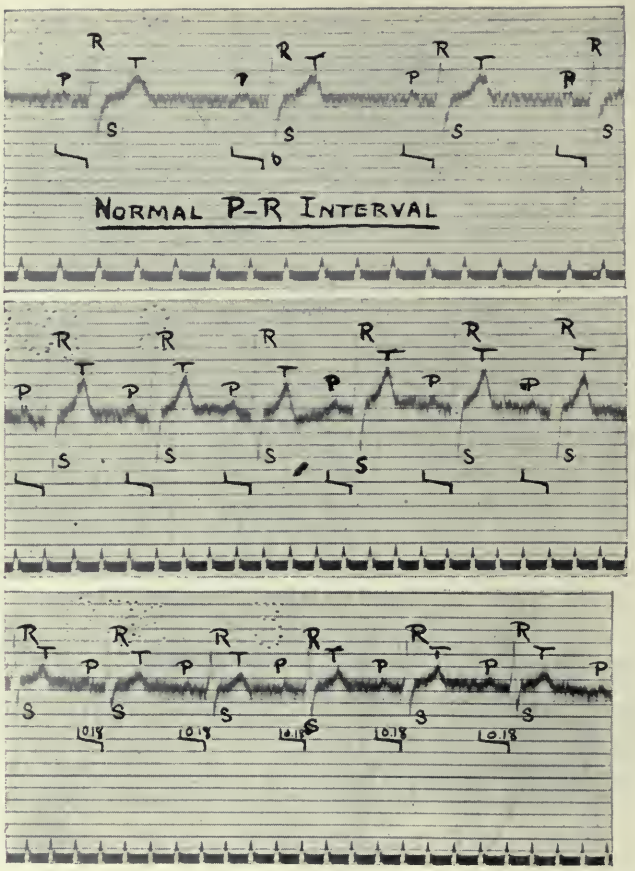

Fig. 15.—Case 4. Electrocardiogram of H. W. B. (three leads) showing normal rhythm with normal P-R interval (0.18 second).

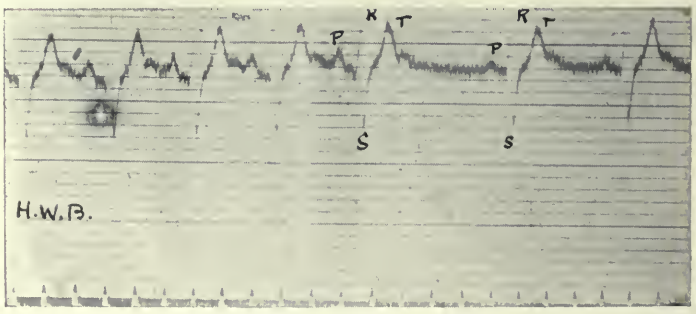

Fig. 16.—Case 4. Electrocardiogram of H. W. B. (Lead 2), showing termination of an attack of paroxysmal tachycardia with retention of the normal P-R interval. Note that the P wave is upright throughout.

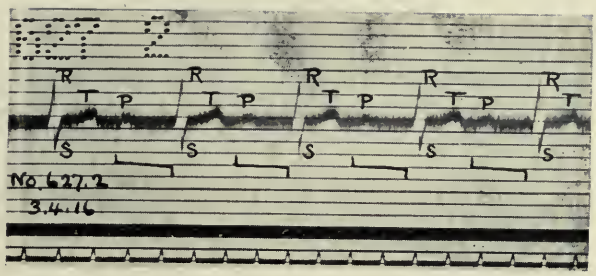

Fig. 17.—Electrocardiogram of H. W. B. (three leads), showing prolongation of the P-R interval one year later (similar to Fig. 12).

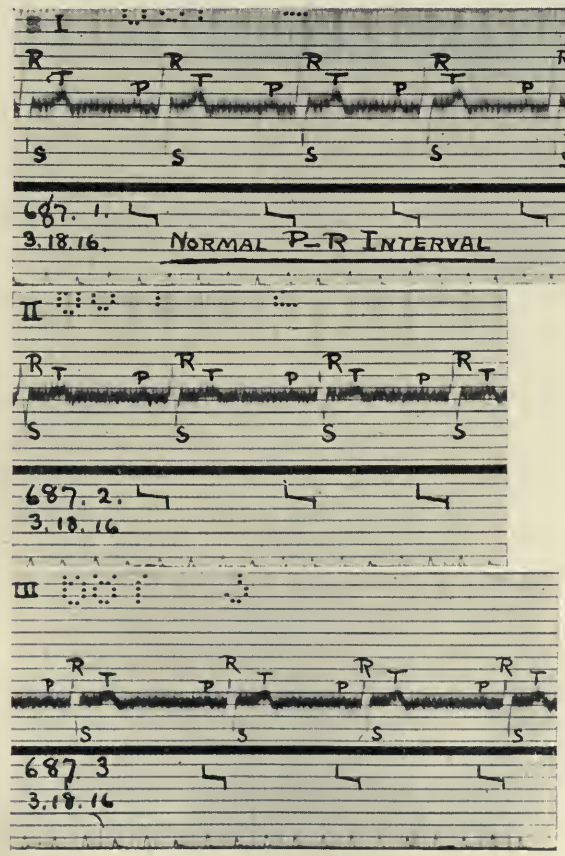

Fig. 18.—Electrocardiogram of H. W. B. (three leads), showing normal P-R interval, two weeks later than Figure 17 (similar to Fig. 14).

(2 mg. hypodermically) produced a tachycardia similar to that produced by exercise, raising the pulse rate from 85 to 128.

One month later, no medicine having been taken since leaving the hospital, an electrocardiogram showed that the P-R interval was now within normal limits (0.18 second). Except for an accentuation of S and T in all leads, the record is otherwise the same as on the previous examination. As the prolonged P-R interval produced no other signs or symptoms, the patient was ignorant of when the change to normal rhythm occurred. Exercise, however, produced a tachycardia similar to that first observed, except that as the P-R interval remained normal P and T were no longer fused. The rapid heart rate slowed to a normal rate during one cycle, as on the previous occasion. The post paroxysmal pause is longer than that of normal cycles, indicating that the P waves of the paroxysm are ectopic.

The patient was not seen for a year, during which time he was in good health. Two days before this visit, for no apparent cause (except a slight increase in cigaret smoking and in the chronic throat trouble), he observed that his pulse rate was 120, and on getting up suddenly, noticed that he would "feel his heart." Electrocardiograms again showed a long P-R interval (0.32 second), with excessive increase in the heart rate after exercise, and fusion of the P and T waves. This persisted for at least a week; but three weeks later, after the cigaret smoking had been stopped and the throat condition had improved, the P-R interval had again become normal (0.18 second). Since then a year has elapsed and the patient has continued in good health, without any cardiac symptoms.

DISCUSSION

A young, neurotic, male adult, with subjective cardiac symptoms only, was observed on different occasions to have an unusually long P-R interval and a tendency to the auricular (or possibly a sinus) form of paroxysmal tachycardia. This latter occurred at least twice spontaneously, and could be produced at will by exercise or with atropin. It occurred independently of the state of conductivity of the A-V system. For no apparent adequate cause, the P-R interval had been observed twice (an interval of over a year elapsing) to be more than 0.3 second. Once this followed a period of hard study and worry, and once an increase in cigaret smoking in the presence of a chronically inflamed throat. On both occasions the P-R interval returned to normal within a few weeks of the cessation of these conditions, and yet it would be presumptuous to assume that they had a causal relationship.

GENERAL SUMMARY

1 Four different types of transient heart block are described, analyzed and discussed:

(*a*) Transient partial A-V block of myocardial origin, occurring during an exacerbation of acute rheumatic carditis, varying with the degree of arthritis, yet responding to atropin.

(*b*) Transient complete A-V block, brought on by digitalis, and temporarily reducible by atropin to a 2:1 rhythm.

(*c*) The development of defective conductivity in the right branch

of His' bundle in an old man suffering with arteriosclerosis, chronic myocarditis and anginoid symptoms.

(*d*) Transient periods of prolongation of the P-R interval (to more than 0.3 second) without adequate cause in a healthy young adult male. He is also subject to paroxysmal tachycardia of auricular (or sinus) origin, which occurs independently of and does not affect the state of the conductive system.

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CHICAGO

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February, 1917, No. 2, vol. cliii, p. 235.

THE ROLE OF THE LEUKOCYTES IN VIRIDANS ENDOCARDITIS AND THE EFFECT OF NUCLEIN INJECTIONS.

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AND

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The work of the past ten years tends to lay more importance on the vital activity of the white-blood cells and their enzymes than was accredited to them during the period of enthusiastic adoption of the humoral theories. While the hopes raised by the work of Petterson, Hiss, Zinsser and others that leukocytic injections would enhance resistance in various diseases have not been fulfilled, these investigations have served the purpose of bringing out the true value of intraphagocytic and extraphagocytic bacterial destruction and the balancing of enzyme and antienzyme. The behavior of leukocytes in frank pyogenic affections is fairly well understood, but in the more chronic diseases, and those with less activity of the leukocyte-producing tissue, the subject is far from clear. The value of the leukocytes as a defense in typhoid has only lately received any approximately acceptable estimation, and in tuberculosis we know practically nothing about it. In no condition would a thorough understanding of leukocytic defensive activity be more helpful than in subacute endocarditis, a pathogenic process whose inception and continuation imply a high degree of adaption of invaders to defenses. It was with the hope of learning something of these phenomena and of means to increase leukocytic value that these studies were undertaken.

Subacute endocarditis is a process consisting of a local pathological lesion in which bacteria are growing and multiplying, surrounded and protected by thrombosis and vegetative masses, and whence they occasionally escape into the blood stream. The organisms in the vegetation are somewhat resistant to the defensive powers in the blood, while those in the blood stream seem to have adapted

themselves completely to both serum and leukocytes. They are relatively resistant to phagocytosis *in vitro* when freshly isolated, but the blood components having to do with phagocytosis are not necessarily reduced, and may, indeed, be actually above the values for normal sera. There is no bactericidal power of any value for the body's defense.

This being the case, immunity phenomena must be found, if present, on the part of the phagocytes. The facts of the matter, as shown by Rosenow, for endocarditis with pneumococci and related organisms, and our own work with Streptococcus viridans, are that opsonins may be subnormal or slightly increased, that phagocytic value in the patient's serum is low, and low or normal in normal serum. This lowered power of leukocytes in the presence of the patient's serum, Rosenow explains by the lack of a substance in the serum, distinct from opsonin, which stimulates intraleukocytic digestion, and probably another substance, possibly derived from the microbes themselves, which render cocci resistant to consumption and destruction. To confuse the worker in this matter, great variations occur in repeated examinations. In general, in our cases, the leukocytic or phagocytic average has been fairly low, the index low, and the patient's cells could be stimulated by normal serum. The suggestions of Rosenow are, therefore, acceptable in our 3 cases of viridans infection, but this is not so in a Streptococcus pyogenes case and one due to a diphtheroid.[1]

One very interesting viridans case gave the following test:

	Per cent. of phagocytic cells.		Phagocytic average.		
(*a*) Patient's serum + normal leukocytes	18	.5	.45	.83	.9
(*b*) Normal serum + normal leukocytes	12		.54		
(*c*) Patient's serum + patient's leukocytes	9	.75	.41	.95	.8
(*d*) Normal serum + patient's leukocytes	12		.43		

While this case shows indices falling within the range of error, it is, nevertheless, evident that the percentage of phagocytic cells is greater in the case of normal ones.

A second test gave practically the same figures, but a third one, two weeks later, resulted quite differently, and gains in importance in this study in view of the fact that the patient had had four doses of nuclein under the skin in the preceding week:

	Per cent. of phagocytic cells.		Phagocytic average.		
(*a*) Patient's serum + normal leukocytes	26.5	.72	1.975	.88	.7
(*b*) Normal serum + normal leukocytes	25.5		2.25		
(*c*) Patient's serum + patient's leukocytes	19	.86	1.56	1.13	.6
(*d*) Normal serum + patient's leukocytes	22		1.38		

It will be seen by this chart that the serum index had not improved beyond the limits of error, yet the interaction of his own combina-

[1] Zentralbl. f. Bakt., lxx, 143.

tion (*c*) and the reactive number of phagocytic cells had appreciably increased. In other words, then, while the serum values have not been seriously below the control, the former low value of the leukocytes, both in the patient's and normal sera, have been replaced by higher values and the former maladjustment of patient's serum and cells has been improved. (Compare *a* and *c* and *c* and *d* on both charts.)

The sum of evidence is, then, that the serum opsonin values may be nearly normal, but the phagocytic values are subject to great variations, usually appreciably below normal.

Complement-fixing antibodies have not been demonstrable in our cases, but in two viridans infections an anaphylatoxin could be found. The details of one case are worthy of citation:

(1) Cocci 0.5 c.c. emulsion, normal serum 0.5 c.c. G. P. complement 0.1 c.c. 37° C., 1 hour.
(2) Cocci 0.5 c.c. emulsion, pat. serum 0.5 c.c. G. P. complement 0.1 c.c. 37° C., 1 hour.
Pigs (all between 240 and 275 grams):
(A) 0.9 c.c., No. 1. No evidence of injection.
(B) 0.8 c.c., No. 1. No evidence of injection.
(C) 0.9 c.c., No. 2. Died in two minutes.
(D) 0.8 c.c., No. 2. Died in five minutes.

No. 3 began scratching almost at once; in a few seconds went into anaphylactic shock. Autopsy: marked emphysema, slight congestion, but no hemorrhage in gastric mucosa. Hemorrhage the size of a pea in pericardium.

No. 4 began twitching in one minute; shock appeared later than in No. 3 but typical. Autopsy same as No. 3, but no pericardial hemorrhage.

Subacute viridans endocarditis is, then, in all probability, a process due to a high pathogenicity locally, without bactericidal serum property, the circulating of bacteria and their anaphylatoxin and a lowered phagocytic power. The bactericidal effect is probably not missed, because antisera with this power are apparently of no value (Horder). Opsonins are powerless to stop the process because they can be raised by bacterin injections, with but little or no effect upon the disease. We are compelled, then, to investigate further the role of the phagocytes and the possibility of increasing their efficiency.

Phagocytosis is a process completed by the mutual relation of chemotactically positive substances and cellular adequacy. It would appear from the work of Marchand, Bail, Rosenow, Gruber and Futaki and others that certain organisms in the process of active parasitism, whether due to virulence, capsule, aggressins, or what not, are distinctly difficult of consumption by phagocytes. Such seems only slightly true of Streptococcus viridans as normal serum and leukocytes, or even infected serum, will act upon them,

there being, therefore, no antichemotactic power but probably antiopsonic power, which, as has already been cited, is accredited by Rosenow to some unisolated serum component. This may possibly be serum protease, for, as an anaphylatoxic state exists, this substance could be greater than normal (if one accept the theory of Jobling and his co-workers). At all events an anaphylactic condition is associated with leukopenia, and while we may not have a reduction of leukocytes in viridans infection, there is at least a very definite inadequacy of their phagocytosis and possibly of their number. Possibly they are kept busy supplying some neutralizing body for the antiopsonic power of the bacteria. It was recognized by Friedberger that in diseases like typhoid, where anaphylaxis can be shown, there is a lowered chemotaxis and a low leukocyte count; such conditions are said to respond to bacterial injection by a rapid and pronounced leukocytosis (Gay and Claypool). Unfortunately this does not occur satisfactorily in viridans infection, and we shall have to use other than bacterins for leukocytic stimulation.

Leukocytes respond to the liberation of protein of any kind, but especially of nucleoprotein; in other words, nucleoprotein has a pronounced chemotactic power. This is well shown in the leukocyte accumulations around bacterial bodies, dead or alive, or when the cells themselves have undergone destruction. This property has been thought of many times and use made of it in injecting nucleins to produce leukocytosis. The clinical use of this substance has fallen into disrepute for several reasons, the best probably being that it has not been thoroughly studied. We have repeated many of the old experiments with the hope of learning more of its properties and to see if it can be of value in viridans infections. The old controversy as to the value of increased leukocytes alone and their content of antibodies, while far from a complete settlement, does not affect the case.

In reviewing the literature germane to this subject we find no experimental or clinical work on the use of nuclein in viridans endocarditis, nor indeed, except bacterins, of anything which is supposed to act upon the leukocytes. The literature on nuclein in its theoretical value and practical therapeutic use, is relatively limited, most of the articles dating from the period in which there was an active controversy about the source of the bactericidal power of the blood. Vaughan, Buchner, and Hahn believe that they increased the resistance of experimental animals to the introduction of various germs when nuclein was administered either before or after the organisms. Vaughan, having isolated the constituent of normal serum to which he ascribed natural protection, and finding it undigested by pepsin, believed it to be nuclein. Then there followed the dispute as to the origin of antibody and complement, a matter now on a working basis, although not settled.

Whether or not leukocytes produced either or both, the results of the work of Petterson, Hiss, Mainwaring and others indicate clearly that some bactericidal power is to be found in the white blood cells and their extract, substances rich in nuclein. The act of phagocytosis is not sufficient to destroy parasitic organisms, and, as is shown by Neufeld and Rosenow, phagocytosis and intracellular destruction do not go hand in hand. Phagocytosis is due to appropriate chemotactic relations, while digestion is due to intracellular enzymes. The failure to complete both functions in every case is not understood. A persistent high number of leukocytes is indicative of a high resistance to organisms which have the chemotactic activity to call forth the cells; but it would seem that in subacute infections there is, despite the fairly high leukocyte count, an inadequacy on the part of the digestive intracellular power. Whether or not a great increase in white cells will supply this power, and whether or not nuclein could be the inciting agent for this purpose, are facts to be settled. The power of nuclein to produce an increase of leukocytes has long been known, and is ascribed in its therapeutic use to Horbaczewsky, a name mentioned by two writers, but we fail to find the original article.

The information about nuclein in the literature may be considered from three stand-points: how nuclein acts, the number and character of the leukocytes in the blood after injection, and the therapeutic effects. The opinion held by most observers is that nuclein exerts a distinct chemotactic effect on the leukocytogenic organs with the appearance of new cells. Others, notably Mendel, consider the new cells as appearing in response to destruction of old ones, this being due to the nuclein introduced. Still others believe there is a squeezing out of leukocytes from tissue spaces by contraction of involuntary muscles, which follows a subsidence of internal vasodilatation, a condition of the vessels shown by Mendel. There is less divergence in the observations upon the kind and number of circulating white cells. Nearly all authors find a decrease in total cells, followed by an increase, and most of them note an increase of the small lymphocytes and decline of the polymorphonuclear neutrophiles. We shall show that this is only a percentage increase and decrease. It is historically interesting to mention that Ames and Huntly cite a primary increase in the lymphocytes as a high percentage of young forms, whereas later the adult or polynuclears are increased. Pankow expressed little faith in nuclein, believing the effect to be due to saline solution; this we shall see is erroneous. The experimental and clinical therapeutics of nuclein begin as far back as 1892 by Löwitt, then by Vaughan in 1896, after which no one seems to have worked much with the substance until the period 1903 and 1907, during which time several articles appeared. The most noteworthy of these are three coming from the Mikulicz Clinic by Mikulicz, Miyake, and Renner. They

found that by the use of yeast nuclein they could increase the resistance of experimental animals to artificial infection and could materially reduce the morbidity and mortality from postoperative peritonitis. They lay stress on the fact that they could get greater protection and greater number of leukocytes (20 to 8) by the use of nuclein than by the use of aleuronat, which they class as a mechanical attractor of leukocytes. Renner, experimenting with dosage in human beings, used 50 c.c. of a 2 per cent. solution, which means 17 mg. per kilo for a man of 70 kilo. There was usually a mild general reaction and a local puffy, sensitive swelling at the site of inoculation. Their own description of the after effects is not in harmony with their nonchalance as to the severity of the reaction. Judging by the reaction we observed in dogs, this injection must be painful. According to Renner, the reaction on the part of the leukocytes takes place anywhere from six to twenty-four hours later, and one cannot foretell when it will appear or when the effect or the decrease will be greatest. The leukocyte increase is usually pronounced, being in his highest case 452 per cent. They say that this procedure, while of distinct clinical value, is not effective against fulminating streptococcal infections.

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EFFECT OF NUCLEIN INJECTION UPON THE LEUKOCYTES OF DOGS.

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The result of work upon the effect of nuclein in experimental animals has not been uniform, probably because under this name both the acid and its sodium salt have been employed and no standardization has been attempted. In approaching this subject, we determined to employ the product accepted by the American Medical Association, nucleinic acid or its sodium salt, and to use the phosphorus percentage as our guide.

In counting blood cells three pipettes were prepared for each count, three chambers counted of each pipette, and the number of leukocytes calculated from the average. Differential counts were made of 400 cells in each case.

Counts were made immediately before the injection, and after the lapse of ½ hour, 1 hour, 2 hours, 4 hours, 7½ hours, 24 hours, 48 hours, 72 hours, and in some cases 96 hours after the injection. The effect of repeated puncturing of the dog's nose was controlled by taking counts on several untreated dogs. The effect of the injection of the menstruum in which the nuclein was to be administered was controlled by making counts after the intravenous and subcutaneous injection of physiological salt solution and 15 per cent. alcohol. The sodium nucleinate solution was prepared in 15 per cent. alcohol and physiological saline, in every case immediately before injection, and an amount of nuclein equivalent to 0.5 mg. phosphorus per kilo of dog weight was injected subcutaneously and intravenously in solution, 1 c.c. of which was equivalent to 1 mg. of phosphorus. The dog with which most work was done was a female of the fox terrier type, varying in weight from 7 kilos to

10 kilos in five months; of 24 counts taken in that time without treatment, the lowest was 7000 and the highest 13,000.

The injection of alcohol or of physiological salt solution, either intravenously or subcutaneously, was followed by no variation that did not fall within normal limits.

After the injection of sodium nucleinate, whether in 15 per cent. alcohol or physiological salt solution, whether subcutaneously or intravenously, there is a primary fall in the whole number of peripherally circulating leukocytes. This reaches its lowest point between the first and second hour, after which it starts to rise. The count is back to normal between four and seven hours, but the rise continues, until at the end of twenty-four hours the highest point is reached. From the twenty-fourth hour there is a gradual decline, the normal being reached between the seventy-second and ninety-sixth hour. In every experiment the curve greatly exceeded any figure for a similar period without treatment. The results of intravenous and subcutaneous injections are comparable, the clearness of the results, however, being greater by the former method.

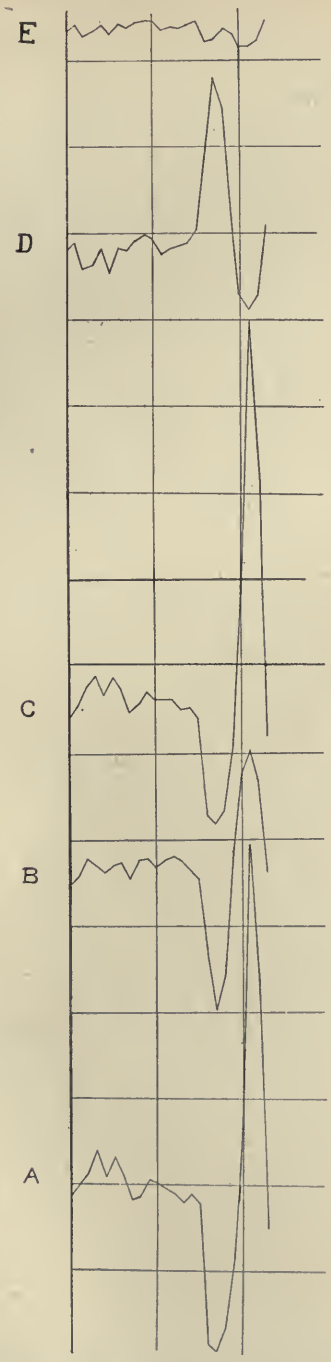
Renner was able to raise the leukocytes 452 per cent. in his highest human case. Our greatest increase in the dog has been 290 per cent. of the original count.

The differential counts show after nuclein injection a relative and absolute decrease of polymorphonuclear neutrophiles, followed by a rise. This fall and rise of the polynuclear is largely responsible for the fall and rise of the whole count. Accompanying this curve of the polynuclears there is a similar downward and upward course of the small lymphocytes, but it is of much less marked degree and more variable in its percentage; so much so, indeed, that no definite curve can be platted which will be followed in every experiment. In every case, however, the percentage of lymphocytes rises as the percentage of the polynuclears falls, and falls as it rises. This in itself shows that the gross variations in the total count must be due to polynuclear fall and rise. We are not now prepared to discuss the variations of the other leukocytes.

In a dog with an orginal leukocytosis and an eosinophilia the effect of the nuclein was the same as in the case of dogs with a normal blood picture.

In order to see if daily or twice daily injections of nuclein in 15 per cent. alcohol subcutaneously in quantities equivalent to 0.1 mg. of phosphorus per kilo would increase the leukocytes continuously and maintain them at a high level, a dog was given this treatment. There was a trifling rise of the whole number and of the percentage (therefore also the absolute number) of polynuclears. There was no continued rise, and the increase was irregular. This line of experimentation will be pursued further.

The Arneth formula in dogs is about as follows: 18, 40, 30, 7, 3. The injection of alcohol and saline has no appreciable effect upon



The curves are drawn to scale. *A*, variations in total number of white blood cells; *B*, variations in the per cent. of polymorphonuclear neutrophiles; *C*, variations in the number of polymorphonuclear neutrophiles; *D*, variations in the per cent. of small lymphocytes; *E*, variations in the number of small lymphocytes.

it. Following an injection of nuclein, especially when given into the vein, there is a great relative and numerical increase of Type 1, cells with a single nucleus, amounting in one case to 52 per cent. of the polynuclears, while Type 2 remains about as normal and Types 3, 4, and 5 decrease. The rise of Type 1 seems to reach a high point in four hours corresponding to the time at which the leukocytes are on the increase and remains during the period in which the polynuclears are rising. Types 3 and 4 experience a fall roughly corresponding to the rise of Type 1, and later, corresponding again to the fall of Type 1, show a distinct increase, this arriving about the time that the leukocytes have resumed their usual numbers.

Nucleated reds have been encountered very occasionally in normal blood. After the injection of nuclein subcutaneously they are slightly increased, but when the substance is given into the vein they are increased quite markedly, in one case rising to eight times the number before injection. This rise appears during the time the leukocytes are falling. From a few observations it does not seem that there is any reduction in the number of red blood cells.

In 4 cases general toxic effects were noted after the intravenous injection of nuclein. The dogs became very restless for a few minutes, then lay quiet but trembling on the floor. After one and a half hours there was vomiting of a frothy yellow material, which vomiting was repeated throughout the day. On one occasion there were no general toxic symptoms.

Tentative conclusions from this work are that after nuclein injection there is a reduction in the circulating peripheral leukocytes, chiefly of the polymorphonuclears, and to a much less degree of the small lymphocytes, followed by a rise in both, but very largely of the polynuclears, even to 95 per cent. of the whole count. The total leukocyte count in dogs may be increased 290 per cent. in twenty-four hours. Repeated small doses of nuclein do not cause and maintain a high tide of leukocytosis. There seems to be destruction of polynuclears because of their great decrease, and the relative and absolute increase of young forms on the left side of Arneth's formula. The appearance of these young forms and of an increase in nucleated reds speaks for an increased activity of the bone marrow, therefore a stimulation of the tissue, but whether by the nuclein injected, as a response to the paucity of circulating white cells, or by excitation by the detritus of destroyed leukocytes we have not yet determined.

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A CLINICAL STUDY OF CHLORIDE EXCRETORY FUNCTION.

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Introduction. The purpose of this investigation has been to study the value, as a clinical test, of the method of measuring sodium chloride excretory function, recently introduced by McLean.[1]

McLean has modified the formula devised by Ambard and Weill[2] to express the laws of excretion of sodium chloride in normal individuals, so that, given the data of rate of excretion of urine, concentration of chloride in the urine and the body weight of the subject, the concentration of chloride which would be found in the blood plasma could be calculated, provided the excretory function was normal. This modified formula is expressed as follows:

$$\text{Plasma chloride} = 5.62 + \sqrt{\frac{\text{Gm. NaCl per day} \sqrt{\text{Gm. NaCl per liter}}}{4.23 \times \text{body weight in kilos.}}}$$

The figure 5.62 refers to the normal threshold for chlorides, or concentration in the plasma at which excretion begins. Ambard and Weill found that excretion began when the concentration of chlorides in the blood plasma had risen above 5.62 per liter. The figure 4.23 is a constant introduced into the formula.

The theoretical concentration of plasma chloride calculated from the formula may then be compared with the actual concentration found by analysis of the blood plasma. The supposed threshold of beginning excretion for any case under study may be calculated from the following formula:

$$\text{Threshold} = \text{plasma chloride} - \sqrt{\frac{\text{Gm. NaCl per day} \sqrt{\text{Gm. NaCl per liter}}}{4.23 \times \text{body weight in kilos.}}}$$

[1] Jour. Exper. Med., 1915, xxii, 234.
[2] Semaine méd., 1912, xxxii, 217.

McLean found in a large series of examinations in normal individuals that the theoretical concentration corresponded fairly closely with the concentration actually found. He then applied the method to the study of pathological conditions[3] and found that relatively increased concentration of chlorides in the plasma occurs especially in certain forms of cardiac and renal disease; that edema is usually accompanied by a relatively increased concentration of chlorides in the plasma, also that chloride and urea functions may be quite independent of one another.

The method of studying chloride excretory function hitherto most generally employed has been the test of the ability of the kidneys to excrete salt added to the diet. The patient is put upon a constant daily chloride intake until equilibrium between intake and excretion is established. Then on a certain day, 10 grams additional sodium chloride are given. If less than 85 per cent. of the added chloride is excreted within forty-eight hours it is interpreted as a retention.

Frothingham[4] studied 40 cases of chronic nephritis, comparing the test of ability of the kidney to excrete added salt with certain other diagnostic and prognostic tests of renal disease. Of the 40 cases, but one showed no impairment of ability to excrete added salt, whereas 14 excreted phthalein in normal amount. Frothingham's table shows that 9 of the cases with normal phthalein had very markedly impaired ability to excrete the added chlorides. Frothingham concluded that the added salt test is the best functional test for the detection of early chronic nephritis, but that it is of not much value in prognosis, since the ability to excrete added chloride may be greatly impaired relatively early in the course of the disease.

O'Hare[5] has studied 15 cases of nephritis by McLean's method of testing chloride excretory function and compared the results with those obtained by the Hedinger and Schlayer two-hour renal test and test of ability to excrete added chloride. He concludes that the McLean test is more satisfactory than the others.

Although the added salt test is undoubtedly of great value in the study of nephritis, it has many obvious disadvantages. The patient should be in a hospital, preferably under the care of a nurse trained specially to give metabolic diets. The test requires five or six days' time to be carried out properly. During this time all the urine must be carefully saved. If, as not infrequently occurs in nephritic cases, the patient vomits after the administration of the added salt the test is lost. Thus its application must be limited almost entirely to the comparatively small proportion of cases that can be studied in hospitals. On the other hand the method of studying excretion of chloride devised by McLean is relatively easy to carry out. It

[3] McLean, F. C.: Jour. Exper. Med., 1915, xxxii, 366.
[4] Am. Jour. Med. Sc., 1915, cxlix, 808.
[5] Arch. Int. Med., 1916, xvii, 711.

is not dependent upon any diet. It requires but two analyses. It does not necessitate having the patient in a hospital subject to the expense and loss of time incident to the added salt test. Therefore, if by the McLean test equally satisfactory information as to renal adequacy for chloride excretion could be obtained, it would be well worth adopting as a clinical test. For this reason we have tried the method in a series of cases, which have received careful clinical study and whose renal function has been tested by other methods.

METHODS. The technic of collecting specimens was carried out as recommended by McLean. The procedure was instituted usually three to four hours after the last meal. The patient was given 180 c.c. of water to drink. Thirty minutes later a seventy-two minute collection of urine was begun. In the middle of this period, blood was withdrawn and immediately shaken with a few crystals of potassium oxalate to prevent clotting. If the patient was unable to void exactly at the end of the seventy-two minute period, the period during which the urine was collected was noted to the nearest minute. The urine was measured to the nearest cubic centimeter. The blood was centrifuged for twenty minutes at high speed. The plasma was drawn off by pipette and analyzed for chloride content according to the method of McLean and Van Slyke.[6] The standard solutions were tested frequently to ensure their accuracy. Two c.c. portions of plasma were used. Nearly all the tests were made in duplicate. The urinary chlorides were determined by a modified Volhard method.

During the course of the work my attention was called by Dr. J. Harold Austin to the variability in the carbon dioxide binding capacity of plasma when no precautions have been taken to prevent escape of carbon dioxide from the whole blood. Gürber,[7] Petry[8] and others have noted that if the carbon dioxide tension of the serum were increased the cells became richer in chloride. If carbon dioxide is permitted to escape from the whole blood the plasma becomes more alkaline, so that in the mechanism of reëstablishing equilibrium of acids and bases between plasma and cells the chloride goes over from cells to plasma probably in the form of hydrochloride acid. Consequently when no attempt has been made to prevent the escape of carbon dioxide from the whole blood the chloride content of the plasma is found 0.200 to 0.500 grams per liter higher than when this loss of carbon dioxide has been prevented. The amount of migration of chloride from cells to plasma is directly dependent upon the amount of escape of carbon dioxide from the whole blood. Thus while the method of taking blood, which was recommended by McLean, is permissible for clinical determination in which small

[6] Jour. Biol. Chem., 1915, xxi, 361.
[7] Sitzungsb. d. phys-med. Gesellsch. zu Würzburg, 1895, 28–37.
[8] Hofmeister's Beitrage, 1902, iii, 260.

variation in threshold is not to be regarded as of great significance, a source of error is excluded by preventing the escape of carbon dioxide from the whole blood. To prevent this escape of carbon dioxide, and consequently the variable amount of migration of chloride from cells to plasma, the method of withdrawing blood under albolene, recommended by Van Slyke[9] for determination of carbon dioxide combining power of the plasma, was used in the latter part of the work. Taking this precaution the normal plasma chloride threshold is found to be approximately 5.30 grams per liter instead of 5.62 grams per liter.

I. *Non-nephritic Group without Chloride Accumulation.* In Table I is presented a group of 10 cases supposedly without nephritis or any circulatory disturbance in the kidneys. In this group the difference between the actual and theoretical concentration of plasma chloride has varied between —0.18 and +0.12 grams per liter. McLean, in a series of seventy-two observations, found slightly greater variations.

TABLE I.—NON-NEPHRITIC GROUP WITHOUT CHLORIDE ACCUMULATION.

Case.	Age.	Sex.	Diagnosis.	Sodium chloride, grams per liter.			
				Actual.	Calculated.	Difference.	Threshold.
I	46	M.	Angina pectoris	5.87	5.88	−0.01	5.61
II	29	M.	Endothelioma of lymph nodes	5.90	5.96	−0.06	5.56
III	25	M.	Traumatic epilepsy	6.17	6.26	−0.09	5.53
IV	23	M.	Luetic aortitis	5.87	5.83	+0.04	5.66
V	63	M.	Tumor of pons	5.63[11]	5.57[11]	+0.06	5.36[11]
VI	30	M.	Gastric neurosis	5.97	6.05	−0.08	5.54
VII	26	M.	Streptococcic bacteremia	5.81	5.96	−0.15	5.47
VIII	57	M.	Chronic myocardial disease; arteriosclerosis	5.81	5.88	−0.07	5.55
IX	16	F.	Bronchial asthma	5.56	5.72	−0.18	5.44
X	57	M.	Arteriosclerosis	5.59[11]	5.47[10]	+0.12	5.42[10]

II. *Non-nephritic Group with Chloride Accumulation.* In Table II are included 8 cases which show elevated chloride threshold, although they were considered after careful study in the hospital to be non-nephritic.

Cases XI and XII.—Entered the hospital with edema, due to cardiac decompensation. On admission both showed high threshold for chlorides. After the edema had subsided the threshold returned to normal.

Case XIII.—The chloride studies were made in this case of luetic cirrhosis of the liver, during a period when the patient had marked ascites, bilateral hydrothorax, and edema of the legs. At that time the phthalein excretion was 20 per cent. in two hours. The urine contained a cloud of albumin. Later when the collections of fluid has disappeared the phthalein rose to 60 per cent.; the urine showed

[9] Personal communication to Dr. J. H. Austin.
[10] Blood taken under albolene.

only a faint trace of albumin. The retention of chlorides was probably due to circulatory disturbances in the kidneys.

Case XIV.—Diagnosis: cardiac decompensation, secondary cirrhosis of the liver. When the chloride study was made the patient's abdomen was steadily filling with fluid. There was almost complete anuria. After withdrawal of most of the fluid a fair urinary excretion was established. Tests made at this time showed the chloride threshold very much lower.

Case XV.—This case apparently belongs to the type described by Christian[11] as having disturbed salt elimination, but not, strictly speaking, actual nephritis. This man gave a history of always having eaten large amounts of salt. To test his statement a twenty-four hour specimen of urine examined for sodium chloride content while he was on the ordinary ward diet showed 29 grams. The chloride threshold was markedly above the normal on two examinations. During this time he had slight edema. He was then placed on a fixed low chloride intake and weighed every day at exactly the same time. On the day 10 grams sodium chloride was added to the diet he weighed 1.5 kilos more than on the preceding day. On that day none of the added chloride was excreted. On the following day, however, 7.9 grams extra chloride was excreted. This man has continued under observation for five months on a somewhat limited salt intake. He has shown no further tendency to edema.

Case XVI.—This case was diagnosed as probable early renal tuberculosis. The urine never showed more than a trace of albumin. The phthalein excretion was 35 per cent. in two hours. Two c.c. indigo-carmine were injected intravenously and the ureteral orifices observed for evidence of excretion. At the end of eight minutes a faint blue stream was seen coming from the left ureter. The amount was not materially increased at the end of twenty minutes. No excretion was observed from the right ureter in twenty minutes. Therefore, whatever may have been the primary disease the case had actual renal inadequacy when these studies were made. The elevated threshold for chlorides may be considered as one of the expressions of this inadequacy.

Case XVII.—The elevated chloride threshold in this case is difficult to explain. The urine examination, blood-pressure readings, phthalein tests, and blood urea analysis gave no evidences of nephritis. The patient was voiding only from 400 c.c. to 660 c.c. urine for several days before the first test of chloride excretory function was made. He was then given theocin, 0.2 gram three times a day for three days and the studies repeated. The plasma chloride, meanwhile, had dropped 0.5 milligram per cubic centimeter. However, the chloride threshold was still elevated above the normal.

Case XVIII.—This case also gave no evidences of renal inade-

[11] Christian, H. A.: Am. Jour. Med. Sc., 1916, cli, 630.

quacy. The diagnosis made was chronic myocardial disease with anginoid attacks. The statement was made by the patient's physician that there had been edema of the legs before admission to the hospital. None, however, was observed during his stay in the ward. No material change in the threshold was observed after giving theocin, 0.2 gram, three times a day for three days or after tincture of digitalis, 0.65 c.c., three times a day for five days. The only suggestive finding in this case was the fact, that the urinary concentration of chloride was always low.

TABLE II.—CASES PRESUMABLY NON-NEPHRITIC WITH CHLORIDE ACCUMULATION.

Case.	Age.	Sex.	Date.	Diagnosis.	Edema.	Sodium chloride, grams per liter of plasma.			
						Actual.	Calculated.	Difference.	Threshold.
XI	63	M.	Oct. 26	Cardiac decompensation	+	6.25	5.86	+0.39	6.01
			Nov. 16		0	6.00	5.92	+0.08	5.70
XII	36	M.	Nov. 2	Cardiac decompensation	+	6.69	5.88	+0.81	6.43
			4		+	6.69	5.95	+0.74	6.36
			16		0	6.43	6.29	+0.14	5.76
XIII	42	M.	Nov. 21	Luetic cirrhosis	+	6.34	5.84	+0.50	6.12
XIV	67	F.	Jan. 7	Cardiac decompensation; secondary cirrhosis	+	6.65	5.68	+0.97	6.59
			Mar. 24		+	6.31	5.88	+0.43	6.05
XV	26	M.	Dec. 20	Bronchial asthma	+	6.62	6.19	+0.43	6.05
			23			6.50	5.99	+0.51	6.13
XVI	28	F.	Feb. 22	Renal tuberculosis (?)	0	6.12[12]	5.63[12]	+0.49	5.79[12]
XVII	50	M.	Nov. 8	Sciatica	0	6.81	5.95	+0.86	6.48
			18			6.31[13]	5.84	+0.47	6.09
XVIII	63	M.	Nov. 8	Myocardial weakness	+	6.37	5.84	+0.53	6.15
			18			6.31[13]	5.77	+0.54	6.16

III. *Chloride Excretory Function in Nephritis.* In Table III are presented the results of study in 22 cases of nephritis, arranged according to the clinical classification employed in the University Hospital.

Advanced Glomerulonephritis: Of 11 cases studied, 9 showed high chloride threshold. Of this group 3 cases (XX, XXIV, and XXVI) showed no lowering of chloride threshold under the influence of vapor baths and salt-free diet.

Case XIX, whose threshold was high when the first studies were made after nineteen days' treatment with salt-free diet and vapor baths, showed a drop of 0.78 gram chloride per liter of plasma. Two days after the administration of 10 grams of sodium chloride, a slight amount of which was vomited, the threshold had again risen slightly beyond its original point. Case XXVIII, in addition to advanced nephritis, on admission had slight cardiac decompensation with edema of the legs. Under rest and digitalis the edema rapidly disappeared, but a hematuria which had been quite marked on admission, persisted. The patient was then put upon salt and protein-low diet and given a hot pack every other day. The hema-

[12] Blood withdrawn under albolene.
[13] Had received theocin for three days.

TABLE III.—GROUP OF NEPHRITIC CASES.

Case.	Age.	Sex.	Date.	Sodium, chloride, grams per liter.				Urine.			Blood-pressure.		Phthalein per cent.	Non-protein blood, mgr. in 100 c.c.	Blood urea, mgr. in 100 c.c.	Eye-ground examination.
				Actual.	Calculated.	Difference.	Threshold.	Albumin.	Casts.	Specific gravity.	Systolic.	Diastolic.				
ADVANCED GLOMERULONEPHRITIS.																
XIX	39	F.	Apr. 17	6.03[14]	5.50[14]	+0.53	5.83[14]	Cloud	Few	1.008–1.011	200	140	1		192	Renal retinitis.
			27	5.69[14]												
			Mar. 6	5.25[14]	...	...	...	....	..	....	250	160				
			Mar. 26	6.03[14]	5.32[14]	−0.71	6.01[14]									
XX	44	M.	Feb. 25	6.25[14]	5.42[14]	+0.83	6.13[14]	Cloud	Many	1.010–1.012	210	135	Trace	.....	142	Angiosclerosis.
			Mar. 4	6.22[14]	5.47[14]	+0.75	6.05[14]	....	..	....	178	120	7			
			8	6.22[14]	5.53[14]	+0.69	5.99[14]									
XXI	40	F.	Dec. 4	6.81	5.90	+0.91	6.53	Cloud	Many	1.009–1.010	204	138	Trace	.....	229	
XXII	47	F.	Dec. 18	6.47	5.75	+0.72	6.34	Cloud	Many	1.012–1.015	262	141	15	.....	83	Neuroretinitis.
XXIII	39	M.	Oct. 26	6.62	5.70	+0.92	6.54	Cloud	Many	1.009–1.013	186	115	Trace	76	....	Angiosclerosis.
			Nov. 1	6.19	5.64	+0.55	6.17	....	..	....	135	97				
XXIV	53	M.	Nov. 30	6.00	5.73	+0.27	5.89	Trace	Many	1.010–1.012	225	165	·12	.....	96	Neuroretinitis.
			Dec. 4	6.02	5.65	+0.37	5.99									
			17	6.22	5.81	+0.41	6.03	....	..	....	....	....	15	.....	115	
XXV	35	M.	Nov. 29	6.47	5.87	+0.60	6.22	Trace	Occas.	1.019–1.027	190	102	25	56	....	Disk, margins blurred; one hemorrhage.
			Dec. 3	6.37	5.81	+0.56	6.18									
XXVI	24	M.	Nov. 4	6.12	5.71	+0.41	6.03	Heavy	Many	1.010–1.025	242	180	33	46	....	Wide-spread retinitis.
			15	6.12	5.82	+0.30	5.92	....	..	....	178	135	37			
			29	6.25	5.81	+0.44	6.06	Cloud								
			Dec. 3	6.28	5.74	+0.54	6.16									
XXVII	45	M.	Feb. 8	6.09	5.69	+0.40	6.02	Light cloud	Few	1.015–1.019	185	135	35	.....	39	Hemorrhages; neuroretinitis
XXVIII	34	M.	Mar. 21	5.53[14]	5.43	+0.12	5.42	Light cloud	Very few	1.005–1.014	182	78	12	.....	80	Negative.
										....	138	49	20			
XXIX	34	M.	Mar. 27	5.48[14]	...	...	...	Cloud	Many	1.009–1.011	175	110	Trace	.....	417	Negative.
			29	5.56[14]												
INTERMEDIATE GLOMERULONEPHRITIS.																
XXX	24	M.	Feb. 1	6.41	5.99	+0.42	6.04	Cloud	Many	1.013–1.015	160	120	30	...	90	
XXXI	48	F.	Dec. 21	6.56	5.74	+0.82	6.44	Trace	Few	1.005–1.020	200–145	110–88	55–68	28	....	Neuroretinitis.
XXXII	24	F.	Feb. 16	5.62[14]	5.54[14]	+0.08	5.38[14]	Faint trace	None	1.010–1.012	190	135	35	.....	31	Angiosclerosis.
XXXIII	39	M.	Mar. 9	5.66[14]	5.55[14]	+0.11	5.41[14]	Cloud	Many	1.012–1.025	176	110	55	.....	37	Angiosclerosis
EARLY GLOMERULONEPHRITIS.																
XXXIV	39	M.	May 27	5.75[14]	5.46[14]	+0.29	5.59[14]	None	None	1.021	155	102	56	.....	25	Proliferative retinitis.
CHRONIC DEGENERATIVE NEPHRITIS.																
XXXVI	29	F.	Apr. 17	6.25[14]	5.62[14]	+0.63	5.93[14]	Boils solid	Many	1.023	150	110	35	.....	36	Negative.
			19	6.12[14]	...	...	...	....	..	....	135	90				
			May 4	6.03[14]												
XXXVII	34	M.	Jan. 22	6.37	5.96	+0.41	6.03	Cloud	Many	1.028	....	....	60	.....	60	Old hemorrhages.
XXXVIII	56	M.	June 4	6.40[14]	5.33[14]	+1.07	6.37[14]	9 gm. per liter	Many	1.015	160	90	Trace	.....	261	Slight angiosclerosis.
RENAL SCLEROSIS.																
XXXIX	55	M.	Nov. 27	6.12	6.02	+0.10	5.72	Faint trace	Few	1.031	....	....	52	.....	48	Negative.
XL	63	F.	Mar. 11	5.78[14]	5.66[14]	+0.12	5.42[14]	Cloud	Many	1.009–1.035	250	130	35	.....	....	Angiosclerosis.
ACUTE PYELONEPHRITIS.																
XLI	37	F.	Nov. 8	5.62	5.51	−0.11	5.51	Cloud	None	1.010–1.015	110	65	10 Trace	187	442	

[14] Blood taken under albolene.

turia gradually diminished until there were no macroscopic evidences of blood in the urine. Erythrocytes could still be found under the microscope, however. A McLean test at this time showed that the threshold was not raised above the normal limit. As a check upon this finding the test of ability to excrete 10 grains additional sodium chloride was made. But 4.9 grams were excreted in forty-eight hours. The urine which had been clear for about two weeks previously, on the day chloride was administered, became quite hemorrhagic in appearance. The red color gradually disappeared in the course of a week.

Cases XIX and XXVIII. These cases illustrate the point that the finding of a normal chloride threshold must not be regarded as conclusive evidence that the kidney function for chloride excretion is normal. In Case XXVIII the sudden return of hematuria after the administration of sodium chloride suggests very strongly that the salt acted as a renal irritant.

Case XXIX.—This patient, who had the greatest waste nitrogen retention of any case in the group, showed no accumulation of chloride in the plasma. A test of ability to excrete added chloride could not be carried out as the patient was bordering on uremia. In this case the diagnosis of advanced glomerulonephritis was confirmed by autopsy a few weeks after the patient had left the hospital.

Thus of 11 cases of diagnosed clinically as advanced glomerulonephritis, 9 showed impairment of renal chloride excretory function by having a high chloride threshold, a tenth showed no elevation of the threshold, but impairment of ability to excrete added sodium chloride, while the eleventh, one of the most severe cases in the group, showed a normal plasma chloride, but could not be studied as to his ability to excrete added chloride.

Intermediate Glomerulonephritis. In this group of 4 cases 2 showed a high threshold for chlorides.

Case XXXI.—This case is of interest because the plasma chloride threshold was high, whereas the ability to excrete added salt was practically unimpaired. On admission to the hospital this patient had advanced neuroretinitis with many hemorrhages. The blood-pressure was 200 systolic and 110 diastolic. The urine showed a faint trace of albumin with only an occasional cast. The specific gravity tended between 1.005 and 1.009, but on a dry diet rose to to 1.028. The blood nitrogen was 28 milligram per 100 c.c. The phthalein excretion was 55 per cent. in two hours. The plasma chloride threshold at this time was 6.44 grams per liter. Of 10 grams added salt, 7.9 grams were excreted in forty-eight hours. The patient was kept quietly in bed on a salt-free low protein diet. The retinitis improved slowly. The blood-pressure fell to 145 systolic and 88 diastolic. Headache, which had been a troublesome feature of the case, disappeared. The improvement has continued over a period of six months, during which time the patient has been on a restricted salt intake.

Early Glomerulonephritis. One case, diagnosed early glomerulonephritis, was studied, as follows:

CASE XXXIV. — This patient came to the hospital, to the service of Dr. de Schweinitz, complaining of dimness of vision. He was found to have a peculiar form of proliferative retinitis, apparently not of renal origin. Physical examination was entirely negative, except for slight enlargement of the heart to the left, accentuated aortic second sound, and slight sclerosis of the peripheral arteries. The blood-pressure was only slightly elevated. Numerous urine examinations failed to show either albumin or casts. The phthalein excretion was normal. There was no accumulation of urea in the blood. The sodium chloride threshold was only slightly elevated. The ability to excrete added sodium chloride was markedly diminished, but 2 out of 10 grams added salt being excreted in forty-eight hours. The degree of impairment of renal function for chlorides revealed by the inability to excrete added chlorides would scarcely have been suspected from the very slight elevation of the threshold.

Chronic Degenerative Nephritis. But 3 cases diagnosed as chronic degenerative nephritis were studied. All showed a high threshold. The chloride excretion seemed more disturbed than the phthalein or urea excretion, except in Case XXXVIII, in whom almost all kidney function had ceased.

Renal Sclerosis. The 2 cases diagnosed renal sclerosis studied, showed no retention of chloride.

Acute Pyelonephritis.

CASE XLI.—This was a case of acute pyelonephritis; the urine contained a large amount of pus and gave pure culture of Bacillus mucosus capsulatus. It is of interest chiefly because of the low chloride threshold in contrast to a tremendous retention of urea and a phthalein excretion varying from 10 per cent. to a trace. The diagnosis of acute pyelonephritis was confirmed by autopsy.

Poisoning by Mercuric Chloride.

CASE XLIII (Table IV).—White, male, age twenty-one years, weight 60 kilos. Took 0.5 gram mercuric chloride, thinking it was a headache remedy. Fifteen minutes later he discovered his mistake, took seven eggs, some starch, and milk. Brought immediately to the hospital, where a modified Lambert treatment was instituted within an hour of the time he had taken the mercuric chloride. During the first twenty-four hours he voided over 4000 c.c. of urine. For several days the urine showed enormous numbers of casts and renal epithelium and a cloud of albumin. The phthalein, which had been low, returned to normal until the Lambert treatment was discontinued, when it dropped slightly again. The blood urea never rose above 0.43 gram per liter.

The concentration of chlorides in the plasma after twenty-four hours of the Lambert treatment dropped to 4.56 grams per liter the

lowest figure that has been found in the laboratory. The urine at the time showed only a faint trace of chlorides, not sufficient to determine quantitatively by the McLean and Van Slyke method for chlorides. Three days later there was a slight rise in concentration of chlorides in the plasma, but still only a trace of chloride in the urine. After the Lambert treatment was discontinued and a diet containing more salt given the plasma chloride rose rapidly until there was evidence of distinct retention.

Case XLIV (Table IV).—White, male, aged twenty-four years, weight 69 kilos. While intoxicated he took 0.5 gram mercuric chloride dissolved in 150 c.c. of water. Vomited about three hours later. Then came to the hospital, where a modified Lambert treatment was instituted immediately. There was good diuresis, 3200 c.c. of urine passed in the first twenty-four-hour period. The phthalein was 7 per cent. at the first examination, but subsequently rose to normal. The urine never showed more than a trace of albumin and a few hyaline and granular casts. Highest blood urea figure 0.300 gram per liter.

TABLE IV.—POISONING BY MERCURIC CHLORIDE.[15]

Case.	Days after taking mercuric chl.	Sodium chloride, grams per liter.				Urine.		Phthalein percentage.	Remarks.
		Actual.	Calculated.	Difference.	Threshold.	Albumin.	Casts.		
XLIII	1	4.56	5.30	−0.74	4.56	Cloud	Many	10	Modified Lambert treatment.
	2	4.62	5.30	−0.68	4.62	"	"	25	
	4	4.92	5.30	−0.38	4.92	"	"	65	
	9	5.50	5.31	+0.19	5.49	Light cloud	None	43	8th day
	14	5.81	5.40	+0.41	5.71	Faint trace	Occasional	48	Lambert treat. dis.
XLIV	1	5.25	5.44	−0.19	5.11	Trace	"	7	Lambert treatment dis. 5th day.
	4	5.27	5.36	−0.09	5.21	"	None	50	
	9	5.87	5.46	+0.41	5.71	"	"	55	

The behavior of the chloride in the case resembled that of Case I. During the Lambert treatment the concentration in plasma was below the normal threshold. After the Lambert treatment was stopped there was distinct retention of the chlorides.

These cases of mercurial poisoning show how a concentration of chloride in the plasma below a normal threshold may be reached by vigorous measures of elimination. In both cases at the time the urine examinations and phthalein excretion showed most damage to the kidney the plasma chloride concentration was well below the normal threshold. Later, when the evidences of nephritis had subsided, the Lambert treatment discontinued and the patient allowed to take a diet containing salt, the concentration of chloride in the plasma quickly rose to a point where it was quite evident that there was distinct retention.

Eclampsia. Through the kindness of Dr. B. C. Hirst it was possible to study the plasma chloride in 5 cases of eclampsia and 2

[15] All blood taken under albolene.

cases bordering on eclampsia. Unfortunately in 2 of the cases the condition of the patient was such that accurate studies of urinary excretion could not be carried out.

The 5 cases of eclampsia all show a high plasma chloride concentration. Three in whom urinary studies could be carried out showed marked elevation of chloride threshold. In Case XLV (Table V), studied while rapid improvement was taking place, the plasma chloride fell 0.22 milligram per cubic centimeter in twenty-four hours. The two preëclamptics also showed chloride retention; in 1 case, quite marked; in the other, which was probably a combination of preëxisting nephritis and threatened eclampsia, not so marked. This chloride retention is in contrast to the very slight tendency for waste nitrogen to be retained in eclampsia.

TABLE V.—ECLAMPSIA.

Case.	Date.	Blood-pressure.		Blood urea. Mgm. per 100 c.c.	Sodium chloride, grams per liter of plasma.			
		Systolic.	Diastolic.		Actual.	Calculated.	Difference.	Threshold.
XLV	Mar. 29	205	135	30	6.00[16] 5.78[16]			
XLVI	Feb. 28				6.37	5.71	+0.66	6.28
XLVII	Feb. 26	138	105		5.76[16]	5.36[16]	+0.40	5.70[16]
XLVIII	Feb. 3			46	6.53			
XLIX	Dec. 30	220	196	26	6.62	5.94	+0.68	6.30
				Threatened Eclampsia.				
L	May 14	180	130	44	6.00[16]	5.32[16]	+0.68	5.98[16]
LI	May 14	220	145	64	5.69[16]	5.33[16]	+0.36	5.66[16]

Discussion. While the McLean method of studying renal chloride excretory function seems to give valuable information in many cases of nephritis, the results in each particular case must be regarded critically. Two cases of advanced nephritis in this series had marked impairment of their ability to excrete salt, yet the threshold at various times was found normal. On the other hand 2 cases with a very high threshold were able to sweep out added salt very efficiently. While a normal threshold does not necessarily signify that renal function for chlorides is unimpaired, a high threshold probably nearly always means impaired chloride excretion, whether this be due to actual renal disease or circulatory deficiency.

The marked effect produced by conditions interfering with the circulation in the kidney, such as passive congestion, must always be borne in mind. The threshold may be quite as high in cases of cardiac decompensation with edema as in the severest cases of advanced nephritis. The excretion of chloride is apparently far more influenced by passive congestion than is the excretion of urea.

The results of the work here presented agree in a general way with the conclusion of McLean that the chloride and urea functions may be quite independent of one another. Retention of chloride without

[16] Blood taken under albolene.

retention of urea occurs very much more frequently than the reverse. But 2 cases were found in the series, in which there was a high blood urea figure and normal chloride threshold. Since both these cases were very ill, so that no other tests of renal adequacy for chlorides could be carried out, it is doubtful whether these cases really had good renal function for chlorides.

SUMMARY. A study of the method of investigating renal excretory function devised by McLean has been made in a series of normal and pathological cases.

The application of Van Slyke's method of withdrawing blood to prevent escape of carbon dioxide and the effect of this procedure upon the chloride content of the plasma were discussed.

A group of individuals with presumably normal renal function was found to conform fairly closely, in the excretion of chlorides, to the laws of excretion formulated by Ambard and Weill.

A group of cases diagnosed as non-nephritic showed elevated threshold for chlorides. Most of these cases showed evidences of circulatory disturbances in the kidneys.

Studies of chloride excretory function were made in 21 cases of nephritis, 7 cases of eclampsia and threatened eclampsia, and 2 cases of mercuric chloride poisoning.

CONCLUSIONS. 1. An elevated plasma chloride threshold, when circulatory disturbances can be excluded, is valuable evidence of the presence of nephritis.

2. A normal threshold may be found in cases with marked impairment of ability to excrete chlorides, particularly if the patient has been kept on a regime including salt-free diet and measures to stimulate elimination.

3. Sodium chloride added to the diet is sometimes excreted as completely by impaired kidneys working under the stimulation of a high chloride content of the plasma as by normal kidneys working under normal threshold; added chloride may be retained when the threshold is normal. Therefore the information gained by both methods of study is desirable,.

4. Chloride excretory function is impaired in nearly all cases of nephritis.

5. Chloride excretory function is much more disturbed in eclampsia then is urea excretory function.

ELECTROCARDIOGRAPHIC STUDIES IN NORMAL INFANTS AND CHILDREN.

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Electrocardiographic studies of congenital and acquired heart disease in infancy and childhood have accumulated with considerable rapidity in the past few years. Furthermore the interpretation of the records obtained has frequently gone beyond the mere analysis of cardiac arrhythmias to include such questions as the relative predominance of one or the other ventricle, the significance of changes in the *T* wave or other portions of the ventricular complex. On attempting, however, to interpret some anomalous electrocardiograms obtained by us in young children, we were surprised to find that the establishment of normal standards for early life has received comparatively little attention. Very few examples were available from infants and these usually in only one of the three customary leads.

Lewis[4] states that "relative preponderance of the right ventricle during the first few weeks of extrauterine life is physiological; the outlines of the electric curves in the new born child are almost constant; . . . . The normal adult forms of initial deflections are assumed between the ends of the second and third month of extrauterine life." His curves from a child two hours after birth, and from the same child six weeks later (showing relatively less right-sided preponderance) are about the only satisfactory data to be had on this subject in English.

Funaro[1] in 1908 studied the electrocardiograms of forty-five infants and children, but many of his subjects were abnormal, and unfortunately only Lead *I* (right arm to left arm) was taken. Although this procedure coupled with a different nomenclature is still upheld by Kraus and Nicolai, it is obviously an inadvisable departure from the method originally recommended by Einthoven and most generally in use. As *S* was greater than *R* in many of Funaro's records, it appears that he too found a preponderance of the right ventricle, though Nicolai at the time attributed this phenomenon either to left ventricle hypertrophy or to the fact that the infant's heart lies more horizontally than that of the adult. Funaro also states that the deflections were very small in the first few days of life, but quickly became

larger in the first month. Such findings, however, are always open to the criticism that the numerous factors entering into the production of a properly standardized electrocardiogram may not have been properly controlled.

The effect of increasing age on the form of the electrocardiogram has been studied by Linetzky[6], who found that *R* increased and *T* decreased with age, whereas *P* was unchanged. Infants and children, however, were not included in this study as a separate group.

The most satisfactory German study of this subject is by Hecht[2]. Included in his exhaustive work on the mechanism of the heart action in childhood, are tables of the values of the *P* (*A*), *R* (*J*), and *T* (*F*) deflections in Lead *I* from 26 newborn infants, 11 under one year of age, 4 young and 26 older children.* These corroborate Lewis' and Funaro's findings that S^1 (*i.e.*, the *S* wave in Lead *I*) is relatively deep in infancy, but in the different subjects of his series it becomes less than R^1, at different periods between the third and seventh month. A split *P* wave he found only once; split *R* and *S* waves occurred more frequently, especially in the older children. The *T* wave he found small in infancy, and inconstant in Lead *III* at all years. The *P-R* interval of the newborn varied from 0·09 to 0·12 sec. (except in two instances of 0·14 sec.), that of the infants from 0·07 to 0·12 sec., that of the young children from 0·11 to 0·15 sec., and that of the older children from 0·10 to 0·16 second. Numerous reproductions of records make this a valuable work for comparative reference.

Methods.

Our studies have been made on 42 normal subjects whose ages range from immediately after birth to 11 years, and on several children exhibiting one or other form of heart disease. (These pathological studies will be reserved for a later communication). Platinum strings (Edelmann galvanometer) of 3120 ohms and 3500 ohms resistance, and of less than 0·02 second deflection time were used. Although the body resistance in ohms was always ascertained, the string tension was so adjusted that 3 millivolts gave 3 centimetres excursion with an arbitrary 2,000 ohms added resistance in circuit. In some cases, the string was so slackened that .1 millivolt gave 2 or 3 centimetres excursion, but in no case did the deflection time exceed 0.02 second. In the following table all figures are calculated on the basis of the standard string tension of 1 cm. for 1 millivolt. Flexible German silver electrodes between pads moistened in strong salt solution were applied to the wrists and left ankle. Later, a copper plate electrode firmly attached to an infant's shoe, with binding post projecting through the sole, was found to be more serviceable and reliable. When the child was too young to recline in a chair insulated by rubber feet, we have laid it upon a table covered with dentist's rubber tissue. In order to obviate any deflection of currents by the child crossing arms or legs, the rubber tissue also was wrapped about arms and legs. The "body resistance" was found to vary between

* Many of these, however, were not healthy children.

TABLE I.—GIVING THE VALUES OF ELECTROCARDIOGRAPHIC DEFLECTIONS IN MILLIVOLTS, THE PULSE RATE AND *P-R* INTERVAL.

Age.	Name.	Sex.	P			Q			R			S			T			Rate.	P.-R. Interval.
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Before Cord.	G.N.	F.	·1			·05			·6			1·4			·2			160	0·11
After Cord.	G.N.	F.	·05	·1	·1	0	0	·6	·4	1·2	1·7	·8	·6	·2	·1	·1	0	130	0·14
10 hours	M.R.	M.	·02	·01	0	0	·2	·1	·5	·1	·2	·3	0	0	0	0	·01	125	0·11
10 ,,	R.H.	M.	·1	·15	·15	0	·4	·2	0	1·0	1·3	·6	1·0	·8	·1*	·15	·2	100	0·12
11 ,,	P.L.	M.	?	·1	?	?	·05	0	·1	·4	·3	0	·1	·1	?	?	?	118	0·12
24 ,,	C.L.	F.	·01	·02	0	0	0	0	·01	·2	·4	·4	·5	·1	·01*	·01	?	130	0·12
26 ,,	F.N.	M.	·05	·1	·05	0	·01	·2	·2	1·0	1·3	·6	·02	·05	·2	·1	·1*	145	0·08
3 days	C.T.	F.	0	·05	·05	0	·1	·2	·05	·5	·7	·3	·5	·4	·05	·1	·05*	96	0·12
5 ,,	G.N.	F.	0	·1	?	0	·2	·3	·2	·5	1·7	·3	·3	·3	0	·1	0	118	0·10
6 ,,	C.L.	M.	·1	·2	·1	0	·2	·2	·3	·3	·5	·6	·3	·1	·2	·2	0	120	0·12
14 ,,	G.N.	F.	·1	·1	·1	·1	·2	·4	·3	·6	1·0	·5	·4	·4	·1	·1	·05	136	0·12
20 ,,	W.E.	M.	·05	·1	?	0	0	·1	·1	·15	·3	·3	·1+	·1	·1	·1	?	166	0·10
3 weeks	S.E.	F.	·1	·2	·1	0	·3	·4	·5	1·2	1·4	·6	·2	·05	·2	·3	·1	120	0·12
3 ,,	O.R.	M.	·1	·1*	·1	·05	·2	·2	·4	·7	·7	·5	·1	·2	·1	·1	·2	132	0·16
6 ,,	D.R.	M.	·1	·15	·1	0	·4	·5	·5	1·0	·9	·6	·5	·3	·2	·4	·3	85	0·12
7 ,,	H.S.	M.	·1	·2	·05	0	·2	·2	·3	1·6	1·0	·5	·1	0	·2	·2	?	150	0·11
8 ,,	C.E.	M.	·1	·2	·1	0	·1	·4	·9	1·2	·9	1·0	·4	·2	·2	·3	·1	100	0·12
9 ,,	M.A.	M.	·15	·2	·1	0	·1	·3	·9	1·4	·6	·8	·2	·2	·2	·3	?	155	0·12
10 ,,	C.M.	F.	·1	·1	·1	·2	·2	·2	·5	·8	·5	·5	·4	·4	·15	·2	·1	140	0·11
14 ,,	C.M.	F,	·1	·2	·05	·2	·3	·1	·5	·9	·5	·4	·4	·2	·2	·2	·1	120	0·12
17 ,,	M.S.	F.	·1	·2	·1	·1	·6	·6	·7	1·8	1·0	·5	·4	1·6	·3	·4	0	148	0·11
5 months	R.H.	M.	·1	·1	·05	·6	·5	·6	1·4	1·6	·9	·3	·4	·2	·6	·4	·1*	134	0·12
5½ ,,	C.L.	F.	·1	·15	0	·05	·05	·4	·8	1·1	·6	·5	·4	·1	·2	·4	·5*	130	0·12
6 ,,	C.D.	M.	·1	·2	·1	0	0	·8	1·8	1·4	1·1	1·1	·4	0	·6	·5	0	130	0·10
6 ,,	R.H.	M.	·15	·2	·1	·6	·4	·5	1·6	1·6	·8	·2	·3	·2	·6	·3	·1	160	0·12
6½ ,,	C.L.	F.	·15	·2	0	·1	·15	·4	·8	·8	·3	·4	·5	0	·5	·3	·1	140	0·12
6½ ,,	G.N.	F.	·1	·2	0	·3	·6	1·0	1·6	1·4	1·4	·8	·3	·05	·5	·5	·3	132	0·13
7 ,,	L.W.	F.	·1	·1	0	0	·3	·6	·7	1·8	1·2	·5	·4	·3	·3	·2	·1	160	0·12
13 ,,	B.Y.	M.	·1	·1	·1	·1	·6	1·	·6	·8	·9	·2	0	0	·4	·2	·2	135	0·15
19 ,,	D.N.	M.	·1	·2	·05	·3	·6	·4	·9	1·3	·8	·5	·4	0	·7	·4	·2*	130	0·13
20 ,,	B.M.	M.	·1	·1	·1	0	·2	·2	·8	1·0	·7	·7	·5	·3	·2	·1	·1	110	0·12
24 ,,	M.S.	M.	·1	·15	·05	0	0	·4	·1	1·0	·6	·3	0	0	·3	·2	·1	112	0·14
25 ,,	G.A.	F.	·2	·2	·1	0	·2	·3	·6	·7	·6	·4	·2	0	·3	·3	·1	135	0·13
5 years	F.A.	F.	·15	·2	?	·2	·1	·3	·9	1·2	·5	·2	·1	0	·15	·2	?	140	0·13
6 ,,	M.A.	F.	·2	·3	·1	0	·05	·3	1·2	1·4	·4	·25	·1	·3	·3	·2	·1	106	0·13
7 ,,	P.I.	M.	·2	·2	·1	·4	·3	0	1·6	1·3	·3	0	0	0	·4	·4	·1	88	0·13
8 ,,	K.M.	M.	·2	·2	·1	·1	0	0	1·1	·8	·5	·15	·4	·6	·5	·4	·1	90	0·14
8½ ,,	C.N.	M.	·1	·2	·1	·4	0	0	1·4	·7	·5	0	·4	·7	·3	·3	0	110	0·16
10 ,,	S.H.	M.	·2	·3	·1	·2	·3	·4	·8	·6	1·0	·7	·2	0	·4	·5	·25	80	0·16
10 ,,	M.E.	M.	·2	·25	·1	·15	·2	·1	1·0	1·2	·6	·15	·4	·3	·25	·2	·17	104	0·16
10 ,,	H.N.	F.	·5	·2	·1	0	0	0	1·0	2·1	1·4	0	0	0	·4	·4	·1	110	0·14
13 ,,	J.N	M.	·15	·2	·1	·3	·1	0	1·6	1·0	·2	0	·8	·8	·6	·7	·1	100	0·15

* Inverted.

700 and 1,400 ohms in different subjects apparently without regard to age. It was frequently difficult to get satisfactory curves on account of the restlessness of the infant under these conditions. This restlessness undoubtedly also affected the heart rate and may have had a slight effect on the *P-R* interval, but as similar records at slower heart rates were obtained when the child happened to be asleep, we do not believe that the form of the complexes or *P-R* interval was materially changed by this increase in rate. As we were unable at that time to employ a rotary time marker, measurement of time intervals was unavoidably less accurate, but the margin of error was far too slight to affect any of our deductions. As Hecht[2], Hoffmann[3], and others have found changes in the form of the electrocardiogram due to changes in position, the infant was placed always upon its back. In a few cases, however, records were also taken with the infant lying first on the left and then on the right side, without materially affecting the form of the complexes. The small changes thus produced (a millimetre or less in the height of *R* or *S*) were less than the changes caused by respiration in the same record.

Results.

The values for the different waves have been arranged according to age in table 1 :—

Preponderance of right ventricle.

It will be seen that the findings of other authors in regard to right ventricular preponderance are here confirmed. It is uniformly present at birth (Fig. 2) and in the early weeks of infancy, and gradually disappears in the second or third month. The various factors, however, change at different periods : thus R^2 becomes greater than R^3 about the sixth week, but S^1 persists abnormally large for several months (Fig. 3). It usually becomes smaller than R^1 (a convenient measuring point) about the eighth to tenth week. By the sixth month, the infant's electrocardiogram has become practically the same as that of the adult (Fig. 4). These changes are illustrated graphically by the accompanying composite curves from the three leads in the different age groups (Fig. 1).

Q wave.

Another striking attribute of most of these curves is the relatively large size of *Q*, especially in Leads *II* and *III*. The average size of *Q* in adults is less than 1 mm., whereas in most of the subjects of this series, it is not only actually larger but larger proportionately to the other waves. This is especially noticeable in Lead *III* of the younger subjects, although in a few cases, *Q* was highest in Lead *II* and four times in Lead *I*. An explanation of these phenomena is found in the recent evidence furnished by Lewis.[5] *Q* in Lead *I* of the human electrocardiogram probably represents spread of activity in the left ventricle, whereas in Leads *II* and *III* of the human electrocardiogram, *Q* probably represents spread in the right

COMPOSITE ELECTROCARDIOGRAMS.

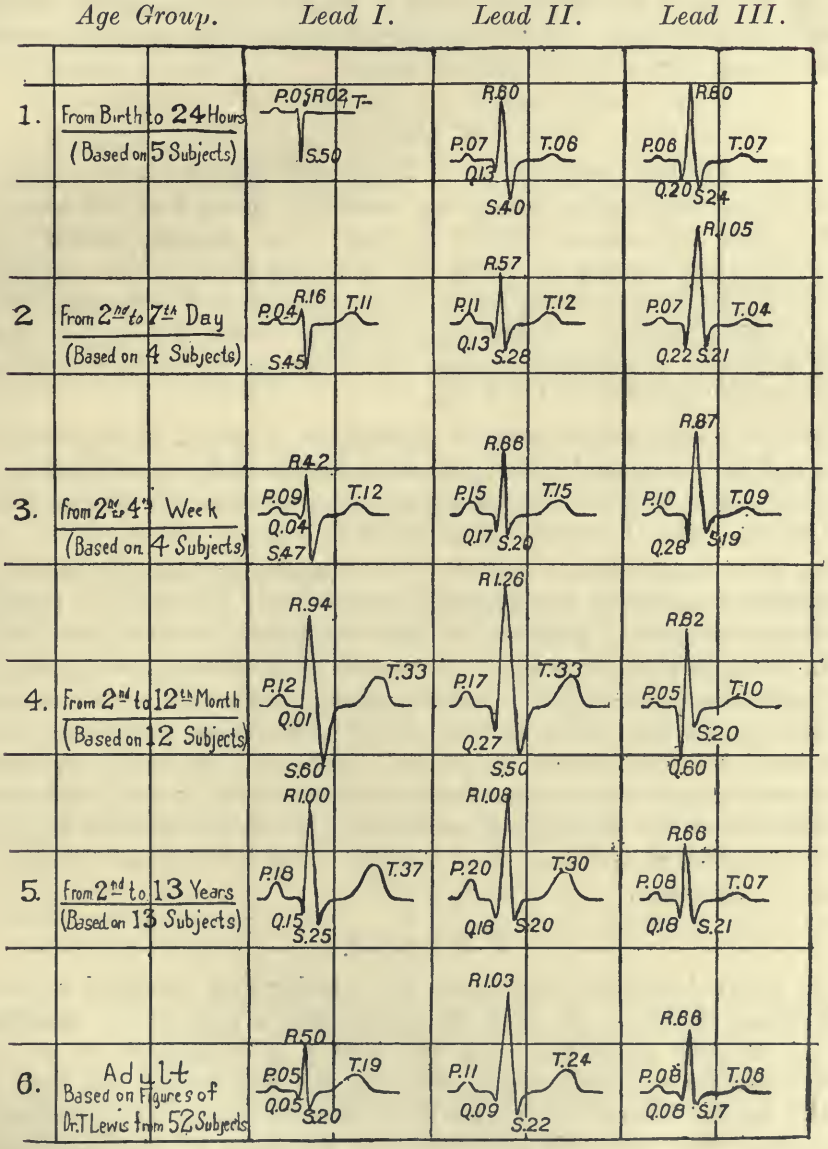

Fig. 1. Composite electrocardiograms arranged according to age groups. The height of each deflection is drawn to scale, from the average figures obtained for each deflection from each group. The numerals opposite each letter refer to the values in millivolts.

ventricle. In the present series, therefore, the behaviour of Q is added to the other signs of right ventricular preponderance in the younger subjects. Confirming Lewis' views, it is interesting also to note that the four cases in which Q^1 is biggest (all between 7 and 13 years of age) were the only ones examined in which the other commonly accepted signs of left ventricular preponderance were also present (see table 1).

T wave.

The T wave has invariably been found absent in the first week of life. In one case in which opportunity was offered to make a record before the umbilical cord was cut, a distinct T wave was present, which almost disappeared after cutting the cord. If this were found to be constant, it would afford interesting opportunity for speculation as to damage done the heart by this procedure. After the first three weeks of life, the T wave reaches a proper proportionate size. It is largest in Lead *II* and lowest and frequently inverted in Lead *III*.

Various abnormalities, such as splitting of R and S, have occasionally been found in the normal infant and child just as in the normal adult. On account of their lack of clinical significance, however, we do not discuss them further. The U wave was but rarely found.

The actual size of the units of the ventricular complex has shown considerable variation in the different subjects, and we have not been able to demonstrate definite relations of these variations to any given factors. In spite of the fact, therefore, that the tension of the string galvanometer was properly standardized, and the resistance of the subject and circuit estimated and allowed for in each case, we are inclined to attribute these variations to factors that we were unable to control. The most probable explanation of the production of the smaller deflections is that after the estimation of the resistance of the infant, its sometimes violent movements may have sufficiently dislodged the electrodes to cause a considerable and unmeasured increase in resistance.

P-R interval.

The interval between the onset of auricular and ventricular activity has also been studied. As Q is unquestionably a part of the ventricular complex, we have taken the P-Q time (when Q is present) as the most accurate measure of this interval, retaining for convenience the term P-R interval. In the absence of Q, as R is then the first sign of ventricular activity, the P-R time has been taken as affording an accurate basis of comparison. From the table, it will be seen that in early infancy the P-R interval is distinctly shortened; being, with but one exception, between 0·08 and 0·12 second in the first six months. After that period, the P-R interval varied between 0·12 and 0·16 second in the different individuals, in other words, was within the lower normal limits of adult intervals.

Sinus arrhythmia.

The number of children that we were able to examine for sinus arrhythmia (juvenile or respiratory arrhythmia) was thirty-seven. Grouping these according to arbitrary age limits, we have the following table showing the average differences between the longest and the shortest heart cycle at the various ages :

First 24 hours	5 cases	Average difference ·006 second.
First month (Exclusive of the first 24 hours)	7 cases	Average difference ·024 second.
Second to twelfth month	10 cases	Average difference ·032 second.
Second to sixth year	9 cases	Average difference ·040 second.
Seventh to thirteenth year	6 cases	Average difference ·103 second.

Except for the children over seven years of age our figures are, in general, slightly less than those given by Hecht,[2] but agree in the gradual increase in the difference between the shortest and longest heart cycles as the age of the child increases (with the concomitant slowing of the pulse rate).

In our study we have considered a child as showing sinus arrhythmia when the difference between the shortest and longest heart cycles was one-tenth of a second or more. This figure is of course purely arbitrary, but it serves as marking a distinction between those children with slight irregularities of a few hundredths of a second and those with evident arrythmia. The first distinct sinus arrythmia occurred in our series at the age of six weeks (heart cycle difference of 0·12 second). Again at two years we encountered sinus arrhythmia with a heart cycle difference of 0·11 second. After seven years of age the condition became more frequent, and was usually, though not always, of the respiratory type. We have observed a case at seven years with a heart cycle difference of 1·2 seconds ; at eight years with a difference of 1·8 seconds ; at ten years with a difference of 1·0 second, and at eleven years with a difference of 1·7 seconds.

Summary.

Electrocardiograms taken on 42 infants and children varying in age from 1 minute to 12 years show that :—

1. Satisfactory records, containing all the peaks seen in the normal adult electrocardiogram, may be obtained from infants at any period of life.

2. The ventricular complexes associated with preponderance of the right ventricle are constantly found in infants from the time of birth up to the second or third month.

3. The modifications of this phenomenon that occur in the different age periods are produced with remarkable constancy in the individuals of each group. Thus the *R* peak of lead *III* ceases to be larger than R^2 between the third and sixth week. In Lead *I*, the *S* depression ceases to be greater than the *R* peak (the other accepted sign of right ventricular

preponderance) between the eighth and ninth week. S^1 continues to be abnormally large, however, in the first two years of life.

4. The initial downward deflection of the ventricular complex, the Q wave, is abnormally large in the infant's electrocardiogram. It is most marked in Lead *III* and from the fourth to the seventh month, but is prominent from the time of birth until the end of our series. The prominent Q^2 and Q^3 should be considered as added signs of right ventricular preponderance; the big Q^1 of four older subjects as an added sign of left ventricular preponderance.

5. Various abnormalities such as splitting of R and S, inversion or diphasicity of T, are found in the normal infant and child, just as in the normal adult.

6. The T wave, present immediately after birth in the single case that was examined before the cord was cut, became smaller after cutting the umbilical cord, and in all cases was practically absent for the first week. After the first three weeks it reaches a proper size proportionate to the other waves. It is always lowest and frequently inverted in Lead *III*.

7. The actual size of the units of the ventricular complex has varied without any definite relation to the different age periods, but the average size for the young has proved actually greater than the average for adult electrocardiograms.

8. The P-R (P-Q) interval is both actually shorter than the P-R (P-Q) interval of adults and also proportionately shorter where allowance is made for the more rapid heart rate of the infant. It varies from 0·08 to 0·12 second in the first year, and from 0·13 to 0·16 second in the rest of the period of observation.

9. Sinus arrhythmia (to a greater extent than 0.1 second between any of the cardiac cycles of a given record) was not seen before the sixth week and seen only once in the first year. From the sixth year up to the age of puberty (*i.e.* the end of the period of observation) sinus arrhythmia became increasingly more frequent.

We desire to thank Drs. J. C. Gittings, B. C. Hirst, J. P. C. Griffith and A. Osthcimer for assistance and for furnishing material.

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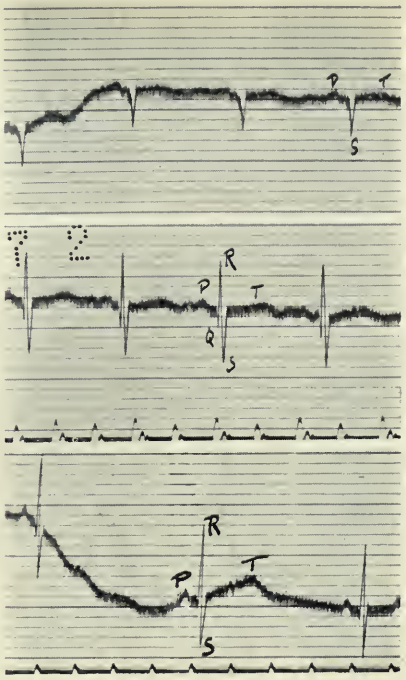

FIG. 2.

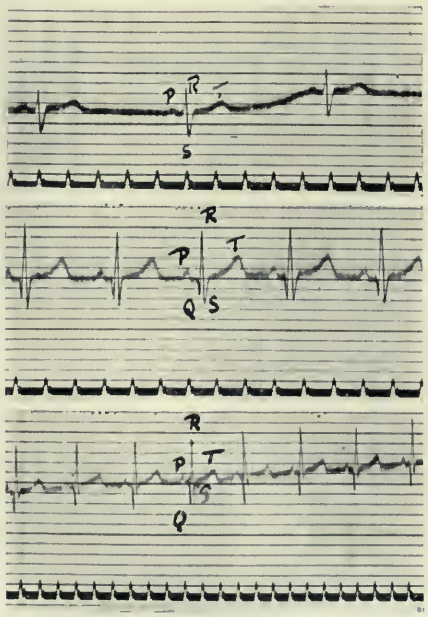

FIG. 3.

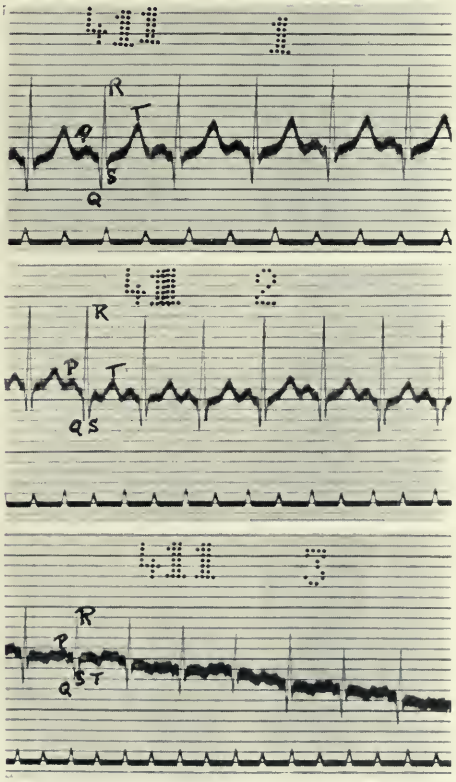

FIG. 4.

Fig. 2. Electrocardiogram from an infant twelve hours old. In Lead *I* note the absence of *R*, the depth of *S* and the small size of *T*. Note also that *R* of Lead *III* is greater than *R* of Lead *II*. The *Q*, *R*, *S* complex in this and the sueeeeding two figures have been redrawn. In all figures the Leads are in their correct order from above down. Ordinates, 5 scale divisions = 1 millivolt. Time in one-fifth sec.

Fig. 3. Electrocardiogram from an infant of six weeks. R^3 is already slightly shorter than R^2, while S^1 is very little bigger than R^1. Note prominence of Q^2 and Q^3, and slight sinus arrhythmia.

Fig. 4. Electrocardiogram from the same infant as in Fig. 2, taken five months later. Except for the prominent *Q*, this is praetieally the same as an adult electrocardiogram.

Acute Diabetes with Enormous Elimination of Nitrogen: Report of Case with at Least Temporary Recovery

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ACUTE DIABETES WITH ENORMOUS ELIMINATION OF NITROGEN: REPORT OF CASE WITH AT LEAST TEMPORARY RECOVERY*

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This case, first, is an excellent example of a certain type of acute diabetes which has received inadequate attention in the literature; secondly, it is of considerable interest on account of the almost unprecedented elimination of nitrogen during the early and fasting days after admission; thirdly, the high figures of ketone elimination deserve mention and emphasize the fallacy of basing prognosis solely on the degree of elimination, and finally, the dietetic and therapeutic measures pursued with prompt success are worthy of brief comment.

REPORT OF CASE

History.—S. W., man, aged 21, Jew, was admitted to the medical division of the University Hospital, Jan. 7, 1917. He stated that until four weeks before he had been in perfect health. Acute superficial pain then developed along the left costal margin, resembling needles being stuck into the skin. Shortly after the onset of the pains, he developed tremendous thirst and also began to lose weight. He stated that he had lost 18 pounds in the four weeks. Polyuria and frequency of urination developed, and the patient also complained of some slight cough and dyspnea. He had never been ill before.

Previous to this illness he drank about five glasses of beer a day. Otherwise his habits and occupation are of no importance. It was difficult to elicit this history, and no further information concerning the onset of his condition could be obtained.

Examination.—The patient was slender, with wasted musculature and a dry skin. He was markedly somnolent. There was a strong "acetone" odor to the breath. Thoracic and abdominal examinations were negative. The blood pressure was: systolic, 100; diastolic, 60. Urinanalysis revealed the urine a corn color; a light flocculent sediment; specific

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gravity, 1.035; acid; a trace of albumin; sugar present; acetone and diacetic acid present; no bilirubin or urobilin. Microscopic examination revealed light and dark granular casts, occasional leukocytes, and calcium oxalate crystals. Blood count revealed: hemoglobin, 100 per cent.; erythrocytes, 5,100,000; leukocytes, 26,400; differential count: polymorphonuclear neutrophils, 88 per cent.; small lymphocytes, 6 per cent.; large mononuclears, 1 per cent.; transitionals, 5 per cent.

The patient was admitted in the afternoon and received no food after 11 a. m. The next morning he was placed on whisky, coffee and sodium bicarbonate, receiving 56 gm. of soda on this day. He was still somnolent, and the breathing was somewhat deep and slow.

Treatment and Results.—January 9, the patient was not improved. The report from the laboratory, as indicated in the table, revealed the severity of the condition. The patient was kept on the same diet and given 87 gm. of soda, of which 30 gm. were given intravenously. The plasma carbon dioxid reading was 49 volumes per cent.

January 10: The patient's condition was worse. The laboratory reports showed no improvement. The diet was increased by the addition of skimmed milk and oatmeal. Sixty gm. of sodium bicarbonate were given.

January 11: The condition was about the same. The patient still showed slight hyperpnea, and was somnolent. The diet was slightly increased, and 75 gm. of glucose were given. Intravenous injection of 1 liter of a 3 per cent. sodium bicarbonate solution was given. The blood urea nitrogen was 13 mg. per hundred c.c., blood plasma chlorids 5.02 gm. per liter, and blood plasma carbon dioxid content 43 volumes per cent.

January 12: The patient was better and brighter. No improvement was shown by the laboratory report over the specimen of the preceding day. The carbohydrate of the diet was increased, and the soda continued.

January 13: There was marked improvement. Soda was continued.

January 14: The patient felt better. The report from the laboratory showed improvement.

January 15: The urine was alkaline for the first time. The plasma carbon dioxid reading was 70 volumes per cent. The Wassermann test was negative.

January 19: There was great improvement. The carbohydrate of the diet was gradually reduced, and proteins and fats were increased. The white blood cells numbered 12,900. On this date, edema of the face was noted, and there was also noted a marked increase in weight. This edema was probably a "soda edema," and it disappeared rapidly on reduction of the amount of sodium bicarbonate administered.

January 24: The urine was sugar-free for the first time.

From this time on the patient continued sugar-free with an increasing tolerance for carbohydrate. On discharge, four weeks later, his tolerance permitted the addition of 40 gm. of bread, 50 gm. of potato and 100 gm. of oatmeal to the diet without the appearance of sugar in the urine.

Since discharge, he has been under observation in the outpatient department and has continued to be sugar-free and is gaining weight. His most recent observation was March 19.

COMMENT

Although the history in this case is indefinite as regards the condition at and preceding the onset of the diabetes, yet there is some suggestion that the early symptoms were due to some acute infection. This is made more probable by the similarity between this case and other cases of acute diabetes following acute infection which have been observed. Such cases usually occur in young persons who have recently been subject to some rather severe acute infection; for example, one of our cases followed scarlet fever and erysipelas. The diabetic symptoms may appear with considerable suddenness, and are rapidly followed by great loss of weight and the symptoms of increasing acidosis. When seen at this stage, the high figures of elimination of nitrogen and of ketonic acids and the general appearance of the patient are apt to lead to a poor prognosis. Further studies, such as the estimation of the carbon dioxid tension of the alveolar air or carbon dioxid content of the blood, however, may show that the degree of acidosis is not so great as the figures of eliminated ketones might suggest, and the results of proper treatment indicate that the prognosis in such cases is better than it might at first glance appear. Under careful treatment, the carbohydrate tolerance is recovered with surprising rapidity and completeness, and the further return to a more or less normal condition is uneventful.

Elimination of Nitrogen.—For the six days after admission, the patient daily eliminated over 31 gm. of nitrogen, the daily average for the six days being 34.8 gm. During this period, the intake as shown in the table was low in nitrogen (24.16 gm.) and the nitrogen loss was therefore extremely great. In the similar case reported by Geyelin and Du Bois,[1] the loss of nitrogen by an acute diabetic, at first on starvation and for eight days after on a moderate nitrogen intake, was enormous (over 281 gm. for the eleven days). They assert that their figures are the highest yet reported for nitrogen elimination under these conditions. Our patient had a higher output for the first six days after admission, and on a

1. Geyelin, H. R., and Du Bois, E. F.: A Case of Diabetes of Maximum Severity with Marked Improvement, THE JOURNAL A. M. A., May 13, 1916, p. 1532.

LABORATORY DATA

Date, 1917	Intake			Analysis of Urine					Blood Carbon Dioxid, Volume per Cent.	Weight, Kg.
	Carbohydrate, Gm.	Nitrogen, Gm.	Sodium Bicarbonate, Gm.	Amount, C.c.	Reaction	Nitrogen, Gm.	Glucose, Gm.	Beta-Oxybutyric Acid, Gm.		
January 8–9	0	0	56	4,740	Acid	35.5	80.8	69.8	..	51
January 9–10	0	0	87	5,170	Acid	32.6	49.0	83.0	49	
January 10–11	35.0	1.9	60	5,515	Acid	38.9	94.4	82.8		
January 11–12	163.5	6.6	90	6,020	Acid	32.3	182.6	85.6	43	
January 12–13	180.0	4.5	60	6,670	Acid	38.5	306.0	85.6	..	47.4
January 13–14	260.0	11.2	60	6,130	Acid	31.3	330.1	76.0		
January 14–15	195.0	8.6	60	3,940	Acid	25.0	182.0	48.1		
January 15–16	206.0	8.3	60	3,570	Alkaline	18.9	178.2	34.7	70	49.5
January 16–17	205.0	9.4	60	3,020	Alkaline	15.8	148.0	22.2		
January 17–18	184.0	9.4	60	3,250	Alkaline	15.2	168.0	8.5		
January 18–19	186.0	9.7	30	3,770	Alkaline	17.6	139.4	4.0	..	52.2
January 19–20	148.0	10.3	20	3,920	Alkaline	14.1	228.0	5.8		
January 21–22	80.0	14.2	20	3,710	Alkaline	14.8	17.6	4.0		
January 22–23	76.0	9.1	20	3,620	Alkaline	17.2	17.2	2.7	..	46.8
January 23–24	0	0	20	1,120	Alkaline	8.5	0	0.8		

fasting treatment his nitrogen loss was even greater than in their case at a corresponding time. This loss of nitrogen represents roughly a loss of about 2½ pounds of flesh per day, if it is assumed that all of the nitrogen came from that source. So far as we know, the figures here reported for this short period are, at least, as high as any on record.

A similar but less prolonged elimination of large quantities of nitrogen has been observed for a few days after admission in a number of our diabetic patients who had been on excessive protein diet before admission. The elimination in such cases continues but a brief period, is not accompanied by loss of weight, and has never been as high as in the present instance.

Elimination of Ketonic Acids.—The figures for the elimination of ketone bodies in this case are high and closely approach those of the case reported by Geyelin and Du Bois. The highest twenty-four hour elimination in our case was 85.6 gm. of beta-oxybutyric acid, while the highest output of ketones in the case reported by Geyelin and Du Bois is 87 gm. of beta-oxybutyric acid.

This degree of elimination is much greater than that seen in many cases which rapidly progress into fatal coma despite our best therapeutic efforts.

Treatment.—This case well exemplifies the value of flexibility in treatment, for no arbitrary outline of treatment can be rigidly applied in every case. In this instance, improvement seemed to commence with the change from a strict starvation treatment to the daily administration of moderate quantities of carbohydrate, and this method was continued until there was a distinct improvement in the acidosis. At the same time, large amounts of sodium bicarbonate were given both by mouth and intravenously.

Once improvement had appeared and the elimination of ketones had somewhat diminished, the carbohydrate of the diet was gradually replaced with protein and fat until the diet became practically our routine carbohydrate-free diet. During this period the urine had become sugar-free and all evidences of acidosis had disappeared.

After the patient had been sugar-free for a short time, carbohydrates were again tentatively added to his diet in small amounts without causing the appearance of the glycosuria. The degree of tolerance exhibited was marked, and on discharge, the patient was receiving such considerable amounts of carbohydrate without glycosuria as to suggest that his tolerance had been restored about to normal. The blood sugar had returned to a normal figure (0.07 per cent.) before discharge.

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ELECTROCARDIOGRAPHIC OBSERVATIONS IN TOXIC GOITRE.

By

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The importance of cardiac signs and symptoms in exophthalmic goitre has been a matter of common knowledge since the earliest description of this disease by Parry[1] in 1786. Tachycardia, palpitation, forcible heart action, and less often cardiac arrhythmia are such important factors in the syndrome that Möbius[2] was led to the dictum that "Basedow patients suffer and die through their hearts." It is only within the past decade or so, however, that the cardiac symptoms have been generally considered as due to thyreotoxic influences, rather than wholly or in part due to embarrassment of the right heart following an often hypothetical pressure on the trachea by the enlarged thyroid gland. As this condition constitutes a relatively simple example of an endogenous intoxication, it has seemed advisable to study the changes produced in the electrocardiogram by the intoxication of this disease, with the additional hope that further light might be thrown both on the resulting cardiac condition and on the changes produced in it by surgical operations on the thyroid.

Method. To this end electrocardiograms were taken on 51 goitre patients seeking surgical relief, in as many cases as possible both before and at short and long periods after operation. Information was sought not only as to the rate and rhythm of the heart action in both simple and toxic goitre cases, but also as to the relative size of the chambers of the heart and to other changes in the form of the ventricular complexes of the electrocardiogram both before and after operation. Endeavor was also made to correlate these findings with the changes observed in the clinical

TABLE I.—CLINICAL DATA IN TOXIC GOITRE.

No.	Name.	Sex.	Age.	Clinical diagnosis.	Blood-pressure.		Symptoms.							Operation.	Pathological diagnosis.	Result.
					Systolic.	Diastolic.	Duration of disease.	Cardiac outline.	Palpation.	Dyspnœa.	Tachycardiac.	Nervous.	Exophthalmic.	Goitre.			
1	B. A.	M	28	Toxic	135	60	3 yrs.	+(Left)	0	0	0	+	+	+	Double ligation	Exophthalmic	Slightly improved.
2	D. A.	F	29	Toxic	110	74	6 yrs.	+(Left)	+	0	0	+	+	+	Single lobectomy	Exophthalmic	Much improved.
3	B. A.	F	24	Toxic	112	53	8 mos.	Normal	0	+	+	+	+	+	One and a half lobectomy	Exophthalmic	Improved.
4	E. B.	F	58	Non-toxic	164	82	14 yrs.	+(Left)	+	0	0	0	0	+	Enucleation	Adenoma	Slightly improved.
5	K. B.	F	13	Non-toxic	....	....	1½ yrs.	+(Left)	0	0	0	0	+	+	Partial excision	Colloid exophthalmic	Improved.
6	R. B.	F	26	Toxic	130	70	1 yr.	Normal	+	..	+	+	0	+	Lobectomy	Colloid	Not improved.
7	C. C.	F	52	Toxic	128	80	15 yrs.	Normal	+	+	+	0	+	+	Ligation	.....	Improved.
8	A. C.	F	21	Toxic	....	....	6 mos.	+(Left)	+	0	0	+	+	+	No operation	.....	Slightly improved.
9	R. C.	F	18	Early toxic	120	85	3 yrs.	Normal	0	+	0	+	0	+	Partial excision	Colloid exophthalmic	Improved.
10	J. C.	M	18	Toxic	115	80	4 yrs.	Normal	+	+	0	+	0	+	Partial excision	Colloid exophthalmic	Much improved.
11	B. C.	F	24	Toxic	125	65	2 mos.	Normal	0	0	+	+	+	+	Ligation	Colloid exophthalmic	Improved.
12	C. C.	F	43	Toxic													
13	M. C.	F	34	Non-toxic	120 145	85 70	4 mos.	+(Left)	+	+	0	+	0	+	Ligation	Exophthalmic	Died.
14	M. D.	F	35	Non-toxic	112	60	15 yrs.	Normal	+	0	0	0	+	+	Enucleation	Colloid adenoma	Slightly improved.
15	A. E.	F	54	Toxic	160	92	1 yr.	+	+	+	0	+	0	+	Double lobectomy	Colloid exophthalmic	Improved.
16	S. F.	F	26	Toxic	135	100	1½ yrs.	Normal	+	+	+	+	0	+	Double lobectomy	.....	Much improved.
17	A. F.	F	24	Toxic	168	68	3 yrs.	+(Left)	+	+	0	+	+	+	Double ligation	.....	Improved.
18	A. F.	F	19	Toxic	120	72	1½ yrs.	Normal	0	0	0	+	0	+	Double lobectomy	Colloid exophthalmic beginning	Improved.
19	A. F.	F	26	Non-toxic	145	105	2½ yrs.	Normal	+	+	0	0	0	+	Partial excision	Colloid cystic	Slightly improved.
20	A. G.	M	48	Toxic	138	72	1 yr.	Normal	+	+	0	+	0	+	Lobectomy	Colloid	Improved.
21	A. G.	F	35	Toxic	148	72	2½ yrs.	+(Left)	+	+	+	+	+	+	Double ligation	.....	Slightly improved.
22	F. H.	F	38	Toxic	....	....	1 yr.	+(Left)	+	+	+	+	0	+	Double lobectomy	Colloid	Died.
23	K. H.	F	17	Non-toxic	120	75	3 yrs.	+(Left)	0	+	0	+	0	+	Partial excision	Colloid	Improved.
24	H. H.	M	32	Toxic	145 135	85 75	10 yrs.	+(Right & left)	+	+	+	+	+	+	Two ligations Two partial excisions.	Exophthalmic	Improved.

25	E. K.	F	19	Toxic	100	65	10 yrs.	Normal	+	0	+	0	Sl.	0	Single lobectomy	Colloid	Improved.
26	A. K.	M	38	Toxic	....	....	Several years	+(Right & left)	+	0	+	0	0	0	Single lobectomy	.....	Not improved.
27	S. L.	F	18	Toxic	145	85	11 yrs.	+(Right & left)	+	0	0	0	0	+	Single lobectomy	Exophthalmic	Slightly improved.
28	M. L.	F	19	Exophthamic	140	....	3 mos.	Normal	0	+	+	0	+	+	Single lobectomy	Exophthalmic	Much improved.
29	I. M.	F	25	Toxic	117	40	4 yrs.	+(Left)	+	+	+	+	+	+	Double lobectomy	Exophthalmic	Improved.
30	M. M.	F	18	Non-toxic	125	70	1½ yrs.	Normal	0	+	0	0	0	0	Single lobectomy	Colloid	Improved.
31	M. M.	F	43	Toxic	129	70	1½ yrs.	Normal	+	0	+	+	+	+	Single lobectomy	Colloid	Improved.
32	J. M.	F	37	Toxic	118	80	3 yrs.	+(Left)	+	+	+	+	Sl.	0	Partial excision	Colloid cystic degeneration	Slightly improved.
33	N. M.	F	17	Toxic	142	70	2 yrs.	+(Left)	+	+	0	+	0	+	Single lobectomy	Colloid adenoma	Much improved.
34	A. M.	F	26	Toxic	148	68	4 mcs.	+(Left)	+	+	0	+	+	+	Double ligation Single lobectomy	Exophthalmic	Improved.
35	M. D.	F	11	Toxic	114	48	2 yrs.	+(Left)	+	+	0	+	0	+	Single lobectomy	Adenoma exophthalmic	Much improved.
36	I. O.	F	37	Early toxic	....	....	1 yr.	+(Right)	+	0	+	+	+	+	Single lobectomy Double lobectomy	Colloid exophthalmic changes	Improved.
37	I. P.	F	19	Toxic	115	82	6 mos.	Normal	+	0	+	+	0	+	Double lobectomy	Colloid exophthalmic	Slightly improved.
38	M. P.	F	16	Toxic	135	70	1½ yrs.	+(Right & left)	0	+	+	+	0	+	Double ligation	Hyperplastic lymphadenitis	Improved.
39	M. P.	F	25	Early toxic	117	72	3 yrs.	Normal	+	+	0	+	0	+	Double lobectomy	Colloid	Improved.
40	F. R.	M	39	Toxic	152	70	2 yrs.	+(Right & left)	+	+	+	0	0	+	Single ligation	.....	Died.
41	R. R.	M	29	Non-toxic	100	60	8 yrs.	+(Right)	0	+	0	0	0	+	Double lobectomy	Colloid exophthalmic	Improved.
42	B. R.	F	28	Toxic	139	65	6 yrs.	+(Right & left)	0	+	+	+	+	0	Single lobectomy	Adenoma exophthalmic	Improved.
43	S. S.	F	32	Toxic	133	56	1¾ yrs.	(+Right) & left)	+	0	0	+	0	+	Double ligation	.....	Improved.
44	A. S.	F	37	Toxic	103	75	12 yrs.	Normal	+	+	+	+	0	+	Partial ligation	Adenoma	Much improved.
45	G. W.	M	35	Toxic	132 140	75 80	11 yrs.	+(Left)	+	+	+	+	+	+	No operation		
46	A. W.	F	33	Toxic	152 170 165	85 65 50	3 yrs.	+(Left)	+	+	0	+	+	+	Ligation	Exophthalmic	
47	G. D.	M	26	Toxic	133	60	1½ yrs.	+(Right)	+	0	0	+	+	+	Partial excision	Colloid	
48	M. N.	F	33	Non-toxic	....	....	14 yrs.	Normal	0	0	0	+	0	+	Partial excision	Colloid adenoma	
49	B. B.	F	50	Toxic	119	62	3 yrs.	Normal	+	0	+	+	0	0	Partial excision	Exophthalmic	Not improved.
50	R. F.	F	51	Toxic	170	105	2 yrs.	+(Left)	+	+	+	+	0	0	No operation		
51	L. N.	F	43	Toxic and mitral stenosis	95 110	60 55	2 yrs.	+(Right & left)	−	+	+	+	+	+	No operation	.....	Improved.

condition of the various subjects examined. On account of the nervous state of many of the patients, fine vibrations of the string were often unavoidable, and occasionally were sufficient to prevent accurate measurement of the P–R interval, but did not otherwise interfere with interpretation of records. A still greater handicap was imposed by ineradicable extraneous electrical disturbances which, together with the limitations of the Edelmann galvanometer, prevented the proper complete standardization of records. For this reason it has been impossible to base any deductions on the actual changes in the size of the ventricular complexes observed in a given case after thyroid removal, and only the relative shapes of the various complexes in the pre- and postoperative records could be considered. Time intervals, the diagnosis of existing arrhythmias, and the detection of right or left ventricular preponderance were, of course, not interfered with. Most of the cases here reported were from the clinic of Dr. C. H. Frazier, whom I take this opportunity to thank for placing them at my disposal.

Results. *Clinical.* The clinical data on the cases studied are included in Table I.

The usual preponderance of females, the relative youth of most of the cases, and the presence of the characteristic symptoms of the disease are all apparent. It is also obvious that almost all cases were of the toxic, hyperthyroid, or exophthalmic goitre type, and that this diagnosis was confirmed by pathological examination. Thirty-five of the patients submitted to partial thyroidectomy, 10 to artery ligation, and 2 to enucleation of adenomata. Of the ligation group 1 was greatly improved, 5 moderately so, 3 showed little or no improvement, and 1 died. In general this group did not tend to show as marked improvement as did those who had a partial excision. Although 3 of the latter failed to show any improvement after operation, it must also be remembered that many of the partial excision group had previously had arteries ligated, without sufficient improvement to obviate further surgical intervention.

Of the 47 cases, 7 were greatly improved by the operation, 23 were moderately improved, 14 only slightly or not at all improved, and 3 died. One of the deaths (Case No. 22) occurred two days after double lobectomy of a colloid goitre and was due to pneumonia complicated by increased thyrotoxicosis; another (Case No. 40) was due to cardiac failure twenty-four hours after ligation of a single artery. It is unfortunately true that in neither case were adequate premonitory signs evident either in the clinical examination or the electrocardiograms taken a few days before operation. The third case (Case No. 13), which had failed to improve after ligation of the superior thyroid artery, developed negative T waves which were still present at the time of the second

operation four months later. Her death from cardiac failure two days after the partial excision undertaken at this time tends to

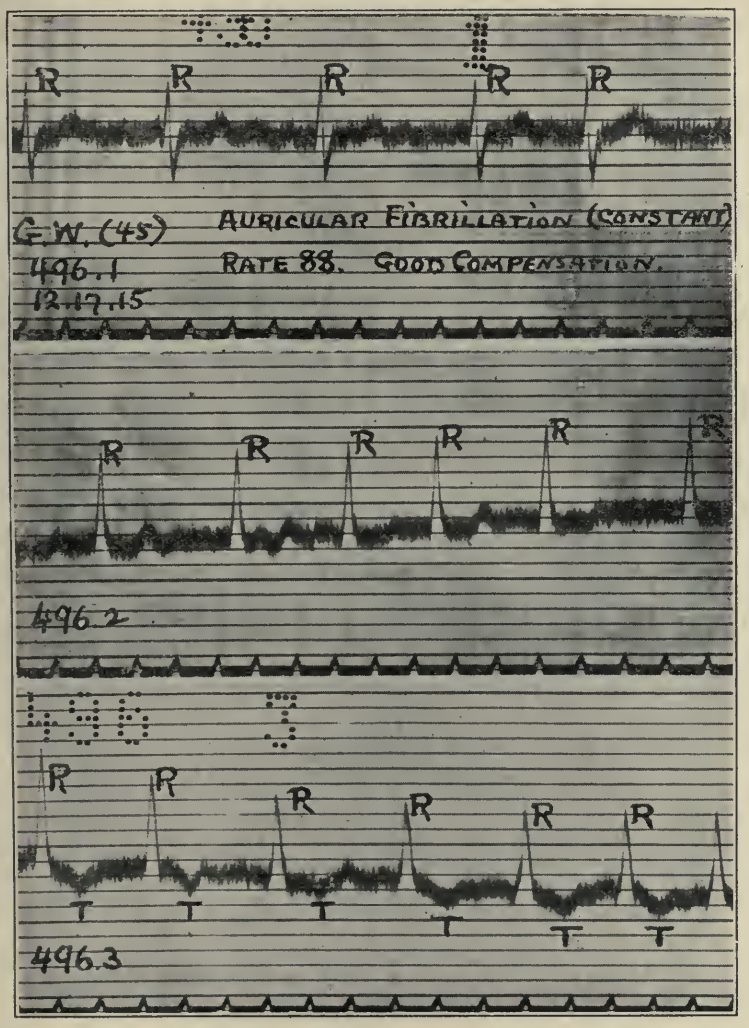

FIG. 1.—Case 45. Electrocardiogram of G. W., showing permanent auricular fibrillation. This and subsequent electrocardiograms were made with the Edelmann galvanometer. As the string could not be standardized with the patient in circuit, 1400 ohms were added as an arbitrary equivalent of the patient's resistance. Platinum strings were used with a resistance varying between 3500 and 5000 ohms. Time intervals are expressed at the bottom of each lead by $\frac{1}{5}$-second intervals. In this figure note (1) absence of sign of auricular contraction (*P* wave); (2) ventricular arrhythmia (irregular occurrence of *R*); (3) occasional fine waves of fibrillation.

confirm the view that negative *T* waves, when not due to digitalis medication, have an unfavorable influence on prognosis. The

electrocardiogram of the patient that died from pneumonia showed abnormal *T* waves in Leads II and III, but similar abnormalities were found in 4 other cases (Cases 2, 3, 5, 35) that were improved by operation.

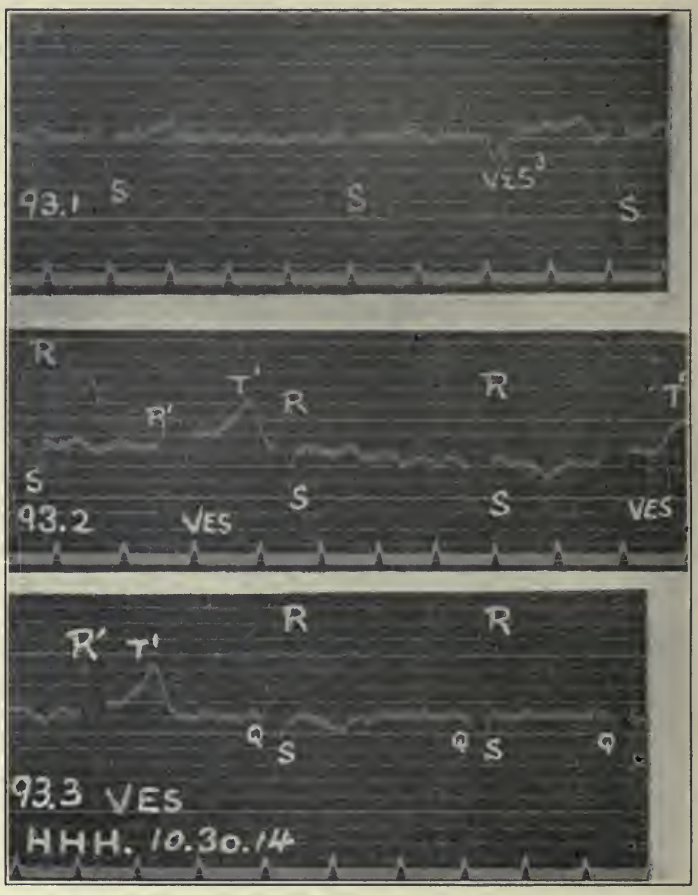

Fig. 2.—Case 24. Electrocardiogram of H. H. H., showing right ventricular preponderance, auricular fibrillation of probably ten years' duration, with occasional ventricular premature contractions arising from several sites. Note (1) that the *S* wave has replaced the *R* wave in Lead I and that the *R* wave of Lead III (R_3) is greater than the *R* wave of Lead II (R_2). Note also that (2) in each lead one or two complexes vary greatly from the normal supraventricular type, and that (3) the same disturbance of mechanism exists as in Fig. 1.

Rhythm. Changes in cardiac rhythm were observed in 11 cases, as follows: sinus arrhythmia, 4 cases; ventricular extrasystoles, 3 cases; auricular fibrillation, 3 cases; auricular flutter, 1 case. The *P–R* interval was prolonged beyond normal limits in 2 cases. As no other adequate cause for such derangement of cardiac

mechananism was given in the past history of all but 2 of these cases, it is fair to assume that the majority, if not all, were caused by the thyreotoxicosis, acting not only by its direct toxic effect, but also indirectly through the cardiac hypertrophy and later degeneration

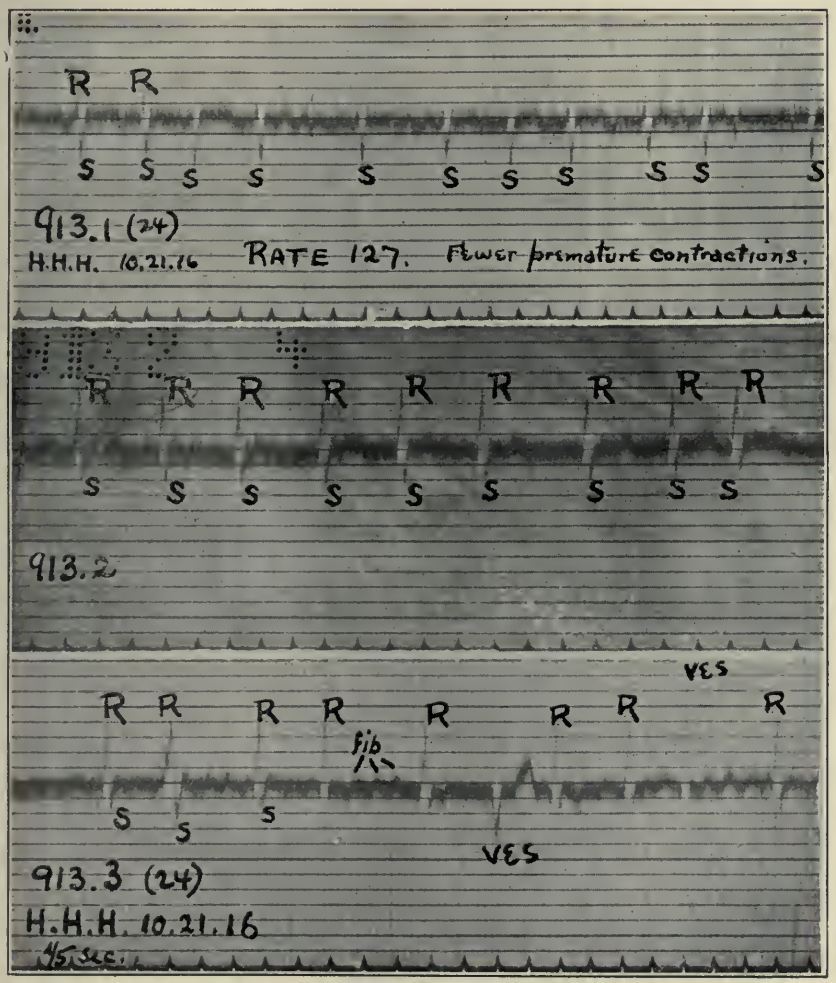

FIG. 3 —Electrocardiogram of same patient as Fig. 2, taken two years later, showing the same picture, except that the premature contractions occur much less frequently.

thus caused. The fact that most of the cases studied received energetic treatment relatively early in the disease will probably account for the comparative infrequency of the more important arrhythmias and of other gross cardiac changes in this series. This in its turn

emphasizes the value of early and thorough medical treatment, followed by prompt surgical intervention in those cases not responding properly to medical care.

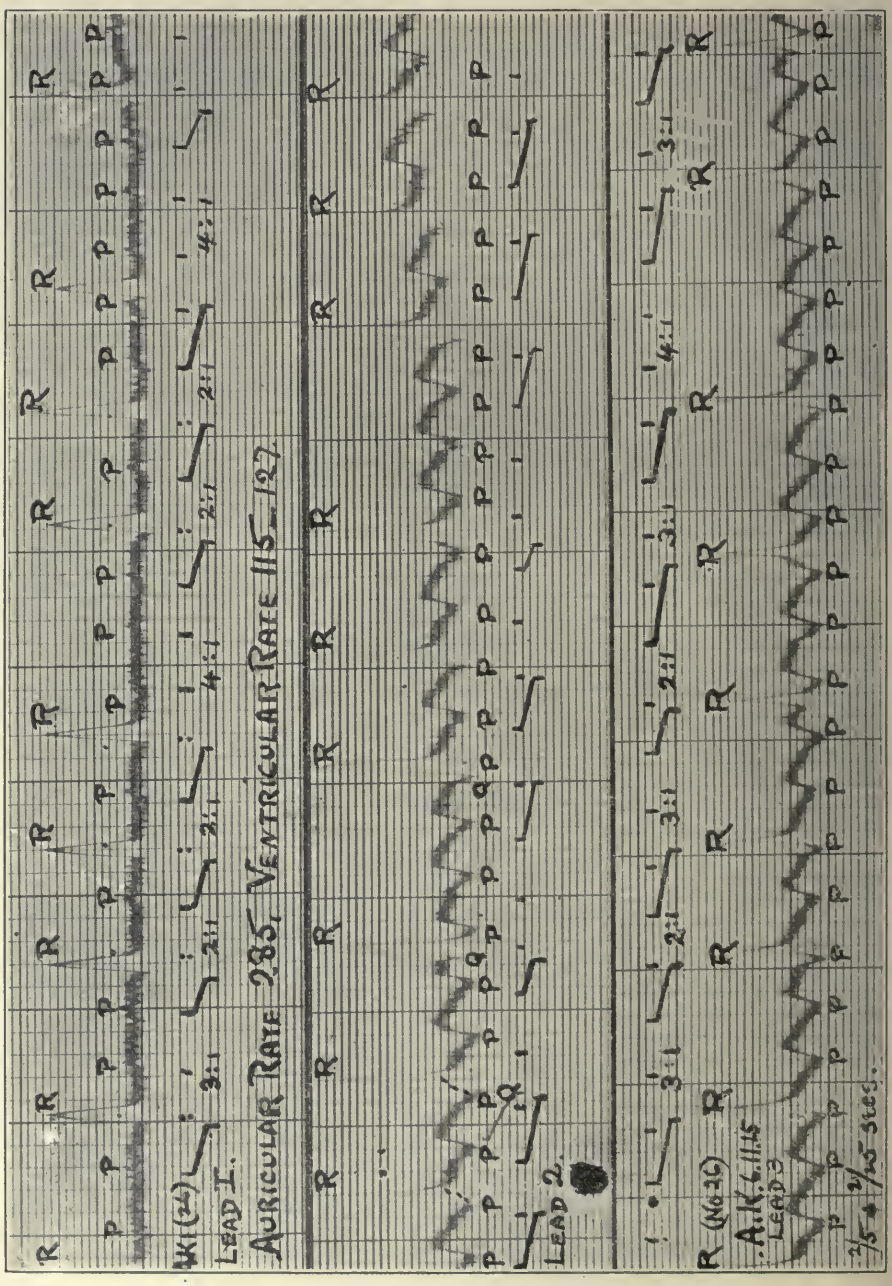

Fig. 4

The small number of arrhythmic cases affords but little opportunity to judge of the effects of operation on the arrhythmia. The case of flutter and two of the cases of fibrillation were undoubtedly permanent, having been followed over several years without change. Although many different attempts were made at different times to change the auricular flutter into fibrillation or normal rhythm all efforts proved unavailing. One of the cases of auricular fibrillation (Case 51) proved to belong to that interesting group of transient fibrillation, to which attention has elsewhere been called.[3] In Fahrenkamp's[4] series of cases of hyperthyroidism in which transient fibrillation was present the arrhythmia occurred in distinct paroxysms of short duration. In the present case, however, fibrillation was present when the patient was first seen eighteen months ago. On account of a coexisting mitral stenosis and the absence of data as to the time of origin of the arrhythmia it was thought at that time that an ordinary case of fibrillation with chronic mitral endocarditis was being complicated by hyperthyroidism. The fibrillation persisted apparently as a constant condition until about one month ago, when it was noted that coincident with general improvement the pulse had become regular. Electrocardiograms taken then and at various periods since then show that normal rhythm has now supervened. As this occurred coincident with improvement in the hyperthyroidism it is fair to assume that the thyroid and not the endocarditis was the chief factor in the production of the fibrillation.

Of the 3 cases exhibiting extrasystoles only 1 accepted surgical treatment, and in this case the extrasystoles disappeared after operation, just as frequently happens in this type of arrhythmia, when the source of toxemia is removed or diminished.

Although sinus arrhythmia is not usually considered to have any clinical importance, it was found to be lessened after operation, along with the signs of clinical improvement. Attention might here be called to the work of Thorne,[5] who considers that, like the other arrhythmias, though in a lesser degree, sinus arrhythmia is never found in a truly normal heart. Whether this belief is accepted or not, any procedure that tends to lessen an existing sinus arrhythmia should be considered a step in the direction of improving the cardiac condition.

EXPLANATION OF FIG. 4.

FIG. 4.—Case 26. Electrocardiogram of A. K., showing auricular flutter, with a varying degree of *A. V.* block, probably of four years' duration. In this as in the other electrocardiograms of this series records were taken from the three customary leads; the tension of the string was so standardized that 1 millivolt caused a deflection of 1 cm. Time intervals in this record are expressed by heavy and light vertical lines across each lead, representing $\frac{2}{5}$ and $\frac{2}{25}$ second respectively (Cambridge galvanometer). Each deflection is lettered according to Einthoven's scheme, and a diagram is added for each lead to illustrate the changing heart block. Note the prominent inverted (diphasic) *P* waves in Leads II and III.

Two cases (Cases 4 and 8) showing distinct prolongation of the *P–R* interval in the absence of digitalis medication are the only

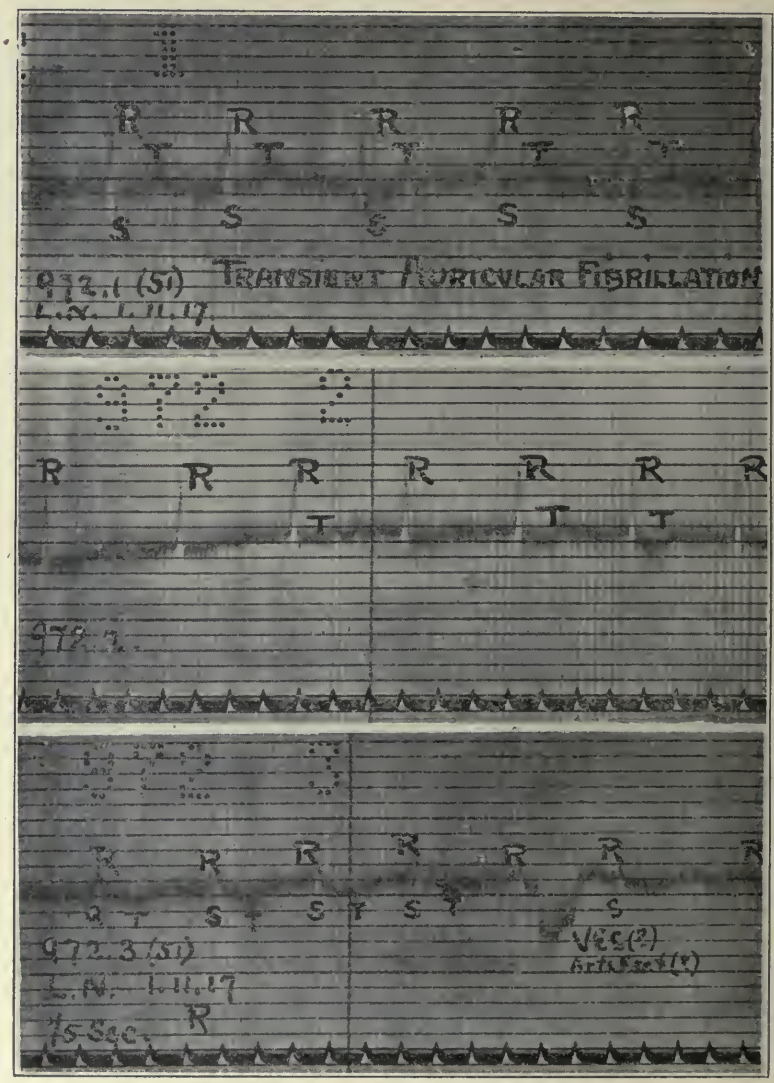

FIG. 5.—Case 51. Electrocardiograms of L. N., showing transient auricular fibrillation. Note that although the ventricular arrhythmia is slight, the same disturbance of mechanism exists as in Fig. 6.

examples of defective conductivity in this series. Reilingh,[6] however, has shown that more advanced degrees, even complete heart-block, may occur in exophthalmic goitre.

Blood-pressure. The systolic and diastolic blood-pressure was estimated soon after admission to the hospital, and in many cases shortly before discharge. The most noticeable abnormality was

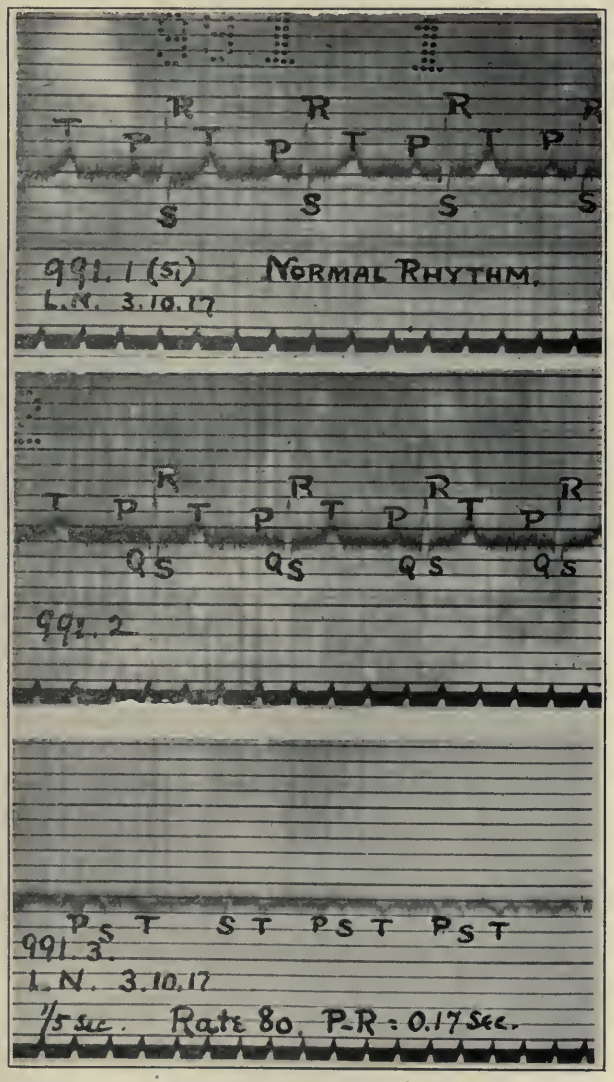

FIG. 6.—Electrocardiogram taken two months later, showing normal rhythm. Note (1) reappearance of *P;* (2) regular ventricular rhythm; (3) absence of irregular waves of fibrillation.

the increased pulse-pressure, which was over 60 mm. Hg. in 11 patients who showed no signs of hypertension. The increase in pressure, which was due both to elevation of the systolic and

depression of the diastolic pressure, is in all probability to be explained as the vascular response to the overacting heart. In 5

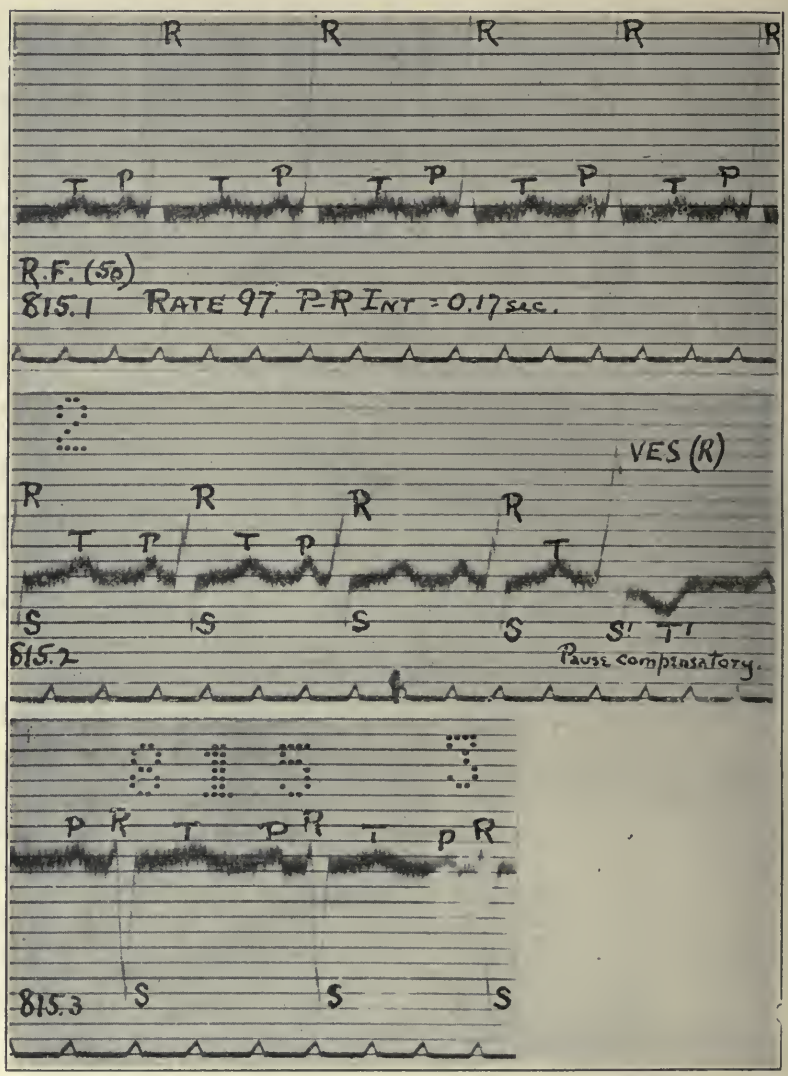

FIG. 7.—Case 50. Electrocardiogram of R. F., showing left ventricular preponderance and one ventricular premature contraction. Note that R_1 is greater than R_2 and that S_3 is greater than R_3. In Lead II note that the last ventricular complex varies greatly from the other complexes of the lead and occurs prematurely (extrasystole).

cases the systolic pressure was above normal limits, but in only 1 were signs of hypertensive nephritis or arteriosclerosis present. In at least 1 case the pressure fell to normal as the patient improved,

so that this, too, should be considered a sign of cardiac intoxication. The 3 cases that showed a lowered systolic pressure (below 110) are difficult to explain, but it should be noted in all of these that a

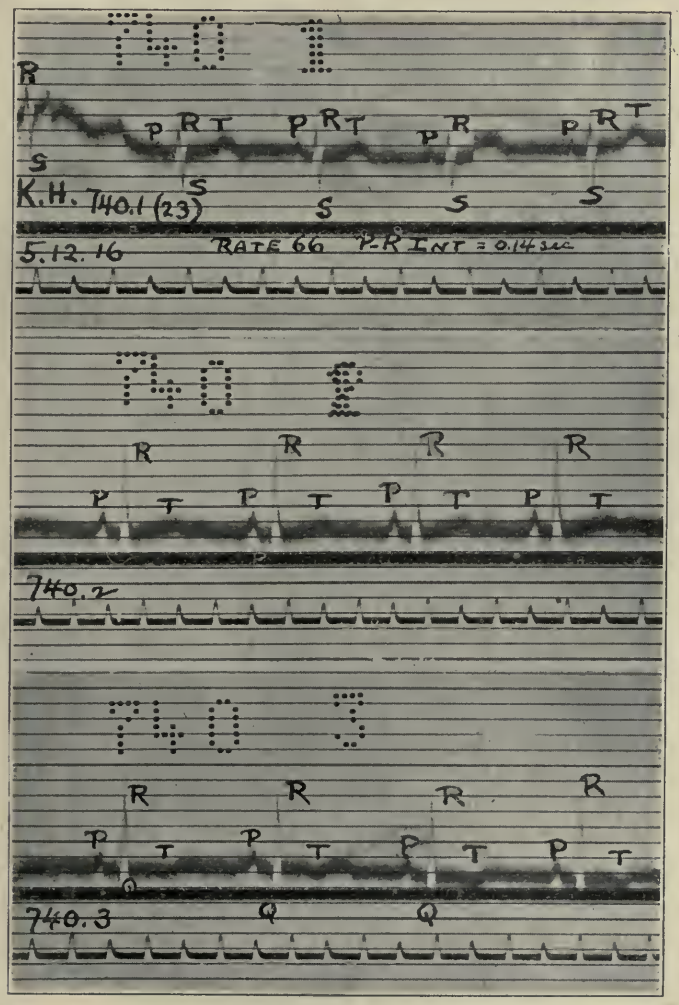

FIG. 8.—Case 23. Electrocardiograms of K. H., showing lessened degree of right ventricular preponderance after operation. Note that whereas S_1 is greater than R_1 in the first record, it is distinctly less in the second. Also R_3, instead of being the same size as R_2, is less than R_2 in the second record.

normal pulse-pressure was preserved. Although due to a different kind of disturbance these changes, as has been pointed out by Taussig,[7] are of the same kind as those found in aortic regurgitation, and may be considered as characteristic of toxic goitre.

Changes in Form of the Ventricular Complex. From the point of view of the cardiographer, one of the items of this study that was approached with the liveliest anticipation was the question as to what effect the thyreotoxicosis and also its treatment by surgical operation would have upon the cardiac function as expressed by

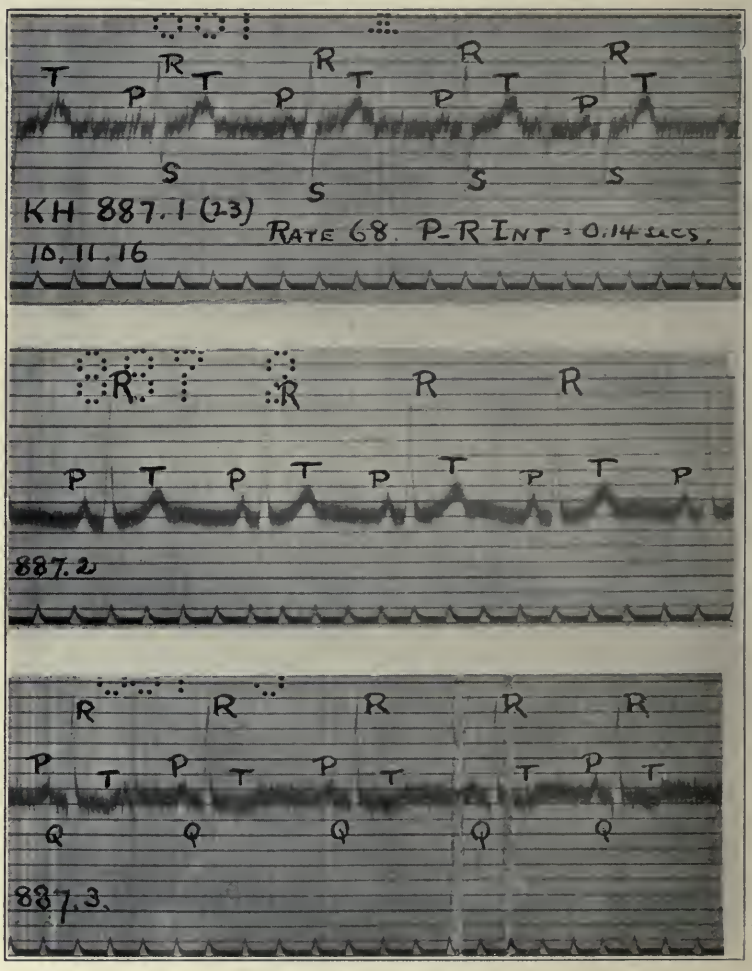

FIG. 9.—Same case as Fig. 8.

the form of the ventricular complex. As an analyzer of cardiac arrhythmias the string galvanometer has already been thoroughly and satisfactorily exploited. As a gauge of the functional capacity of the heart, however, in demonstrating abnormalities in form of the ventricular complex, its possibilities are still far from being realized. Lewis[8] has called attention to the fact that "if in any

subject electrocardiograms which show considerable divergence from what is regarded as normal are obtained it is probable that the heart is abnormal." This has corresponded to our experience

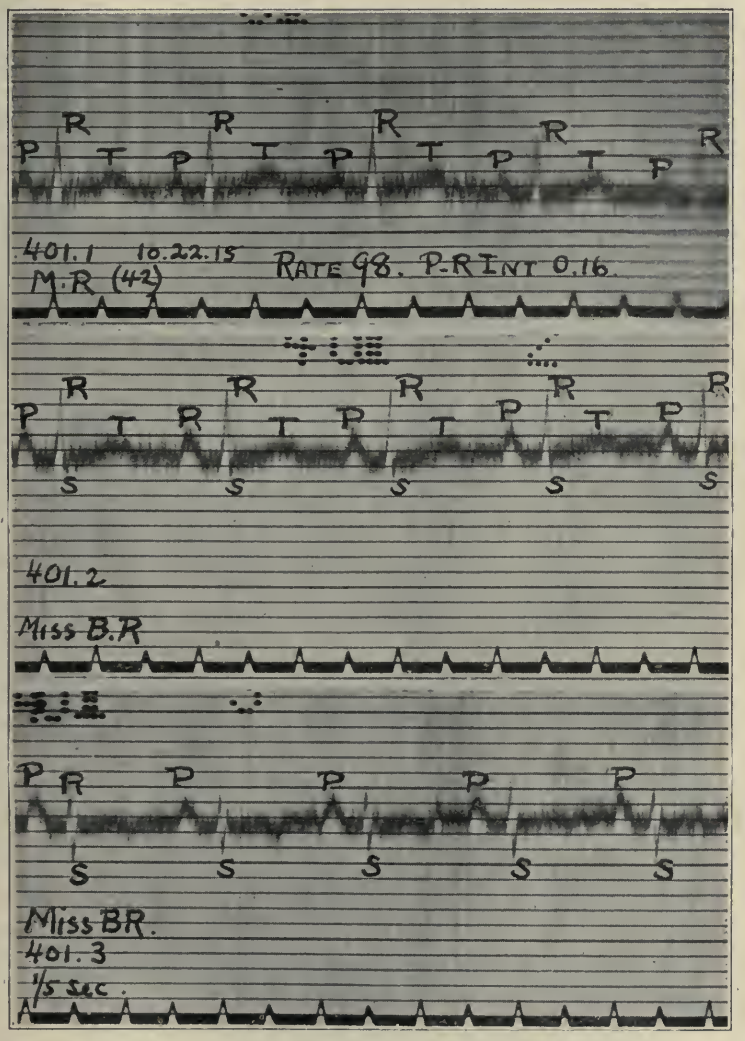

FIG. 10.—Case 42. Electrocardiograms of M. R. before and after operation, showing lessened T wave after operation and a more marked degree of left ventricular preponderance. In the second record note that the T waves are less than in the first (especially in Lead I); also that R_1 has become slightly longer than R_2 and that S_3 is proportionately longer than R_3. (See Fig. 11.)

in this clinic, although we cannot subscribe to "the converse proposition that if the summits fall within the normal limits of amplitude the heart is probably normal." We have seen in 2 cases

of this series, as well as in several other cardiac cases examined electrocardiographically, that the electrocardiogram was well within normal limits shortly before death from cardiac failure, and that even the form of the complex might be nearer normal shortly before

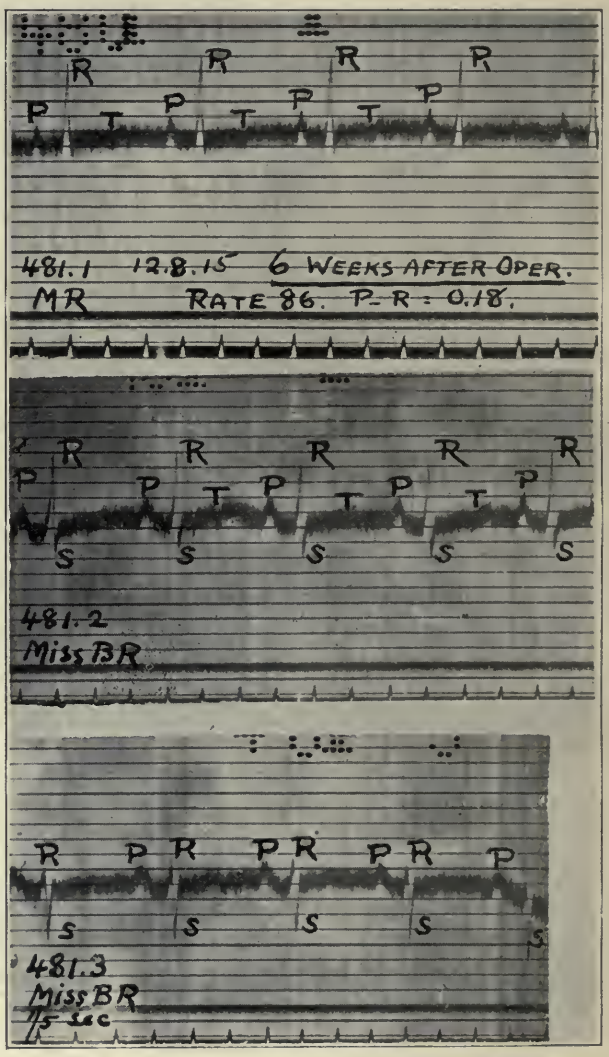

FIG. 11.—Same case as Fig. 10.

or during acute cardiac failure than it had been when the patient was clinically in better condition.

Preoperative variations from the normal form of the ventricular complex, notice of which has been included in Table II under the

TABLE 2.—ELECTROCARDIOGRAPHIC DATA ON GOITRE PATIENTS.

No.	Preoperative electrocardiogram.			Postoperative electrocardiogram.			
	Rate	P-R Int.	Form of egg.	Time after.	Rate	P-R Int.	Form of egg.
1	114	0.14	Normal	6 wks.	120	0.14	S less in all leads.
2	130	0.11	T_2 and T_3 inverted	7 days	109	0.14	S increased in all leads. T less (inverted or upright).
3	130	0.14	T_2 and T_3 inverted				
4	66	0.22	L. V. H.	5 days	78	0.20	P and T less in all leads.
				3 mos.	72	0.22	Same.
5	60	0.18	R. V. H.	4½ mos.	80	0.12	R. V. H. much less marked.
6	90	0.12	T_2 diphasic, T_3 inverted	5½ mos.	80	0.12	T_1 diphasic, T_2 and T_3 inverted. Not improved.
7	89	0.18	S_2 and S_3 very large.				
8	95	0.22	Tendency to L. V. H.				
9	98	0.14	Normal	7 days	100	0.14	T less in all leads.
10	68	0.14	Normal				
11	150	0.12	Q_2 and Q_3 very large.	5 days	112	0.16	T less in all leads.
12	127	0.16	Normal				
13	73	0.15	T_1 and T_2 diphasic	7 days	125	0.16	All T_2 inverted, Q_2 and Q_3 increased.
..	..	..	.....	3 mos.	95	0.14	Same. Died two days after second operation.
14	102	0.16	Normal	8 days	111	0.18	Very little change. Q_2 more marked S_2 less so.
15	58	0.16	Normal	7 mos.	68	0.16	Same. One ventricular extrasystole recorded.
16	88	0.14	Big notch low on R_3	3 days	109	0.14	S less in all leads. Notch on R_3 less and higher.
17	120	0.15	Tendency to R. V. H.	9 days	125	0.14	Tendency to R. V. H. more marked.
18	96	0.14	Deep S in all leads	8 days	105	0.14	No change.
19	130	0.14	Normal	3 mos.	108	0.13	No change.
20	87	0.14	Tendency to L.V. H.	9 days	100	0.14	L. V. H. more marked.
21	130	0.14	Tendency to R.V. H.	8 days	125	0.14	R. V. H. more marked.
22	103	0.14	T_2 and T_3 inverted	....	..	..	Died two days after operation.
23	66	0.16	Tendency to R.V. H.	5 mos.	68	0.14	Less tendency to R. V. H. T waves increased.
24	..	..	.....	5–7 yrs.	120	..	Auricular fibrillation, ventricular extrasystoles R. V. H. constant.
25	93	0.15	Tendency to R.V.H. Sinus arrhythmia	9 days	94	0.15	Less tendency to R. V. H. Sinus arrhythmia less.
26	..	..	.....	2–4 yrs.	127	varying	Auricular flutter. A–V Block (2 : 1–4 : 1).
27	100	0.13	L. V. H.	5 days	130	0.14	L. V. H. unchanged. T_1 and T_2 less marked.
28	90	0.14	L.V.H. T_1 negative	1 yr.	..	..	L.V.H. unchanged. T_1 upright.
29	100	0.17	Tendency to L.V.H.	9 mos.	..	..	L. V. H. more marked.
30	102	0.16	Normal	8 days	90	0.16	Less T.
31	100	0.15	Tendency to L.V H.	6 days	109	0.15	Tendency to L. V. H. less marked.
32	103	0.16	L. V. H.				
33	120	0.14	Tendency to R.V.H. T_2 and T_3 inverted	6 days	90	0.13	T less in all leads. R. V. H. unchanged.
34	117	0.14	Normal	7 days	88	0.14	S and T in all leads.
35	102	0.11	Tendency to R.V.H.	8 days	110	0.12	No change.
36	105	0.15	Normal	4 days	130	0 16	No change.
37	118	0.14	Tendency to R.V. H. Notched P_3 sinus arrhythmia	4 mos.	107	0.16	Less tendency to R. V. H. Notch in P_3 absent.
38	140	0.12	R. V. H. T_3 inverted	1 yr.	134	0.12	T_1 and T_2 increased T_3 less, R. V. H. no change.
39	75	0.18	Normal	3 days	102	0.16	T less in all leads.
40	101	0.14	Tendency to R.V.H.	....	..	..	Died 24 hours of heart failure.
41	63	0.16	L. V. H.	4 days	66	0.18	Less T in all leads.
42	102	0.16	Tendency to L.V.H.	6 wks.	80	0.18	L. V. H. more marked.
43	146	0.11	Normal R_3 notched	3 days	102	0.12	T less in all leads. R_3 notched deeply at peak.
44	130	0.12	Normal	8 days	94	0.14	S increased; T less in all leads.
45	109	..	Auricular fibrillation. T_2 diphasic, T_3 inverted	....	..	..	Improved without operation AF constant.
46	..	..	.....	5 mos.	64	0.14	Normal.
..	..	..	.....	1 yr.	60	0.14	S increased in all leads.
47	105	0.14	R. V. H.	7 days	115	0.15	R.V. H. disappeared. Normal.
48	..	..	.....	9 days	88	0.12	Normal.
49	127	0.14	Occasional ventricular extrasystole	7 days	130	0.14	Less T in all leads. No extrasystoles seen.
50	90	0.17	L. V. H. Occasional ventricular extrasystole	....	..	..	Improved without operation.
51	85	..	Auricular fibrillation T_2 diphasic, T_3 inverted R_3 M shaped	No operation	..	..	Improved without operation. Normal rhythm developed coincident with general improvement. T_2 upright. R_3 still anomalous.

R. V. H. and L. V. H. refer to right and left ventricular preponderance respectively.

heading of "Form of egg," not only include such changes as those showing hypertrophy of one or other ventricle but also notchings and

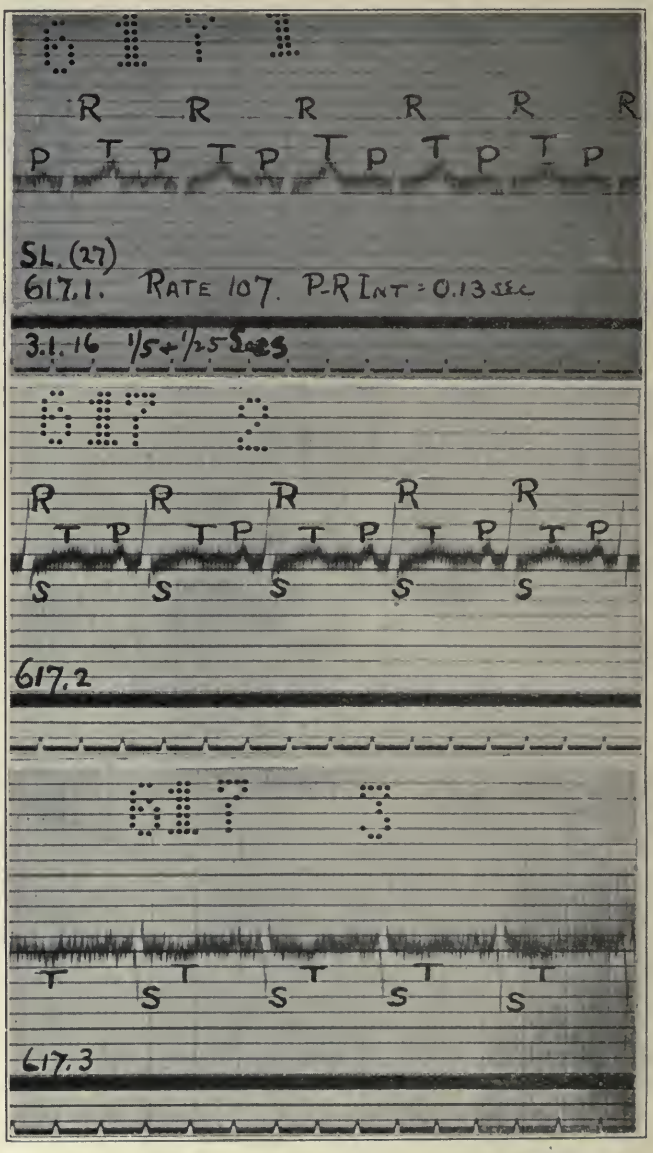

FIG. 12.—Case 27. Electrocardiograms of S. L., showing lessened T wave after operation. Time intervals in this record are expressed in $\frac{1}{5}$ and $\frac{1}{25}$ second, by vertical lines, as well as the Jaquet time marker. (See Fig. 13.)

undue prominence of single waves of the Q, R, S group (the clinical significance of which changes is as yet unknown) and also

various changes in form of the *T* wave. Essentially normal electrocardiograms in both form and rhythm were found in 22 of the 51 cases of the series.

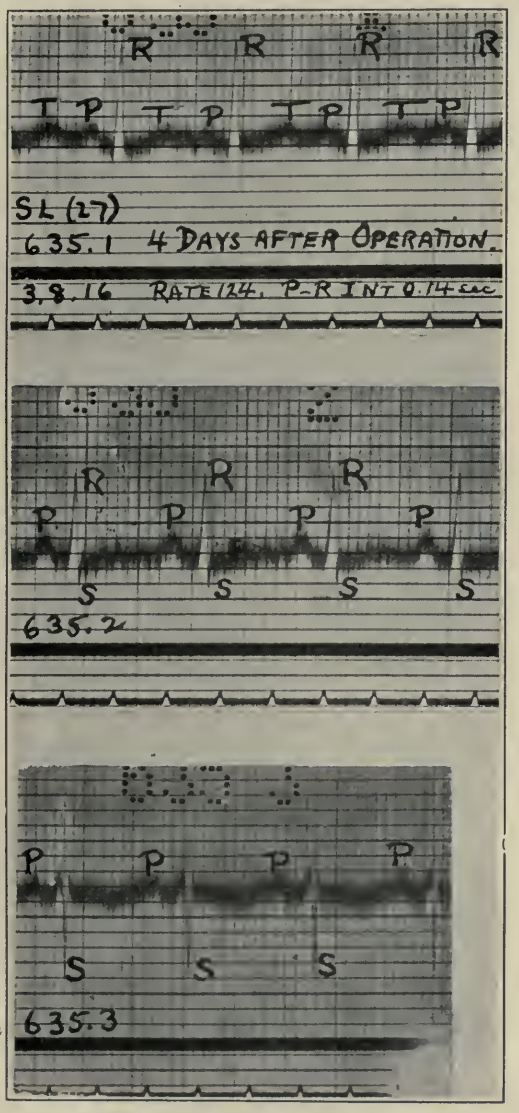

FIG. 13.—Same case as Fig. 12.

Hypertrophy of Auricles and Ventricles. Although the electrocardiogram registers action currents whose intensity depends on the strength of stimulus production, certain characteristic changes in the form of the ventricular complex have been recognized as indicat-

ing a preponderating hypertrophy of one or other ventricle. The evidence as to hypertrophy that is obtained in this way is more accurate and reliable than either the results of percussion or orthodiagraphic examination. The deduction as to the size of the muscle mass is nearly always true, because the stimulus going to

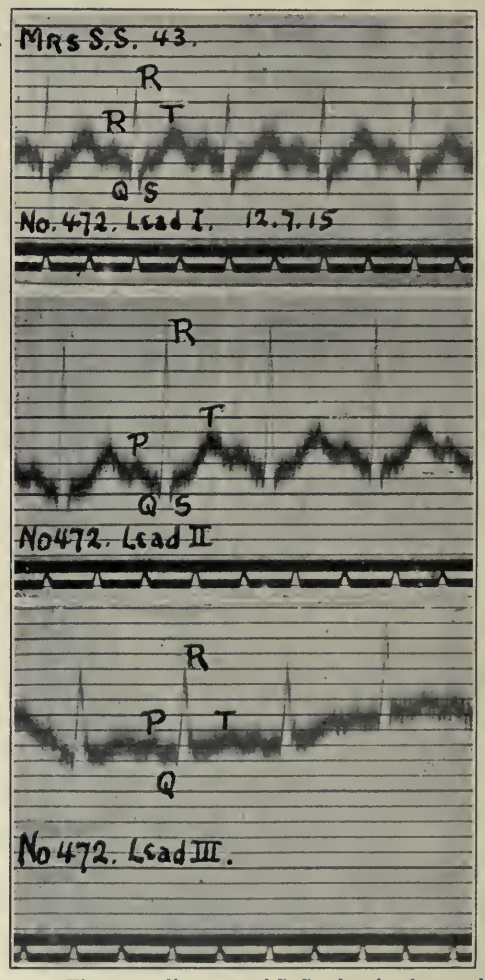

FIG. 14.—Case 43. Electrocardiograms of S. S., showing lessened *T* wave after operation that persisted for at least six months after operation.

the hypertrophied muscle mass is nearly always correspondingly increased. Some of the cases of this series, however, and also experimental work still in progress, indicate that the form of the ventricular complex (*i. e.*, the intensity of the stimulus production) may be noticeably changed without the occurrence of equivalent

change in muscle mass. Alterations occur within a few days after operation, when it would have been impossible for the muscle mass to have changed materially. The change in form that follows change in the axis of the heart has been shown to be relatively slight and clinically negligible, and is therefore not considered in this series.

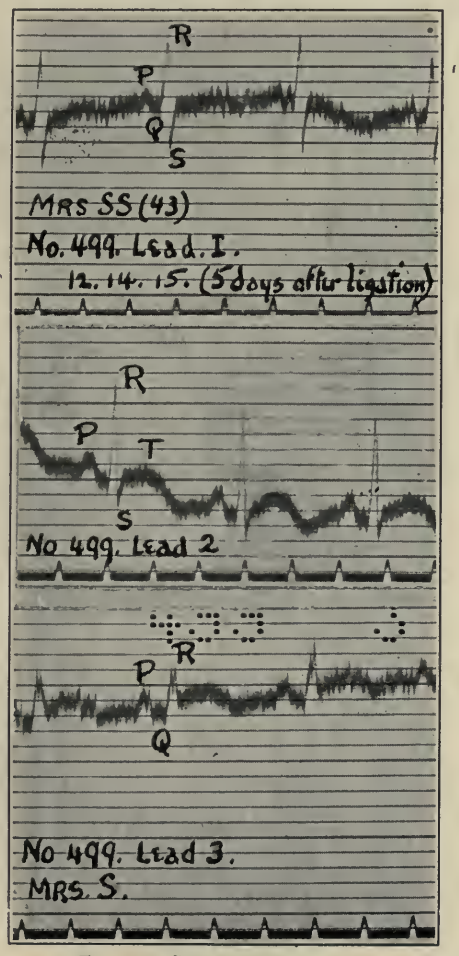

FIG. 15.—Same case as Fig. 14.

The state of right ventricular preponderance (R. V. H.) has been considered as present when both the S wave of Lead I (S_1) is greater than the R wave of Lead I (R_1) and when R_3 is greater than R_2 and when Q_2 and Q_3 are unusually large. When one of these factors is present or when the waves in question approach equality the condition is spoken of as a "tendency to R. V. H." Similarly

when R_1 is greater than R_2 and S_3 greater than R_3 and Q_1 prominent the state of left ventricular preponderance is recognized, and when this condition is approximated it is spoken of as a "tendency to left ventricular preponderance."

In the present series the majority of cases betrayed no signs of ventricular hypertrophy in the electrocardiogram. This is in accord with the experiences of other writers. Contrary to the general

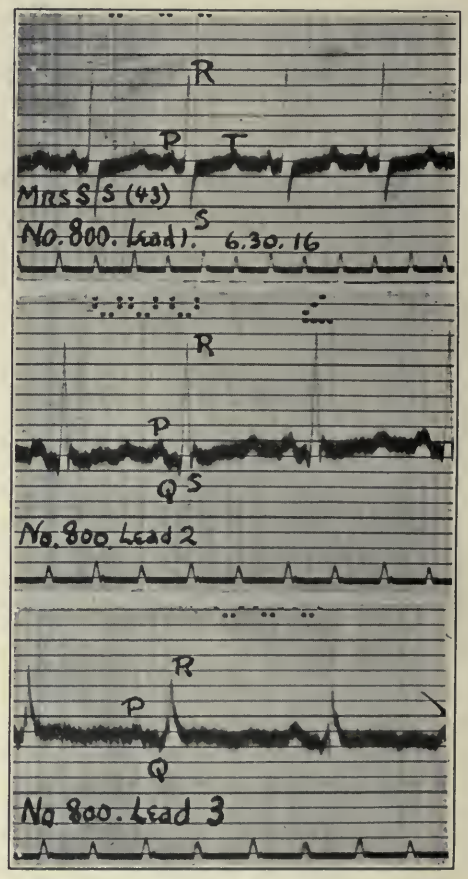

FIG. 16.—Same case as Fig. 14.

opinion, however, when hypertrophy was present, right ventricular preponderance has proved to be as common as left. It was distinct in 3 cases and a tendency to it existed in 8 cases. Left ventricular preponderance occurred in 6 cases and a tendency to it in 5 cases. It is difficult to explain why some cases of uncomplicated toxic goitre should develop right and others left ventricular preponderance. The most probable explanation would be that the left pre-

ponderance is due to whatever causes are responsible for the increased systolic pressure; whereas in the right preponderance cases other factors, such as relatively or actually incompetent mitral valve, would be considered as playing a more important

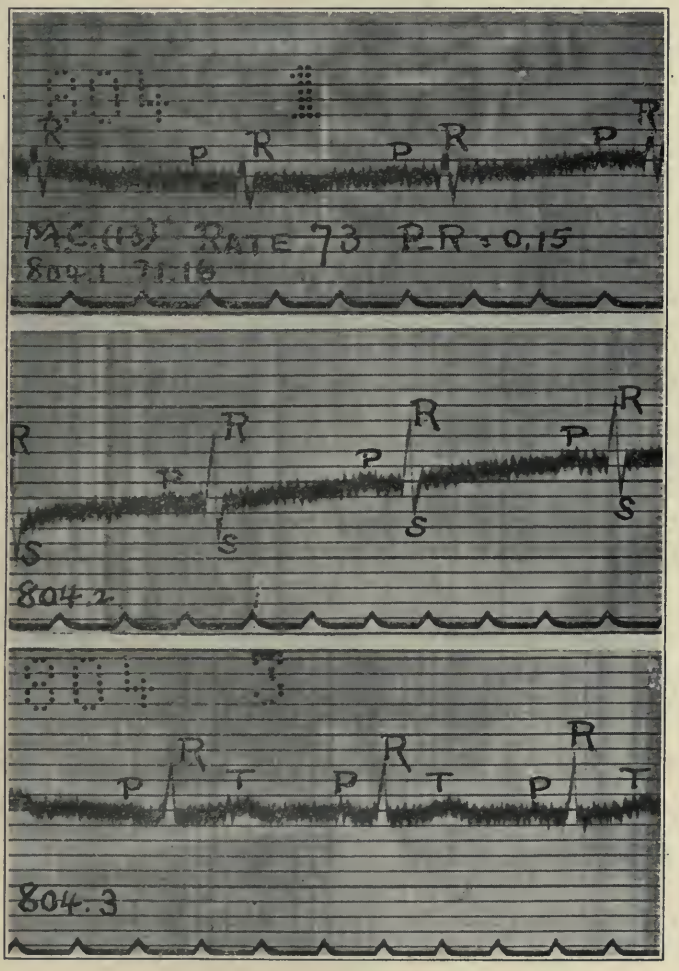

FIG. 17.—Case 13. Electrocardiograms of M. C., showing development of a negative *T* wave after operation. That this may be considered as a bad prognostic indication is supported by the death of this patient from cardiac failure after a second operation.

part. Some significance may be attached to the fact that the average duration of the disease in the right ventricular cases was under two and a half years, whereas in the left ventricular cases it was over four and a half years.

After surgical operation 5 of the right ventricle cases showed

less preponderance, 3 showed no appreciable change, and 2 an increased amount. Of the L. V. H. cases after operation, on the other hand, 4 showed no change, 6 showed an increased amount,

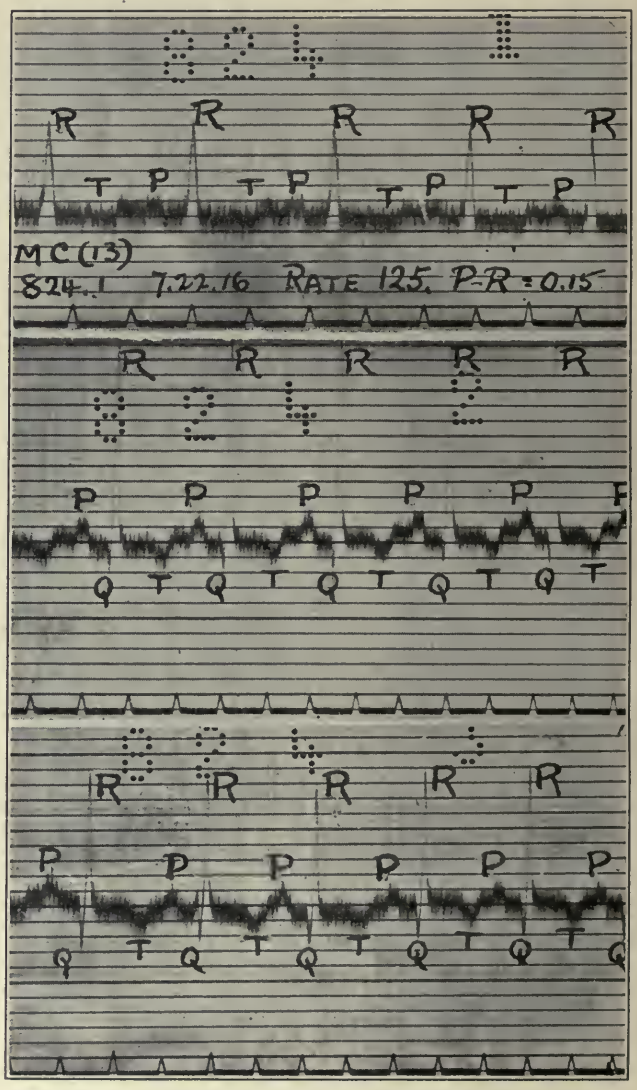

FIG. 18.—Same case as Fig. 17.

and only 1 showed a lessened degree of preponderance. This probably indicates that the left ventricular cases are due to a muscular hypertrophy that has already been accomplished and cannot be relieved by treatment even though signs of improvement are present.

The same reasoning may be applied to those right ventricular cases that do not change or progress. For those that showed lessened

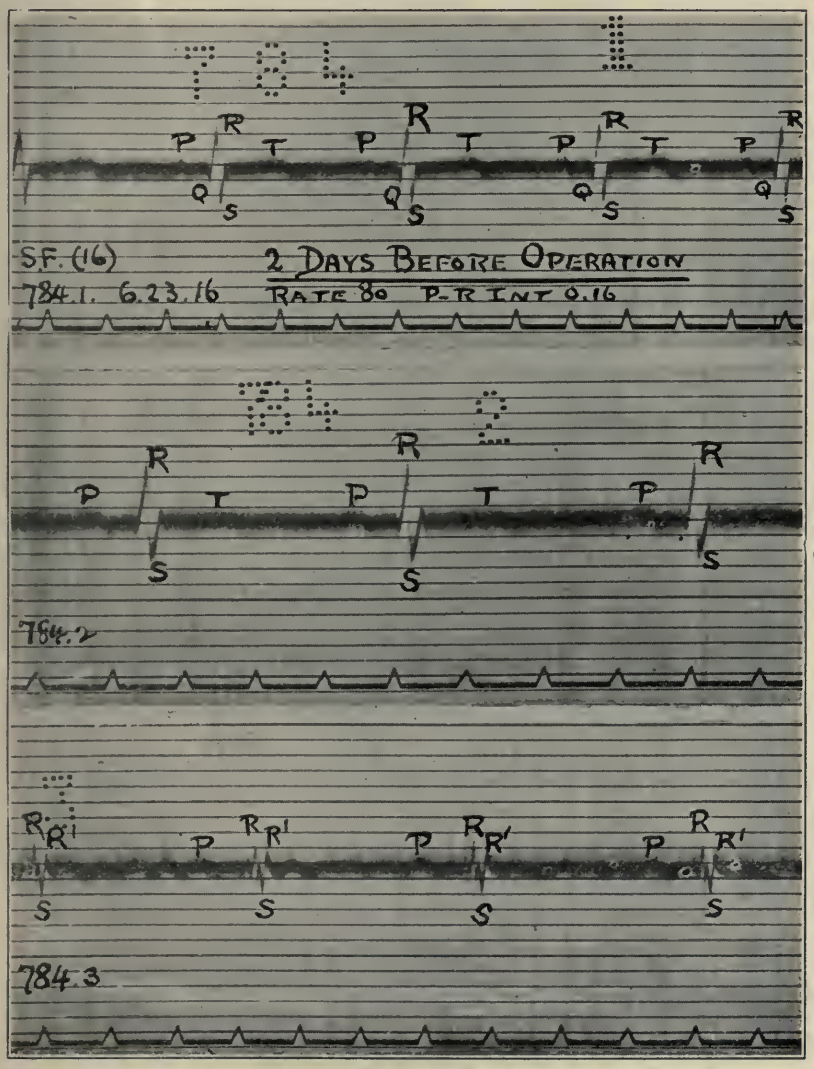

FIG. 19.—Case 16. Electrocardiograms of S. F., taken two days before, three days after, and twelve days after surgical operation, showing approximation of R_3 to normal in the last records. Note that the deep notch of R_3 in the first records becomes less and less noticeable in the second and third records. In this case the small postoperative T wave had already become larger by the twelfth day.

hypertrophy after operation a lessened impulse due to diminution of the intoxication might be assumed or possibly a nearer approach to competency of the mitral valve.

Changes in T Wave. In most of the cases of this series the T wave was well developed. This was especially noted in the more toxic cases and was even true in the 5 cases in which T_2 was either diphasic or inverted and in both cases in which T_1 was diphasic or

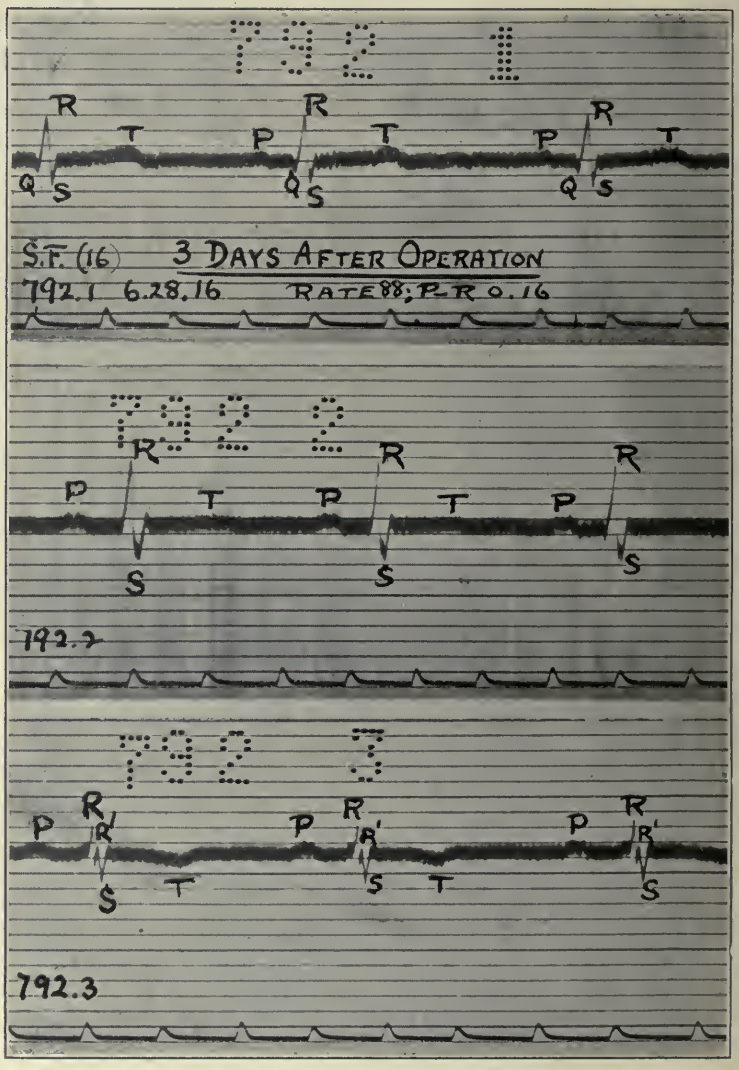

FIG. 20.—Same case as Fig. 19.

inverted. If in the absence of digitalis medication the T wave may be considered, as many authorities believe, as an index of the force of the cardiac contraction, such a result would be expected from the overacting goitre heart.

It is significant that out of the 23 cases that were examined within ten days of operation 11 showed a distinctly lessened *T* wave in all leads, coincident with general clinical improvement. One case also that improved greatly without operation exhibited

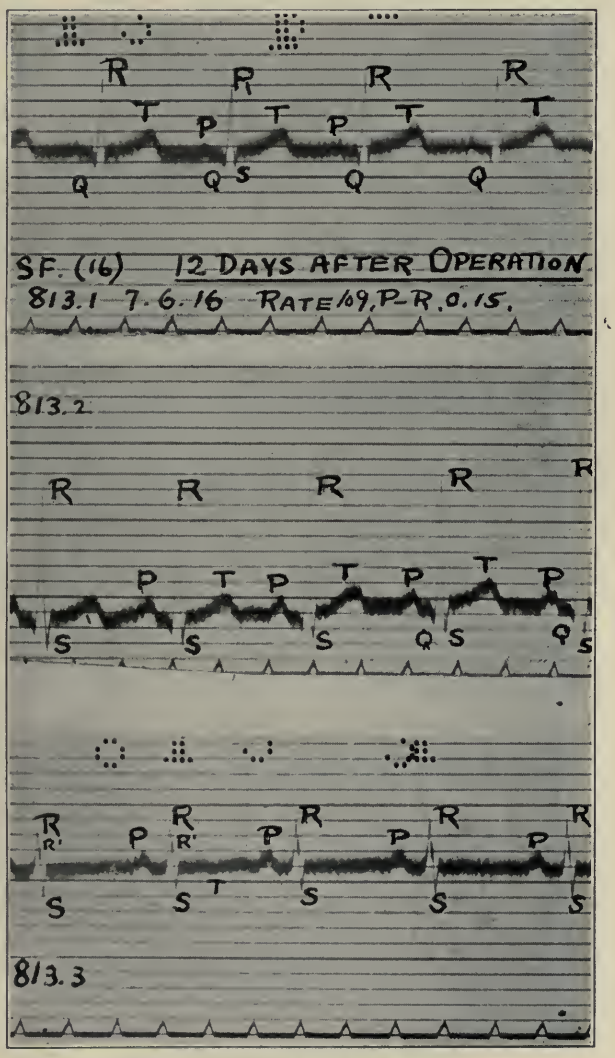

FIG. 21.—Same case as Fig. 19.

a diminished *T* wave in all leads during the improved period. The welcome deduction that could be drawn from these facts, to wit, that the smaller postoperative *T* wave indicated a quieting down of the overactive heart, was unfortunately complicated by

another observation, namely, that three control studies of three normal hearts showed the same tendency to a postoperative diminution of the *T* wave. This is a detail that in itself needs further

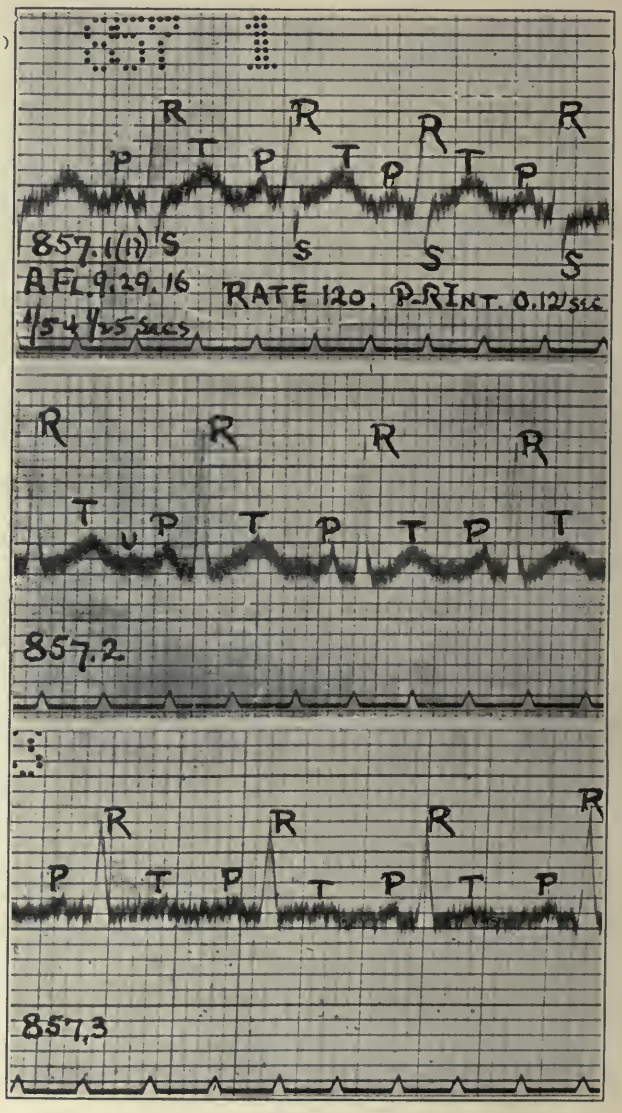

FIG. 22

investigation; at present, however, we are limited to the statement that such diminution is less in the normal than in the goitre cases and only lasts a few days. The diminution of the *T* wave of the

goitre heart is so much more accentuated and durable, often lasting three or more months, that it may be taken as highly probable that the heart's overaction has really been lessened and the treat-

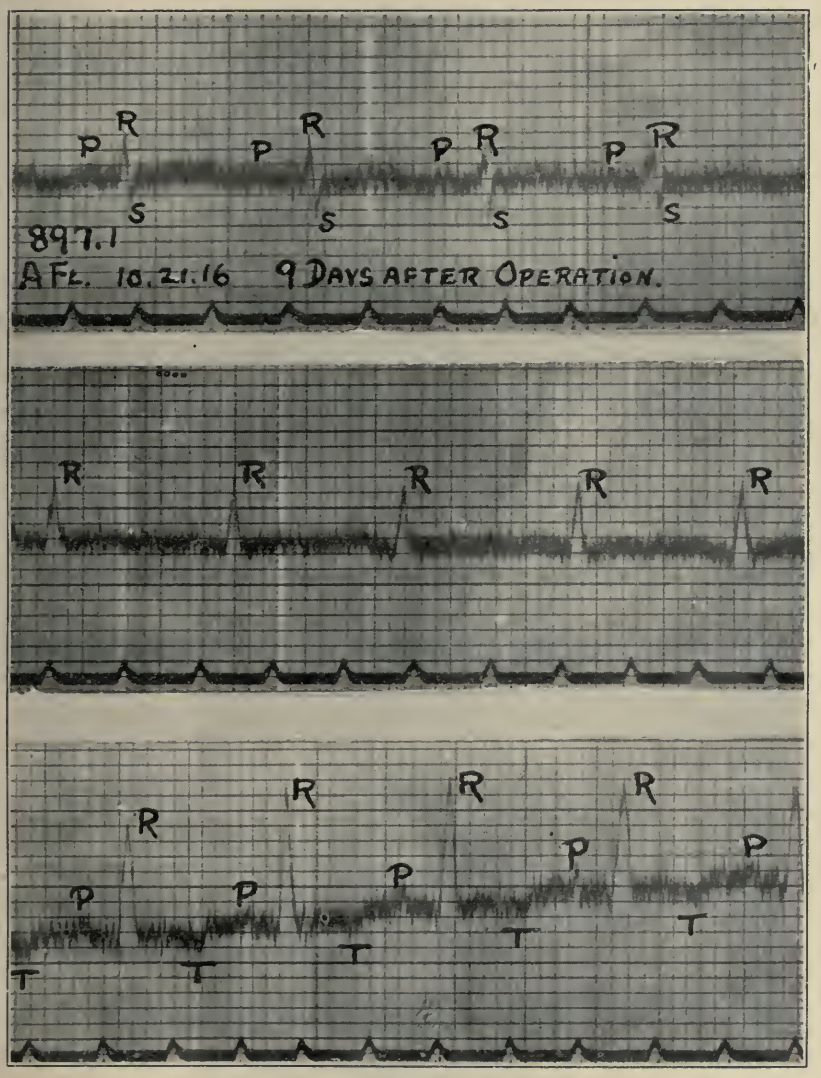

FIG. 23

ment been proved beneficial in so far as the electrical changes may be taken as a guide.

The persistent postoperative inversion of the *T* waves in one case (Case 13), with a subsequent fatal outcome to a second opera-

tion, has already been commented upon. In another case (Case 6) a failure to improve after operation was accompanied by the development of a negative *T* wave. In 2 other cases (Cases 28 and 51)

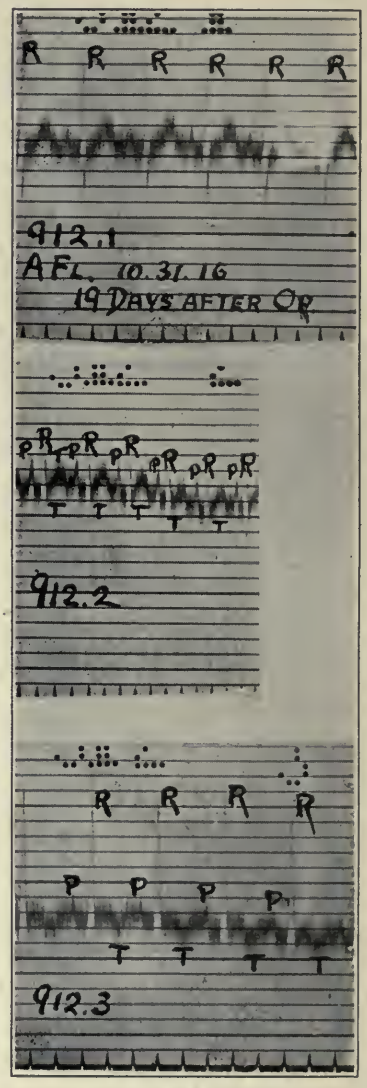

FIG. 24

general improvement during observation was accompanied by a change from negative to positive *T* waves. To this extent support is given to the idea that inverted *T* waves are of bad prognostic significance.

Other Changes in Form of the Ventricular Complex. In the form of the preoperative electrocardiograms various small changes from the established normal have been observed such as would be found in any similar series of cardiac cases. I refer to such details as unusually deep *S* waves in one or more leads, notching of the *R* wave and so forth. These were frequently changed after operation, or appeared after operation when they had previously been absent. As the significances of such changes, however, is not yet understood, and as they were often contradictory, no deductions can be drawn from them. Thus in Case 1 the *S* wave of an otherwise normal record was decreased in all leads after operation, whereas in Case 2 the *S* waves were all increased after operation. Case 16 (Fig. 11) presents an item of interest. In the preoperative electrocardiogram the *Q*, *R*, *S* group of Lead III was a bizarre collection of three upward and two downward peaks. Three days after operation, while the patient's general condition was improving, Lead III showed these peaks in such modified form that they could be explained as a deep notching of an *R*, *S* group. Nine days later, coincident with further improvement, Lead III had almost approached a normal complex, the notch being higher on the *R* wave and barely visible. While such anomalies are common in Lead III, and while their underlying cause is unknown, nevertheless it is reasonable to assume that the nearer approach of the electrocardiogram to a normal form is indication of an improvement in the cardiac mechanism.

SUMMARY. 1. Electrocardiographic studies have been made of 51 cases of goitre, mostly of the toxic type, in as many cases as possible both before and after surgical operation.

2. In the series of 47 patients that submitted to surgical operation (ligation, partial excision, and enucleation of adenomata) 3 deaths occurred; 2 of these offered no premonitory signs in either clinical or electrocardiographic examination; in the third case the development of negative *T* waves might have served as an adequate warning.

3. Electrocardiograms, essentially normal as to form and rhythm, were found in 22 cases (43 per cent.). Preponderating hypertrophy of the right ventricle (or a tendency thereto) was found in 11 cases, and of the left ventricle (or a tendency thereto) also in 11 cases. After operation, however, one-half of the right ventricular cases showed a diminished degree of preponderance, whereas all but one of the left ventricular cases showed either no change or an increased amount of preponderance.

4. Cardiac arrhythmia was found as follows: sinus arrhythmia, 4 cases; ventricular extrasystoles, 3 cases; auricular fibrillation, 3 cases; auricular flutter, 1 case; delayed conductivity, 2 cases. Two of the cases of fibrillation and the case of flutter proved constant over several years. The other case of fibrillation was of the

transient kind and disappeared coincident with the improvement that followed medical treatment.

5. The *T* wave was found to be unusually prominent in most cases. In about half the cases it was markedly and persistently diminished after operation. This was found to be true to a lesser degree in postoperative cases with normal hearts. Other changes in form of the ventricular complex after operation indicated an approximation to normal cardiac mechanism.

6. Blood-pressure estimation showed an increased pulse-pressure in most cases that was diminished with the improvement that followed surgical relief. Systolic pressure was also high in those cases that showed left ventricular preponderance, but never exceeded 170 mm. Hg.

Conclusions. 1. In early cases of toxic goitre the characteristic tachycardia is not accompanied by any signs of myocardial change that are demonstrable with the string galvanometer.

2. With persisting overaction of the heart, hypertrophy of either ventricle may become manifest.

3. Progressive hypertrophy and overaction results in myocardial degeneration that may be manifested by any type of cardiac irregularity: sinus arrhythmia, premature contractions, auricular flutter, auricular fibrillation, heart-block, etc.

4. If the existing intoxication is the chief factor in the production of the arrhythmia this may disappear with removal of the intoxication.

5. Successful treatment, whether medical or surgical, improves the cardiac condition by this means. This is shown not only by the occasional disappearance of an arrhythmia but also by diminution in the size of the *T* wave and in the pulse-pressure as well as by the general clinical condition.

6. The development of diphasic or inverted *T* waves, especially in Leads I and II, should probably be considered as influencing prognosis unfavorably.

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THE BACTERIOLOGY AND MICROSCOPY OF THE CONTENTS OF THE SEMINAL VESICLES POST-MORTEM. A STUDY OF FIFTY-TWO CASES[1]

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In view of the unprecedented interest manifested by urologists today in spermatocystitis, and in realization of the focal sepsis occasioned thereby, too little suspected and investigated by the average medical man, be he internist, surgeon or specialist, but nevertheless characterized by a retinue of urinary, arthritic, neurological, mental and even systemic diseases, it behooves the profession to welcome any attempt to study this obscure affection.

This paper is merely the forerunner of a more important one directed to the microscopy and bacteriology of spermatocystitis, during life, as revealed at the time of seminal vesiculotomy. Thus we hope to establish on a firmer basis certain indefinite and conflicting ideas respecting: (1) the presence of spermatozoa in the seminal vesicle, (a) normally and (b) when inflamed; (2) the life or death of spermatozoa in spermatocystitis and (3), the identification of the invading bacteria, gonorrhoeal and non-gonorrhoeal.

Normally, the seminal vesicles elaborate a specific yellowish albuminous secretion, which according to Virchow is a proteid compound, and claimed by Walker to belong to the group of histones. It is insoluble in water, but dissolves readily in acetic acid and a solution of ferrocyanide of potassium; it is not coagulated by heat, unless salts, particularly sodium chloride, are present; liquid when warm, it becomes gelatinous in consistence when cold. Much discussion has prevailed in the past respect-

[1] Read before the Philadelphia Pathological Society, January 25, 1917.

ing the function of the seminal vesicles, both in the capacity of secretory structures and as reservoirs for semen. The opinion is generally held at present that the spermatocystic secretion is derived from the epithelium of the vesicular mucosa, that it bears a definite though unknown relation to the spermatozoon, and can be differentiated by chemical analysis from the testicular secretion. This view is held by Akutsu, Casper, Fürbringer, Rehfisch, Quinby and many others. In addition to its own peculiar secretion, the seminal vesicle, under normal conditions harbors epithelial cells, occasional leucocytes, lecithin granules, sago bodies and numerous spermatozoa.

Singularly enough there is no unanimity of opinion even today respecting the point as to whether or not the seminal vesicles subserve the function of reservoirs. Hundreds of years ago Fallopius and Regnerus de Sraof contended that the vesicles were containers of semen, while opposed to this view were Wharton van Hornes, Swamerdam and Hunter. The Royal Society of London left the decision with Sraof, but he was unable to form a conclusion on this scientific matter. More recently Rayer states "In the contents of the seminal vesicles there are found some seminal threads, although not so regularly and not so numerously that the seminal vesicles can be considered as storage places for the spermatozoa." Lenhossek and Weisz conclude that the "seminal vesicles positively do not serve as reservoirs for the testicular secretions."

Irrespective of these statements, alleged to be based upon investigations, we believe consensus of opinion favors the belief that the seminal vesicles, in addition to their own secretory function, also serve as reservoirs for the testicular secretion. Evidence to this effect is furnished by the fact that practically all vesicles normally contain varying numbers of spermatozoa, also in our post-morten study of fifty-two cases, many vesicles were found to harbor spermatozoa. Thus the dual function of the vesicles can scarcely longer be contested.

It is not our intention at this time to differentiate the acute, subacute and chronic types, nor to enter into a discussion of the clinical aspects of spermatocystitis. It is sufficient to recall,

(1), the frequency of the affection, hence its importance, and (2) the gross pathology associated with the bacteriology. Inflammation of the seminal vesicles may be divisible into the following forms: (1) catarrhal; (2) suppurative, with or without loculations of pus or abscess formation; (3) interstitial and (4) pericystic. On rectal examination, these may vary from an impalpable lesion to a pyovesiculosis of 50 cc. content or a mass the size of a goose egg (Kocher, Petersen). The perivesicular form leading to a chronic infiltration of the trigone and neck of the bladder may lead to symptoms identical with those of prostatism and associated in one of our cases with a residual urine of 15 ounces. In a considerable number of our cases studied post-mortem, atrophy, obliterative changes and inflammatory enlargement of the vesicles were demonstrable.

The frequency of spermatocystitis is yearly becoming more widely recognized, some investigators regarding it as the commonest complication of gonorrhoea (Lloyd, Fuller, Collan, Weisz). It is probably true that epididymitis does not exist without an associated seminal vesiculitis, and the belief is steadily gaining credence that inflammation of the spermatic vesicles is as common, if not more frequent than prostatitis, as a gonorrhoeal complication (Lucas, Colombini, Guépin and Kennedy). Mayer found 60 per cent of patients with posterior urethritis to have involvement of the seminal vesicles. Lewin and Bohm encountered spermatocystitis in 35 per cent and prostatitis in 29 per cent of 1000 gonorrhoeal cases.

Although gonorrhoea is the usual cause of spermatocystitis, it is seldom, in our experience, that the gonococcus can be identified; the vast majority of cases presenting a mixed infection or at least some other superimposed pyogenic bacterium. Post-mortem study in our series failed to demonstrate the gonococcus in a single case. In the living subject, Reich was the first to demonstrate gonococci in a spermatocystic abscess, and v. Sehlen found them in the expressed secretion of the vesicles. Since then this observation has been repeatedly made in 14 to 60 per cent of cases (Collan, Mayer, Möller, Feleki, Finger, Lewin and Bohm). Recognizing the prevalence of spermatocystitis due to

gonorrhoea, and conscious of the opinion of some authorities that men who have had repeated attacks of gonorrhoea, invariably are sufferers from chronic inflammation of the seminal vesicles, it should be borne in mind that occasionally other causes are responsible. Among these should be mentioned: (1) bacterial invasion from contiguous structures; (2) urethral instrumentation; (3) circulatory disturbances; (4) systemic infections; (5) perineal trauma according to Kocher and (6) masturbation and venereal excesses. Tuberculosis, an affection of not uncommon occurrence is of such special interest and importance that it deserves separate attention and has been excluded from consideration in this paper.

The content of the inflamed vesicles is a muco-purulent or purulent material mixed with a pellucid filamentous stroma or colloidal brownish liquid containing conglomerated fat globules, desquamated and degenerated epithelial cells, pus, erythrocytes, lecithin granules, concretions, bacteria and possibly spermatozoa. In a case of gonorrhoea complicated by bilateral epididymitis, pyelonephritis and spermatocystitis, Duhot found in the hemorrhagic vesicles a thick, cloudy content consisting of numerous leucocytes and erythrocytes, hematoidin crystals, pigment granules, epithelial cells, extra and intracellular gonococci, a few bacilli, but no spermatozoa. Guelliot and Collan likewise found the inflammatory content devoid of zoösperms. Lewin and Bohm saw either dead or living spermatozoa in 11 per cent of cases; in the remainder none was visible.

The precise bacteriology of the seminal vesicles has always been a matter of much uncertainty and conjecture. There is reasonable ground to doubt the occurrence of a pure gonococcal spermatocystitis, just as there is of a cystitis, prostatis, epididymitis or even a posterior urethritis. The differentiation of the gonococcus from the M. catarrhalis is at times a matter of great difficulty. It will be remembered that although the posterior urethra normally contains no bacteria, the anterior urethra furnishes at least a dozen or fifteen varieties. During the course of a siege of gonorrhoea, invasion of the posterior urethra and its adnexa by the mixed infection from the anterior urethra

becomes a simple matter, and then as a rule the gonococcus speedily becomes implanted in the tissues or supplanted by the associated more rapidly growing and virulent pyogenic bacteria. Thus, frequently, the complications and sequellae of gonorrhoea arise.

Huet finds that bacteria may be present in the seminal vesicles of healthy animals, as horses, cattle, pigs and laboratory animals, and attempts to apply his observations to man. He has also found that in animals dying of acute septicemia, the specific organism (anthrax, pneumonia) can be recovered in the vesicular secretions. Furthermore, it was shown definitely that an infection with which the male was suffering could be transmitted to the female during the act of copulation.

Marchildon reports two cases of typhoidal spermatocystitis, that were autopsied and pure cultures of the B. typhosus obtained from both vesicles and prostate.

Fuchs was able microscopically and culturally to demonstrate the B. coli in association with gonococci.

Barney reports a few cases in which sterile cultures were obtained, although in one vesicle the microscopical smear showed a large colony of cocci, possibly diplococci, some of them intracellular. Voelcker reports finding the pneumoccocus in vesicles removed at operation. Picker has repeatedly shown not only this organism but also the gonococcus. Fuller states that in many chronic cases no gonococci can be demonstrated definitely to exist in the urinary tract or in the material expressed from the seminal vesicles; organisms apparently streptococci, however, have been found. Belfield relegates the infection to the colon bacillus and the gonococcus. Schmidt says that the "gonococcus is not the germ which is the one most often found. In the acute stage the gonococcus may pave the way for secondary infection, but it soon becomes extinct and the secondary infectious germs are the cause of the resultant condition and symptoms. In the order of frequency these are the staphylococcus, streptococcus and the colon bacillus. I do not mean, however, that the gonococcus is ever absent in the chronic cases." Smith sums up the evidence and claims that the sem-

inal vesicles are infected "principally by Neisser's diplococcus, and its associates, the staphylococcus, the streptococcus and the colon bacillus."

Squier, in 1913, in spite of negative findings in repeated cultural and bacteriological tests of the vesicular contents, believes in the theory of a mixed infection. In 1915, in view of the multiplicity of results, he forms two conclusions: (1) with the exception of the suppurative cases, the gonococcus is regularly absent; (2) there is almost constant growth of pyogenic bacteria. Referring to the work of Rosenow and Buerger with pneumococci in 1907, he suggests that the gonococcus may undergo a mutation depending upon its environment and declares that "it is not too much to presume that the gonococcus may mutate, and what is in the beginning a Neisserian seminal vesiculitis, is latterly a streptococcic infective process."

From the foregoing diversity of opinion it is apparent that little is definitely known respecting the exact nature of the bacterial flora of spermatocystitis. Moreover many reported bacteriological results have been based upon secretion or material obtained after expression or massage of the seminal vesicles and prostate. Even were it possible by this means to keep the two secretions separate, the method, so far as accurate findings are concerned, is open to serious criticism on account of unavoidable contamination from the bacteria prevalent in the urethra. Absolute indisputable results can be obtained only by incision of the vesicles and direct bacteriological examination of their contents.

Believing that a study of the spermatocystic secretion, postmortem, might be of interest, if not instructive, we submit the following table of fifty-two cases, reserving the more important series, namely those cases studied during life in conjunction with seminal vesiculotomy, for a secondary report.

The cases here tabulated were all autopsied in the Philadelphia General Hospital, and we wish to express our deep appreciation to Dr. Randle C. Rosenberger for his courtesy in placing this material at our disposal. Few, if any, of the bodies were as fresh as might have been desired. Some were seen in a few hours, but many not until after they had been in cold storage

for a day or more. In most instances, the bladder with attached seminal vesicles had been removed and placed in a refrigerator for a few to twenty-four or more hours before bacteriological culture was attempted. The age of the subjects ranged from eighteen to seventy-four years. The diagnoses are noted for the respective cases but have no definite significance with respect to the seminal vesicular findings, even in those few cases where a history of gonorrhoea was obtainable, which was the case in only 13.4 per cent, of whom practically one-half showed no growth, and the remainder the colon bacillus, with one exception when the M. albus was isolated; 15.3 per cent denied gonorrhoeal infection, and of these one-fourth demonstrated the colon bacillus. In thirty-seven cases or 71.3 per cent no statement, negatively or positively, of venereal infection was obtainable.

The technique of examination was as follows: The vesicles were sufficiently dissected to expose their exterior. A prominent area of the surface was rendered sterile by searing with a hot blade. The vesicle was then incised with a sterile knife and its lumen and content exposed. Cultures were taken on blood agar or serum, on plain agar and in bouillon. If no growth appeared in two days under the usual cultural conditions, the incubation was continued anaerobically for a few days more. Another portion of the vesicular secretion was then spread on the glass slide, stained and studied microscopically. Finally a hanging drop preparation was made and observed under the microscope.

In sixteen cases B. coli was cultured, but little importance attaches to this, since it was found in a number of subjects who denied venereal infection. Moreover, in view of the high percentage of times this bacterium was cultured as compared with the other organisms, the thought naturally arises, whether this could not have been a post-mortem invasion of the vesicle due to the proximity of the rectum. At any rate the finding supports the belief that intestinal flora may permeate the tissue spaces or minute cellular perforations, and thereby in this manner gain entrance to the urological tract. It is significant, how-

Table of autopsied cases showing microscopical and bacteriological contents of seminal vesicles

NO.	NAME	AGE	DIAGNOSIS	GONORRHOEA DURING LIFE	HANGING DROP	CULTURE	SMEARS MICROSCOPICALLY
1	Brokel	69	Hemiplegia chronic myocarditis		None obtained—vesicles atrophied—lumen could not be found	No culture taken	No fluid could be expressed and no smears made
2	Reaves	28	Pulmonary tuberculosis	−	No spermatozoa seen	No growth	Fluid thick and inspissated, almost gelatinous—no pus cells
3	Featis	51	Acute dilatation heart—chronic myocarditis	−	Well defined and large vesicles — spermatozoa seen	No growth	Spermatozoa
4	Barnes	28	Spinal pachymeningitis	+	Spermatozoa seen	No growth	Spermatozoa
5	Rockmack	60	Senile dementia		Spermatozoa seen	No growth	Spermatozoa
6	Bakas	41	Paresis		Spermatozoa seen	No growth	Spermatozoa
7	Richardson	59	Chronic interstitial nephritis; chronic myocarditis		No spermatozoa	No growth	Very little—practically no fluid in the vesicles—smears = negative
8	Jasper	57	Senile dementia; chronic myocarditis		No spermatozoa	No growth	Negative
9	Nothenius	39	Malignant endocarditis		No spermatozoa	No growth	Few pus cells—no organisms seen—no spermatozoa
10	Hatzfeld	34	Chronic myocarditis paresis		Very thick seminal fluid—no spermatozoa seen	M. aureus	Pus cells—no spermatozoa
11	Fay	60	Diffuse nephritis	−	No spermatozoa	No growth	Negative

12	Prince	46	Pulmonary tuberculosis—left pyopneumothorax		Blood clot in vesicles—no hanging drop	No growth	Red blood corpuscles and a few white blood corpuscles—no spermatozoa seen
13	Marioshelli	65	Bronchiestasis		No spermatozoa	No growth	Negative
14	Keating	47	Pernicious anemia		No hanging drop	No growth	Vesicles atrophied, no lumen found nor fluid expressed, no spermatozoa seen in smears
15	Petrofski	44	Paresis—horse shoe kidney		Spermatozoa seen	B. coli	Few pus cells—spermatozoa
16	Cornery	49	Acute cardia dilatation—chronic nephritis—alcoholism	+ Also syphilis	Spermatozoa seen	No growth	Spermatozoa
17	Bond	50	Pyothorax—myocarditis		Spermatozoa seen	B. coli	Spermatozoa—pus cells—epithelial cells
18	Dean	18	Miliary tuberculosis—tuberculosis meningitis		Spermatozoa seen	No growth	Few pus cells—spermatozoa
19	Weikle	53	Tabes—aortic regurgitation myocarditis		Spermatozoa seen	No growth	Spermatozoa
20	Monaghan	63	Aneurism — nephritis — emphysema — pneumothorax	+ Also syphilis	Spermatozoa seen	B. coli	Spermatozoa—pus cells
21	McGonigle	56	Epilepsy — pulmonary tuberculosis—chronic interstitial nephritis	+	No spermatozoa—left testicle absent—old healed scar over scrotum—right testicle O. K.	B. coli	Pus in prostate—pus in smears—no spermatozoa

Table of autopsied cases—continued

NO.	NAME	AGE	DIAGNOSIS	GONORRHOEA DURING LIFE	HANGING DROP	CULTURE	SMEARS MICROSCOPICALLY
22	Vloerburg	57	Tabes—paresis		No spermatozoa	No growth	No pus cells—no spermatozoa
23	McClain	28	Pneumonia		No spermatozoa	M. aureus	Few pus cells—no spermatozoa
24	McNama	43	Granuloma—fungoides	—	No spermatozoa	No growth	Large and well defined vesicles—no pus cells—no spermatozoa
25	Monocelli	46	Cerebro-spinal lues—hemiplegia		No spermatozoa	B. coli	Pus cells—no spermatozoa
26	Williams	26	Lobar pneumonia		No spermatozoa	No growth	Negative
27	Wagner	74	Hemiplegia—senile broncho-pneumonia		Hypertrophy of prostate — vesicles atrophied — no spermatozoa	B. coli	Only small amount of fluid—few pus cells—no spermatozoa
28	Bryant	50	Intra-cranial hemorrhage	—	No spermatozoa	No growth	Negative
29	Printz	42	Cerebral lues—hypostatic pneumonia		Spermatozoa seen	B. coli	Few pus cells—spermatozoa
30	Howard	30	Chronic interstitial nephritis		Spermatozoa seen	No growth	Negative
31	Buggey	61	Cerebral thrombosis		No spermatozoa	No growth	Negative
32	Maloney	50	Myocarditis — hemiplegia		No spermatozoa	B. coli	Few pus cells—no spermatozoa
33	Shaw	59	Paresis—myocarditis		No spermatozoa	No growth	Negative
34	Prendergast	50	Chronic endocarditis—anemia—cirrhosis of liver		No spermatozoa	No growth	Negative

35	DeSondro	49	Perforation intestinal typhoid fever		No spermatozoa	No growth	Negative
36	Banks	42	Aneurism of thoracic aorta	+ Also syphilis	Spermatozoa seen	No growth	Spermatozoa
37	Harris	56	Myocarditis—nephritis hypertrophic prostate	−	Thrombosis in left seminal vesicle — right shows spermatozoa	No growth	Few pus cells—spermatozoa with tails off and broken
38	Erickson	50	Myocarditis—diabetes nephritis		Very thick and inspissated seminal fluid—no spermatozoa	B. coli	No pus cells—negative
39	Fagen	44	Pulmonary tuberculosis		Vesicles well defined—large lumen — no spermatozoa	B. coli	No pus cells—no spermatozoa
40	Dempsey	46	Paraplegia — bronchopneumonia — mitral disease		No spermatozoa	No growth	Crystals—triple phosphates—very little fluid in vesicles—no pus cells
41	Wharton	37	Chronic myocarditis		Well defined vesicles—spermatozoa seen	B. coli	No pus cells seen—spermatozoa
42	Bowen	44	Chronic myocarditis		Not much fluid present —few spermatozoa	No growth	Red blood corpuscles and white blood corpuscles—few spermatozoa
43	Komszey	64	Lobar pneumonia		Vesicles and prostate atrophied—no lumen of vesicles found —no hanging drop	No culture taken	No pus cells—spermatozoa seen
44	Welsh	44	Aortic regurgitation—mitral regurgitation —hydrothorax	+	Well defined vesicles—spermatozoa seen	M. albus	No pus cells—spermatozoa seen

Table of autopsied cases—concluded

NO.	NAME	AGE	DIAGNOSIS	GONORRHOEA DURING LIFE	HANGING DROP	CULTURE	SMEARS MICROSCOPICALLY
45	Steventon	47	Delirium tremens—lobar pneumonia	–	Spermatozoa seen	B. coli	No pus cells—spermatozoa seen
46	Conway	28	Myocarditis — morphine and heroin habitué		Gelatinous substance in vesicles and practically no fluid—no spermatozoa seen	B. coli	Few pus cells—no spermatozoa — mucoid substance
47	Fuchs	59	Myocarditis — choelethiasis — esophageal obstruction		Very little fluid in vesicles—few spermatozoa	No growth	Spermatozoa
48	Chester	66	Senile dementia — chronic nephritis		Very little fluid—no spermatozoa seen	B. coli	Crystals—triple phosphates—no pus cells—no spermatozoa—red blood corpuscles
49	Ball	54	Transverse myelitis—cystitis—pyelitis	+ Also syphilis	Very little fluid in vesicles—no spermatozoa	B. coli	Crystals—no pus cells—no spermatozoa
50	Powell	31	Pulmonary tuberculosis—hypomania		Many spermatozoa	No growth	Occasional pus cells—many spermatozoa
51	Long	49	Pulmonary tuberculosis—tertiary lues	–	Very like inspissated fluid in vesicles—no spermatozoa	B. coli	Pus cells—crystals—no spermatozoa
52	Michael	44	Broncho-pneumonia—pulmonary tuberculosis—appendicitis		Spermatozoa seen	No growth	Spermatozoa—no pus cells

ever, that in all save six cases of colon infection, pus cells were demonstrable in the vesicular secretion, evidencing the probability of an ante-mortem spermatocystitis due to this bacterium.

In two cases, one of myocarditis and paresis, the other of pneumonia, M. aureus was found in association with pus cells in the vesicles.

In one case with a history of gonorrhoea, M. albus was cultured.

In a few cases B. subtilis was obtained in the culture. This was undoubtedly the result of contamination.

The high percentage of sterile cultures is not surprising, when it is realized that the subjects and vesicles were refrigerated for considerable periods previous to the time of taking cultures, and helps to explain the failure to obtain any bacterial growth in a number of cases presenting pus in the vesicles.

A review of the tabulated cases reveals the fact that in twenty-one or over 40 per cent, spermatozoa occurred in the presence of evidence of inflammation, either bacteria or pus or both. Obviously, the zoösperms were all dead. In fourteen cases exhibiting signs of spermatocystitis, or approximately 27 per cent, no spermatozoa were discoverable.

CONCLUSIONS

1. The seminal vesicles harbor spermatozoa after death, and therefore presumably during life.

2. This function is exercised in the presence of inflammation (spermatocystitis), although in a large percentage of inflammatory cases no spermatozoa can be found (post-mortem).

3. The determination of the viability of the zoösperm in the presence of seminal vesiculitis, the exact identification, classification and relative frequency of the invading bacteria, particularly with reference to gonorrhoea, are questions impossible of solution by post-mortem investigation, and will constitute a secondary and final report after bacteriological and microscopical study conducted in conjunction with seminal vesiculotomy on the living subject.

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