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STAFF

In this volume are collected the more important papers published by the Department of Pathology and Bacteriology of the University of Illinois, College of Medicine, Chicago, during the years 1920 and 1921.

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FOOD ACCESSORY FACTORS IN BACTERIAL GROWTH

III. FURTHER OBSERVATIONS ON THE GROWTH OF PFEIFFER'S BACILLUS (B. INFLUENZAE)

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In a previous article,[1] I called attention to the rôle of two substances in the growth of Pfeiffer's bacillus; one, hemoglobin or a derivative and the other, a substance obtainable from plant tissues (carrot, potato), animal tissue, bacteria, yeasts, etc. There are two methods by which these processes have been studied—one, by adding the substances in question to plain blood agar or broth in test tubes and observing the growth after inoculation with Pfeiffer's bacillus; the other by making a poured blood unheated or heated) agar plate, seeding heavily with Pfeiffer's bacilli and then inoculating here and there with bacteria, yeasts, pieces of tissues, etc. About the latter, Pfeiffer's bacilli will develop large or "giant" colonies, in this manner forming a cluster of colonies about the central foreign colony or tissue and known as the "satellite" phenomenon.[2] Further experiments have been made as to the mechanism and the nature of the substances involved in the growth processes of this organism which I wish to report now.

In the following experiments at least two strains of Pfeiffer's bacilli were used; one isolated from a pneumonic lung during the 1919 epidemic, the other from the spinal fluid of a case of so-called influenzal meningitis in a child. No differences of behavior were noticed between these organisms. In certain experiments other respiratory strains of Pfeiffer's bacilli were used with comparable results.

Blood mediums when moderately heated will give a profuse growth of Pfeiffer's bacillus; when heated in the autoclave for 30 minutes it yields practically no growth. The autoclaved medium now may be made to yield excellent growth by adding thereto certain substances which in themselves do not allow growth when added to plain medium.

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[1] Jour. Infect. Dis., 1917, 21, p. 392.
[2] Davis, D. J., Ibid, p. 178.

Such substances include tissues of various kinds, or their extracts or filtrates, such as carrot, potato, and animal tissues. However, when these tissues or their extracts are heated to the boiling point for a time (1 to 2 hours) or are autoclaved they will no longer activate the autoclaved blood. Table 1 illustrates these points.

TABLE 1

GROWTH OF PFEIFFER'S BACILLUS

Plain medium + autoclaved blood	=	0
Plain medium + carrot or potato filtrate	=	0
Plain medium + autoclaved blood + carrot or potato filtrate	=	+++
Plain medium + autoclaved blood + autoclaved filtrate	=	0
Plain medium + washed heart muscle (guinea-pig)	=	0
Plain medium + autoclaved blood + heart muscle	=	+++
Plain medium + autoclaved + autoclaved heart muscle	=	0

Various plant tissues may be used in this experiment and also various animal tissues, such as liver, heart muscle, kidney, brain, spleen, etc. There is an advantage in using plant tissues because they contain no hemoglobin. However, in using animal tissues for activating purposes, by washing small pieces for a long time one can remove this substance. Furthermore by employing the satellite test one can observe the activating effect even on medium containing unheated hemoglobin, so there is no doubt but that animal tissues behave in the same way as plant tissues.

In addition to the plant and animal tissues, various lower organisms may be used in the same manner, either in the form of suspensions or as extracts or filtrates (Berkefeld). Table 2 shows this fact.

TABLE 2

GROWTH OF PFEIFFER'S BACILLUS

Plain medium + autoclaved blood	=	0
Plain medium + bacteria	=	0
Plain medium + autoclaved blood + B. coli	=	+++
Plain medium + autoclaved blood + B. coli filtrate	=	+++
Plain medium + autoclaved blood + heated B. coli (60 C.-30 m.)	=	+++
Plain medium + autoclaved blood + heated B. coli (100 C.-5 min.)	=	+++
Plain medium + autoclaved blood + heated B. coli (autoclaved) (30 min.)	=	0

In this experiment other organisms, like staphylococci, streptococci, sporotricha, blastomycetes, yeasts, etc., may be used, little difference being noted, provided, of course, the reaction is not appreciably altered or is properly adjusted. It will be seen that here again bacteria and other organisms or their filtrates activate the autoclaved blood medium.

By heating the organisms this activating power is gradually reduced so that exposure to the temperature of the autoclave for 30 minutes will cause it to disappear entirely.

It seems clear that we are concerned with two substances, and we may now in the light of these experiments examine further into the behavior of Pfeiffer's bacillus grown on blood and serum medium. Table 3 reveals the facts in condensed form.

TABLE 3

GROWTH OF PFEIFFER'S BACILLUS

Plain medium + crystallized hemoglobin	=	+ (slight)
Plain medium + unheated blood	=	+ (slight)
Plain medium + blood (heated to 60 C. for 5 hours)	=	+++
Plain medium + blood (heated to 100 C. for 5 minutes)	=	+++
Plain medium + blood (autoclaved 30 minutes)	=	0
Plain medium + autoclaved blood + serum	=	+++
Plain medium + autoclaved blood + autoclaved serum	=	0
Plain medium + serum	=	0

From this experiment it is seen that crystallized hemoglobin or ordinary fresh blood plus plain medium is not a good medium though definite growth will occur. When corpuscles are heated to a certain point, however, their value to a medium is markedly enhanced. When heated beyond this point their value is destroyed.

Serum, when added to autoclaved blood medium, will reactivate it promptly, yielding a medium very favorable for growth. Pure serum alone added to plain medium does not yield a growth. Ascitic fluid, if fresh and of high specific gravity, behaves like serum but when of low specific gravity or old it has little or no action. When heated to boiling for 2 hours or autoclaved for 30 minutes the reactivating power of serum and ascites fluid is destroyed. Evidently, then, the serum and the ascites fluid behave quite like plant and animal tissues and also like bacteria and their filtrates referred to.

When ordinary unheated blood (defibrinated or whole blood) is added to plain medium the growth though definite is not abundant. When heated to 55 C. even indefinitely growth also is slight or at times absent. At 60 C. growth is not profuse unless this temperature is applied from 2 to 5 hours. If continued for 2 to 3 days no growth results. At 100 C. a few moments' exposure or simply bringing the medium to this temperature is sufficient to allow profuse growth and exposure for 1 to 2 hours destroys its growth producing value. At 120 C. (autoclave) a few minutes' exposure of the blood mediums

renders it valueless. Thus with increasing temperature the time necessary to obtain a favorable medium becomes less and less and also with increasing temperature the time necessary to destroy its growth value becomes gradually less.

These facts are interpreted as meaning two things. In the first place, the hemoglobin in itself is not a good medium for Pfeiffer's bacillus, perhaps will not support growth at all, and only when it has been changed by heat to hematin or some closely related derivative can it cooperate with a second substance in the blood. This change of hemoglobin appears to take place slowly at 60 C. but more rapidly at higher temperatures. This amount of heat as shown, however, does not destroy the second substance in the blood or the serum; therefore we have the two substances within certain ranges of heating operating together and yielding a profuse growth. If the heating is continued, the second substance is destroyed and no growth takes place without reactivation.

A medium in the form of albumin or peptone appears to be necessary for, as I have shown in previous papers, hemoglobin or hematin alone does not support growth of this organism. The heat resistant substance appears to be hematin or hemin since the action of heat on hemoglobin results in the formation of these substances.

One other point should be mentioned. At or even below about 55 C. blood is coagulated and becomes chocolate in color. However, such blood medium, even though heated for many days (3 weeks), does not yield a favorable medium. When activated with sterile carrot juice the growth is profuse. Blood medium heated at 60 C. or above for 2 or 3 hours yields a good growth without the addition of carrot or tissue juice. My interpretation of this fact is that the temperature of 55 C. or thereabouts is not sufficient in a certain time to cause the change in the hemoglobin resulting in the formation of the derivatives necessary to maximum growth. On the other hand, this temperature continued long enough renders inactive the second substance. There is therefore this interval in the heating of blood in which a profuse growth does not result. Beyond this temperature the hemoglobin is rapidly changed, and heating at the boiling point or at autoclave temperature for hours does not destroy the heat resistant substance (hematin) formed.

As to the second substance: Its heat relation has already been presented. It readily passes through Berkefeld filters and appears to be a product intimately related to living cells. The question as to the

possible relation of this body to growth factors or vitamines naturally arises here. It has been discussed by me in a previous paper [1] and, as then pointed out, there are features about it that suggest that we are dealing with a body of that nature. However, so little is known of the real character of these substances and the criteria for their identification are so indefinite, that little more can be done now to raise the question. I think that both of these bodies may be spoken of as growth or food accessory factors for this organism, using the term in the sense that growth processes depend on them. Whether the mechanism here involved is the same as the mechanism of vitamines in animal growth is not known. However, through the study of such bodies which, as I have detailed, are found in bacteria, yeasts and other tissues, we may be able to throw light on the real mechanism by which vitamines operate. Possibly the ultimate source of these substances may be found in the realm of these lower bacterial organisms. As pointed out,[1] this phenomenon, so far as the growth of Pfeiffer's bacillus is concerned, would seem to center about the metabolism of iron, and this would suggest that the processes are in the main concerned with oxygen or its transfer.

Other agents appear to be able to alter hemoglobin in the same way as heat. The decomposition or digestion of blood by bacteria of various kinds, if not prolonged, yields a product which gives abundant growth. This may be shown by adding putrified blood filtrate to medium in a test tube and inoculating with Pfeiffer's bacilli. If the decomposition of the blood has gone on for a long time (2 to 3 weeks by B. coli for example) no growth of Pfeiffer's bacillus will result on medium to which it has been added. Such medium can be reactivated, however, by adding to it fresh carrot or potato juice or fresh unheated blood, serum or animal tissue. The so-called peptic digest used by Fildes [3] no doubt contains the same substances, the pigment portion being reactivated by the supernatant fluid.

It was shown many years ago by Ghon and Preyss [4] that pure hematin alone would not support the growth of this bacillus but when used on hematin medium with another organism, good growth would result. This has been confirmed by others, including myself, and is in entire accord with the observations detailed now on heated hemoglobin, assuming that hematin results during this process. Hemin also

[3] Brit. Jour. Exper. Path., 1921, 2, p. 16.
[4] Centralbl. f. Bacteriol., 1902, 32, p. 96.

is stated by Olsen [5] to support growth along with other organisms and this substance, too, may result during the process of heating. I have not tested this point myself.

I have attempted to activate many iron and other compounds with substances like carrot juice and bacteria, but I have not been able consistently to do so. The iron derivatives of hemoglobin appear to be the only ones that will so react. I have gone into this point in considerable detail in another paper,[2] and I shall discuss it here no further than to state that for this purpose tests may be readily, and I think delicately made by the plate method used for determination of satellitism.

The relation of this process to the guaiac reaction has been discussed recently by Olsen,[5] who points out that a parallelism exists between the ability of Pfeiffer's bacillus on medium containing hemoglobin and its derivatives and a positive guaiac test. A derivative not containing iron, like hematoporphyrin, will give neither. He does not discuss the question of the reactivation of heated blood by different substances. Fildes [3] also discusses this question but does not explain the fact that many iron and other compounds give a positive guaiac test but do not promote the growth of Pfeiffer's bacillus. He also raises the question as to the possibility of the second substance being a peroxide of such a nature that through the catalytic action of hematin the transfer of oxygen to the bacillus from the peroxide is accelerated. He was led thus to the conclusion to which I was led some years ago [6] through a study of the behavior of blood pigments in high dilutions, namely, that the nature of this process is catalytic.

SUMMARY

Pfeiffer's bacillus grows feebly on mediums containing unheated blood.

Blood mediums heated to 60 C. or higher for definite periods of time yield profuse growth of the bacillus.

Heating in the autoclave (120 C.) for a few minutes or at lower temperatures for longer periods renders blood medium incapable of growing Pfeiffer's bacillus.

This superheated blood medium may be reactivated by adding to it plant, animal and bacterial extracts and filtrates which by them-

[5] Ibid., 1920, 85, p. 12.
[6] Davis, D. J.: Jour. Infect. Dis., 1907, 4, p. 73.

selves do not support growth of this organism. The latter substances lose this property on heating at autoclave temperature for a few minutes or at a lower temperature for longer periods.

The growth process of Pfeiffer's bacillus may be represented thus: plain medium + heat resistant substance (hematin or derivative) + heat labile substance = growth of Pfeiffer's bacillus.

THE ACCESSORY FACTORS IN BACTERIAL GROWTH

IV. THE "SATELLITE" OR SYMBIOSIS PHENOMENON OF PFEIFFER'S BACILLUS (B. INFLUENZAE)

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If one makes an ordinary blood-agar plate by pouring a tube heavily inoculated with Pfeiffer's bacillus (B. influenzae), colonies will appear which are very tiny; often apparently many of the organisms do not grow at all. However, if some other organism, a staphylococcus for example, is inoculated onto the plate and the plate incubated for 24 hours, the Pfeiffer colonies close to the staphylococcus colonies will appear very much larger than those in other parts of the plate and also apparently more numerous. Thus, there appears a prominent central colony (staphylococcus) surrounded by a cluster of relatively large Pfeiffer colonies. This has been termed the "satellite" phenomenon.

In 1897, Grassberger[1] first pointed out that close to the margins of colonies of Staph. aureus the Pfeiffer bacillus tended to form giant colonies. He also showed that if the staphylococcus cultures were killed by heat (¼ hour duration) and mixed with blood medium, the growth of the Pfeiffer bacillus was markedly enhanced. He therefore concluded that the bacterial products and not the symbiosis favorably influenced the growth of Pfeiffer's bacillus. In 1898, Meunier[2] noted the relatively large colonies of Pfeiffer's bacillus about other colonies grown in plate cultures with it, and referred to the phenomenon as "cultural satellitisme." Allen[3] observed it on plates when isolating bacteria from certain cases of chronic influenza. He grew Pfeiffer bacilli on medium to which heat killed staphylococcus albus had been added and noted a growth far more profuse than on ordinary blood mediums, attributing it to a toxin highly adjuvant to the growth of Pfeiffer's bacillus. He made identical experiments with Pneumococcus, Staph. aureus, B. coli, B. acidi-lactici and Microc. paratetragenus, all of which behaved alike.

The favoring action of organisms on the Pfeiffer bacillus has been observed and noted by many other observers. For years it has been customary to smear the entire slant surface of blood agar with Pfeiffer's bacilli and then make a light streak with staphylococcus

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[1] Ztschr. f. Hyg. u. Infectionskrankh., 1897, 25, p. 453.
[2] Semana méd., 1898, 18, p. 268.
[3] Lancet, 1910, 1, p. 1263.

through the center in order to enhance the growth of the former. The favoring action is also evident when plating out material, such as sputum, containing a mixed growth of Pfeiffer's bacillus and other organisms, especially if the former are numerous, in which case the cluster of large Pfeiffer's bacilli colonies about colonies of the other bacteria offer a striking appearance.

Further than to note the phenomenon, little has been done to analyze the factors concerned in its production. For this reason, I have thought the results of certain observations, made to determine its nature, worthy of presentation.

No organism other than Pfeiffer's bacillus is known that behaves in this way when grown with other organisms. At any rate, this statement is true when organisms are tested on blood or hemoglobin plates as described. A somewhat similar appearance is presented at times by certain motile bacteria on plates. A large central colony may become surrounded by a cluster of smaller and younger colonies which have arisen from bacteria moving out from the central colony. This phenomenon can easily be differentiated from the satellitism of Pfeiffer's bacillus because the central colony and those clustered about it are the same organism. Since the Pfeiffer bacillus is unique in this regard the phenomenon has become a most valuable means of differentiating it from other closely related bacteria.

The reaction does not appear to be a mutual one. That is, while the Pfeiffer bacilli are favorably influenced in their growth, the central or foreign colony is not affected either favorably or unfavorably. This may readily be shown by planting a portion of a plate with Pfeiffer bacilli and then inoculating with other bacteria. No difference is noted in the size of the latter colonies in the two halves of the plate. Owing to this fact, the question arises as to whether the term symbiosis should be applied to this phenomenon. Since it is not a mutual process it would seem that the term commensalism would be the proper one to use.

While the favoring action of an organism on the growth of Pfeiffer bacilli on solid blood medium is thus definitely manifested, the same action is not so clear on blood medium when the organisms are intimately mixed and transferred in this condition. Here Pfeiffer's bacilli multiply along with certain feebly growing cocci or bacilli (streptococci, diphtheroids) through several generations, gradually becoming less and less numerous. With Staph. aureus it dies out in two or three generations. There would appear to be in mixed culture

another factor inhibiting in character and which quite rapidly overcomes Pfeiffer's bacillus. So, too, in fluid blood cultures Pfeiffer's bacillus may grow through a few generations with a streptococcus or a staphylococcus, soon, however, becoming extinct. Further evidence of an inhibition or antagonistic action of bacteria on Pfeiffer's bacillus will be referred to later. In this connection, too, may be mentioned the fact that on nonhemoglobin medium Pfeiffer's bacillus will live through many generations when growing with another organism. I have grown it mixed with a diptheroid through 7 generations on plain mediums which without this bacillus would not grow at all. Neisser grew it through 20 generations with the B. xerosis on plain mediums and others have confirmed these results. The question always enters here as to the possible influence that blood in the medium may have since we now know that heated blood plus bacteria will yield a growth. It would appear, then, that a favorable as well as an unfavorable effect is exerted, by staphylococci at least, and also presumably by other organisms.

The zone of favorable influence about a foreign colony varies considerably in width even for one and the same organism. It may be as narrow as 1 mm. or may be 1 cm. or more in width. It is usually clearly visible for a distance of 2 to 3 mm. There is little difference apparently between the width of the zone of influence about different bacteria. It seems wider when the number of colonies is few.

The zone is symmetrical and most intense usually at or near the margin of the central colony. There are, however, certain exceptions to this. Often about staphylococcus colonies one sees a narrow zone of inhibition which may be a fraction of a millimeter in width. Beyond this the colonies of Pfeiffer bacilli are large and prominent. In this narrow zone the colonies are small or entirely absent. About certain other bacterial colonies, too, zones of inhibition appear which may be very wide. I have a strain of B. subtilis which is strongly hemolytic and about which there is a zone of inhibition so wide that it apparently neutralizes the favorable influence that might be exerted by the colony. At any rate, that is the interpretation I have given the observation. The zone of hemolysis is nearly 1 cm. in width; the medium is there alkaline and colonies composed of bacteria other than Pfeiffer bacilli (Staph. albus, for instance) are also here inhibited. This is the only organism I have met with thus far that does not reveal a favorable action on the Pfeiffer bacillus; though probably others could be found if systematic tests were made with a great number of bacteria.

When using for central colonies certain bacteria that tend to form large spreading growths, one may observe that the adjacent Pfeiffer colonies may be covered by the spreading margins of the central colony. Hence, one might conclude on superficial examination that such a colony does not act favorably on Pfeiffer's bacillus. However, by more careful inspection one may see the enlarged colonies of Pfeiffer bacilli underneath, or apparently in, the large central colony, showing clearly that a satellite zone was early formed and had later been covered or overgrown by the spreading colony. Strains of sarcina lutea and B. mucosus will at times behave in this manner.

With the exception of these instances, I have not found an organism that will not enhance the growth of Pfeiffer's bacillus. I have examined the following with positive results: Staph. albus and aureus, Strept. hemolyticus and viridans, Pneumococcus types 1, 2 and 3, also many strains of group 4, Meningococcus, Micrococcus catarrhalis, B. diphtheriae, B. pseudodiphtheriae (many strains of diphtheroids), Sarcina lutea, B. coli, B. typhosus, B. paratyphosus (A and B) B. dysenteriae, Sp. metchnikovii, gonococcus, B. mucosus (Friedländer), B. pyocyaneus, B. prodigiosus, B. sporogenes, B. enteritides, B. fecalis-alkaligines. Fifteen different organisms were isolated on plates from the air. These were chiefly chromogenic organisms and were not definitely identified. All without exception favorably influenced the Pfeiffer bacilli. I also tested on plates many sputum bacteria, and all colonies that grew revealed the satellite phenomenon with the exception of the strain of B. subtilis mentioned. No differences were noted between pathogens and saprophytes.

Strains of yeasts stimulate the growth of Pfeiffer's bacillus very well, provided the strain grows appreciably in 24 to 48 hours. With slow growing organisms in general, the phenomenon is not observed because during the short growth period of Pfeiffer's bacillus the former have not had time to multiply sufficiently to exercise any appreciable influence. This is true of tubercle bacilli, blastomyces, sporotricha and achorion quinckeanum. The tests were all made using at least two strains of Pfeiffer's bacillus, one from a case of influenzal meningitis and the other from a case of bronchopneumonia. In some instances many more strains of Pfeiffer's bacillus were used in the tests.

Pfeiffer bacilli are influenced favorably about to the same degree by both hemolytic and nonhemolytic bacteria. However, there is an advantage in using as a central colony one that causes hemolysis like Strep. hemolyticus or a hemolytic colon bacillus because in the clear

zone of hemolysis the Pfeiffer colonies can be readily seen, and their variation in size easily ascertained. Furthermore, the phenomenon is equally well brought out by using blood corpuscles hemolyzed by such agents as water. Satellitism, then, is not dependent on, or due to, hemolysis or to liberation of the hemoglobin from the corpuscles because the phenomenon is observed equally well in blood, unlaked or laked by bacteria or by water.

The reaction is noted on plates about both alkali and acid formers. At least, this is true unless large amounts of these substances are generated. The instance cited of a strain of B. subtilis inhibiting the influenza colonies may be an example of a strong alkali former preventing the development of the bacilli as already discussed. On 1% fermentable sugar mediums pneumococci and streptococci still exert their favorable influence.

In connection with the experiments with laked corpuscles some of the blood laked by the addition of a large amount of water was passed twice through a Berkefeld filter. The filtrate, distinctly tinged red and proved to be sterile, was added to the plain medium plates which were inoculated with several strains of Pfeiffer bacilli. Not only did the bacilli grow well on this filtered hemoglobin medium, but the satellite arrangement appeared quite as well as on ordinary blood plates. It is clear from this that the growth factor is not held back or absorbed appreciably by the process of filtration.

The variety of blood used in plating appeared to make no appreciable difference. The phenomenon appears equally well with human, sheep, horse, rabbit, guinea-pig, dog and pigeon bloods. Pigeon blood yields the most profuse growth, and for that reason the satellitism is not so evident.

Washed red cells do as well as unwashed in the medium in revealing the phenomenon. This is an important fact because it excludes serum inhibition as a possible factor in the reaction. Pfeiffer bacilli are to some degree inhibited in their growth by blood serum. Several experiments were made by adding increasing amounts of serum to ordinary chocolate blood medium (heated to 90 C. for 5 minutes), on which an abundant growth appears. As the amount of serum increased, there was noticed some diminution in the amount of growth. In other words, chocolate agar to which unheated serum has been added is not as favorable a culture medium as chocolate agar without the serum. One might postulate that in the zone about the central colony the serum has been altered so that it has lost its inhibiting effect.

While this may or may not be true, it probably has little or nothing to do with this phenomenon since the experiment in which washed corpuscles were used in the medium conclusively demonstrates that the presence of serum inhibition plays no rôle in the reaction.

Experiments were designed to test the influence of other substances on Pfeiffer's bacillus. Masses of dead bacteria killed by heating or drying, or dying naturally in a test tube, were prepared, and a small amount placed on a blood plate seeded with Pfeiffer bacilli. Strains of staphylococci colon bacilli and streptococci, all of which when alive and growing on Pfeiffer plates revealed satellitism, showed no favoring influence when thus tested. This experiment was made many times, and no stimulating effect was ever noted by these dead bacteria. This merely indicates that from the dead bacteria the favorable substance or influence is not disseminated or diffused to any appreciable extent. When dead bacteria or filtrates of cultures are intimately mixed with blood medium, Pfeiffer bacilli will grow on this far more profusely than on medium without the bacteria. From the living and growing bacteria, however, the favorable substance is diffused into the surrounding medium for some distance.

Next sterile animal tissue was tried. Small pieces of guinea-pig and rabbit liver, kidney, myocardium, voluntary muscle, spleen and brain were freshly prepared and placed on a blood plate seeded with Pfeiffer bacilli. About such tissue definite satellitism was seen after 24-hour incubation, all tissues behaving essentially alike in this respect. Curiously enough, a drop of blood placed on a hemoglobin plate does not reveal this reaction, or at least only to a slight degree. For this reason, one can exclude the blood or hemoglobin that may be present in these tissues as the determining factor. When one heats these animal tissues in the autoclave or to boiling for some time, they will no longer show satellitism with Pfeiffer bacilli. So, too, small pieces of fresh carrot and potato obtained under sterile precautions will also reveal the phenomenon on blood plates, but when autoclaved for a short time will no longer do so. One may therefore state that the factor responsible for this satellite phenomenon is destroyed by heat. In all the experiments, of course, proper precautions were taken to avoid any bacterial contamination of the animal or plant tissue. They must be absolutely sterile.

I have tested many chemicals, including iron compounds, both organic and inorganic, oleic acid, etc., without finding one that will consistently do what the living colony or tissue will do. It should be

said that no chemical will act as a substitute for blood or hemoglobin in growing Pfeiffer bacilli.[4] Ghon and Preyss [5] showed that on hematin agar Pfeiffer bacilli will grow when associated with another organism but not alone. However, when placed on a blood plate, hematin will not show satellitism. Oleic acid has been added to mediums to advantage in growing Pfeiffer bacilli, especially in the last few years. When small droplets or pieces are placed on a blood plate which has been inoculated with Pfeiffer bacilli, a wide clear zone of hemolysis soon appears around it. Usually the bacilli are not stimulated to grow in this zone, but I have noted at times that near the outer margin of the hemolytic zone large Pfeiffer colonies may form quite comparable to those about a bacterial colony. Whether or not this phenomenon is comparable to that which appears about bacterial growth I am not able to say. Presumably, it changes the hemoglobin into a derivative (hematin), thereby permitting another substance in the blood, serum or in other bacteria to act with it and enhance the growth of the Pfeiffer bacilli. This substance I will discuss further.

The question arises as to the relation of this favoring influence of a foreign organism and the favoring influence of heated hemoglobin on the growth of Pfeiffer bacilli. It has long been known that heating blood medium—that is, chocolate medium—improved it for growing this bacillus. I have studied this phenomenon,[6] and it appears that in order to obtain the most favorable medium the heating of blood must be done between quite definite limits—55 and 120 C., and for definite periods of time. Blood heated to 55 C. indefinitely will not yield a maximum growth. Heating to 60 C. for 2 to 4 hours is necessary, but if continued for 3 or 4 days, the medium is rendered useless. Boiling the blood medium for a moment will yield a favorable medium, but boiling for 1 to 2 hours will destroy its value for Pfeiffer bacillus cultures. Autoclaving for 30 minutes will also destroy its value. These results may be plotted in the form of a curve.

The fact that superheating or autoclaving will render blood mediums valueless for the culture of Pfeiffer bacillus permits one to analyze the satellite phenomenon further. On an autoclaved blood plate Pfeiffer bacilli will not grow. Mixed with another organism it grows in the zone about the foreign colony very well, thus showing satellitism on the autoclaved medium even better than on unheated blood medium. Moreover, by adding a bacterial filtrate or a carrot or potato filtrate

[4] Jour. Infect. Dis., 1907, 4, p. 73.
[5] Ztschr. f. Hyg. u. Infectionskrankh., 1897, 25, p. 45.
[6] Jour. Infect. Dis., 1921, p. 169.

(Berkefeld) or blood serum to such filtrated medium, Pfeiffer bacilli will grow profusely, an excellent medium thereby being furnished for the cultivation of this organism.

The appearance and characteristics of the zone on this medium are identical with those described on unheated plates. Owing to the clots of blood formed in the heated medium, in order to observe the satellitism, it is necessary to remove them before plating. This can readily be done by passing the medium through filter paper when hot. Using this filtered blood medium and inoculating with Pfeiffer's bacilli, the phenomenon is satisfactorily observed about a foreign colony or other material used for this purpose.

From this last observation we may conclude that we are dealing with two substances, one of which is heat stabile and is present in the heated blood, the other a substance less heat resistant and found in extracts and filtrates of animal and plant tissues of bacterial cultures. These two substances when operating together in plain medium will allow a profuse growth; either one alone will not permit growth in plain medium. This conclusion has been arrived at and developed in a previous article [6] through a study of the growth of this organism on autoclaved blood medium in tubes to which various activating substances were added. The results there presented agree entirely with those reported here and enable us to explain satisfactorily the satellite phenomenon. On the autoclaved blood medium plates seeded with Pfeiffer's bacilli growth will appear only around a foreign colony where the heat labile body necessary to complement the heat stabile body of the heated blood is generated. This substance from the foreign colony or from a piece of carrot or animal tissue is a diffusible substance, for it exerts its influence to a distance the width of the satellite zone. On unheated blood plates the process is more complex because, in addition to the hemoglobin and its derivatives, there are other compounds in the serum and even in the corpuscles. It appears, however, that the foreign colony not only furnishes the heat labile substance necessary, but also breaks up the hemoglobin into its derivatives (hematin and hemin) with which the former acts. It is difficult to be certain of this last point because hemoglobin is an unstabile substance, and it is difficult to know when it is free from its derivatives. Probably in blood plates there is always some hematin, and it may very well be that pure hemoglobin or oxyhemoglomin will alone (in plain mediums) not support the growth of Pfeiffer bacilli. This would seem to be indicated by the fact that in crystallized hemoglobin medium

or in fresh blood medium the growth of Pfeiffer bacilli is scant and even at times negative. In all probability, then, a foreign bacterium or animal or plant tissue on unheated blood plates breaks down the hemoglobin into its derivative hematin, at least to some extent, and also furnishes the heat labile substance referred to. Thus, in a uniform zone about bacteria, tissue, etc., exist the two substances the cooperation of which is necessary for the profuse growth of Pfeiffer bacilli.

A final point deserving a word of comment is the possible influence of another organism on the growth and possibly on the virulence of Pfeiffer's bacillus in the animal body. The statement has been made that in mixed culture Pfeiffer's bacillus is more dangerous and more pathogenic for animals and possibly for man. Some years ago I made experiments on animals which seemed to indicate that this is true, and it may be true to some degree. However, we now know that various living tissues of the animal will do exactly what an associated organism will do in the way of stimulating the growth of Pfeiffer's bacillus, so that apparently this favoring influence is being exerted by various tissues of the body on this bacillus during an infection, and the additional influence of an associated organism, as for example streptococci or staphylococci in the respiratory tract, would presumably make little difference in the final result.

SUMMARY

The phenomenon of satellitism as observed in connection with Pfeiffer's bacillus is described in detail.

It is observed in association with bacteria, yeasts and fungi of various kinds and their filtrates; also with plant and animal tissues and their extracts and filtrates.

Heating in the autoclave (120 C. for 30 minutes) will destroy the activities of these substances. Heating for longer periods at lower temperatures will do likewise.

On clarified autoclaved blood medium Pfeiffer's bacillus will not grow. On plain medium, to which organisms or tissues or their filtrates (hemoglobin free) are added, it will not grow. When these substances are mixed good growth results.

The explanation of satellitism therefore would seem to be: Diffusible products or extracts of bacteria, fungi, tissues, etc., stimulate the growth of Pfeiffer's bacillus in conjunction with hematin or with hemoglobin. Thus, profuse growth of Pfeiffer's bacilli occurs immediately around colonies of organisms or pieces of plant or animal tissue.

THE ACCESSORY FACTORS IN BACTERIAL GROWTH

V. THE VALUE OF THE SATELLITE (OR SYMBIOSIS) PHENOMENON FOR THE CLASSIFICATION OF HEMOPHILIC BACTERIA

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I have pointed out[1] that the satellitism of Pfeiffer's bacillus may be observed with many diverse micro-organisms. This suggests the possible value of this phenomenon for purposes of classification and identification of the hemophilic bacteria. Indeed, in my own work I have used this test for some time and I long since concluded that it was one of the most reliable and uniform criteria we have for this group.

The value depends on two facts: First, apparently all strains of the Pfeiffer type of hemophilic bacilli will reveal the satellite phenomenon when grown with another organism on blood plates. I have tested many hundreds of strains and have yet to find an exception. Second, the important point, which I have only recently observed, that Pfeiffer bacillus does not favorably influence itself. That is, if one prepares a blood plate seeded diffusely with Pfeiffer bacilli and then inoculates here and there with the same organism, after incubation one will observe no favorable influence on the colonies of the organisms last inoculated.

It seemed to me that in view of certain slight differences between strains of this group, cultural and otherwise, that possibly here was a method of further differentiation. Accordingly, using as central colonies various strains of Pfeiffer bacilli, blood plates were seeded with homologous and heterologous strains. Strains thus tested included 3 from the lungs of patients with epidemic influenza pneumonia, 1 from influenzal meningitis, 3 from excised adenoids and 1 from an infected frontal sinus; also 3 indol and 3 nonindol forming strains obtained from Dr. E. O. Jordan. In no instance, when such "cross" satellite tests were made, did there appear any favoring influence of one strain

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[1] Jour. Infect. Dis., 1921, 29, p. 178.

on another, though all were influenced favorably by staphylococci and other bacteria. Various strains were mixed on the same medium and cultivated together to find whether the growth was enhanced to any degree. The results were negative. It would appear, then, that the differences that have been noted by agglutination, cultural reactions and virulence tests between strains of Pfeiffer bacilli cannot be detected by this test.

However, for differentiating from closely related groups, the test becomes of real value. This applies especially to the organisms that grow well on blood and now commonly classed as hemophilous. The Committee on Classification of the Society of American Bacteriologists has recently placed provisionally in the genus Hemophilus, the Pfeiffer bacillus, B. pertussis (Bordet), the Morax-Axenfeld bacillus, B. ducreyi and the Koch-Weeks bacillus. Strains of all these organisms were subjected by me to this test of satellitism. They were used as central colonies on plates seeded with Pfeiffer's bacilli and about all, a favorable influence on the Pfeiffer bacilli was noted. Three strains of B. pertussis, one of Morax-Axenfeld and one of B. ducreyi were used. On the other hand, none of the bacilli just mentioned is favorably influenced by other bacteria. It appears then that here is a definite and sharp method of differentiating the Pfeiffer group of bacilli from other hemophiles.

It should be stated that apparently none of the latter organisms is strictly speaking hemophilic as is the Pfeiffer bacillus. While they grow better on blood or hemoglobin medium, they can all be cultivated on special mediums without blood. Another difference between the Pfeiffer bacillus and the other bacteria mentioned is that the former grow and indeed grow better on medium containing small even minute quantities of hemoglobin, whereas the latter require large amounts of blood or tissue fluids.

Several strains of bacilli from the conjunctiva, which, I think, commonly would be called Koch-Weeks bacilli, were tested and none revealed satellitism with Pfeiffer bacilli. On the other hand, they were favorably influenced by other organisms (staphylococcus and streptococcus) exactly as are typical Pfeiffer's bacilli. I am therefore inclined to the view that they all are strains belonging to the Pfeiffer group. This test would not exclude, of course, the possibility of their being differentiated from other strains by finer methods.

SUMMARY

The satellite phenomenon is of value in identifying and in classifying members of the hemophilic group. Its value depends on the fact that Pfeiffer's bacillus is not favorably influenced in its growth by homologous or heterologous strains, while apparently all other strains of hemophilic organism reveal this phenomenon.

Food Accessory Factors in Bacterial Growth

VI. Further Observations on the Substances Necessary for the Growth of Pfeiffer's Bacillus

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FOOD ACCESSORY FACTORS IN BACTERIAL GROWTH

VI. FURTHER OBSERVATIONS ON THE SUBSTANCES NECESSARY FOR THE GROWTH OF PFEIFFER'S BACILLUS *

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Several years ago I [1] called attention to the fact that in the cultivation of hemophilic bacteria (*B. influenzae*) there are necessary two substances, one being a heat stable substance and closely identified with the iron containing pigments of the blood, namely, hemoglobin and hematin; the other factor residing in fresh animal and plant tissues and in many bacteria, including yeasts, blastomyces, sporotricha, etc. The latter substance is more heat labile than the first factor mentioned, autoclaving for fifteen minutes or boiling for a longer time being sufficient to destroy it. The interaction of these two substances is somehow necessary for the growth of this organism. Presumably the second factor in some way renders the iron more available and, in view of the nature and function of this element in life processes, one is tempted to interpret the phenomenon as related to oxidation, and possibly catalytic in nature. In 1907, I [2] pointed this out, basing my conclusion on the fact that very minute quantities of blood (1-180,000) were sufficient to induce growth of these bacteria.

This work was done some years before the recent epidemic of influenza. The strains of so-called influenza bacilli then used in the investigations by me were isolated from a great variety of respiratory infections, including measles, whooping cough, bronchitis, meningitis, varicella and pneumonia; and since all efforts to

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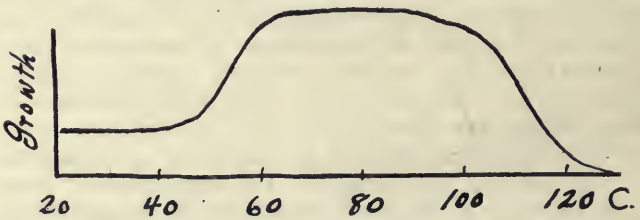
1. Davis, D. J.: Food Accessory Factors (Vitamines) in Bacterial Cultures, with Especial Reference to Hemophilic Bacilli, J. Infect. Dis. **21**: 392 (Oct.) 1917.

2. Davis, D. J.: Hemophilic Bacilli: Their Morphology and Relation to Respiratory Pigments, J. Infect. Dis. **4**: 73, 1907.

demonstrate an epidemic strain of influenza bacilli during the last epidemic failed, and since it is now quite generally conceded that epidemic influenza is not caused by Pfeiffer's bacillus, I think the results obtained with these hemophilic organisms isolated before the influenza epidemic are comparable with the results obtained with similar organisms isolated during or since the epidemic. My own data,[3] as well as the results of many others, do not indicate that these bacilli now are in any way different either in their biologic properties or in their distribution from what they were before the epidemic.

The work referred to above regarding the growth accessory factors in the cultivation of Pfeiffer's bacillus has now been corroborated by a number of workers both here and abroad. Fildes,[4] Thjötta and Avery [5] and Rivers [6] have all found that the growth of Pfeif-



Growth of Pfeffer's bacillus on blood medium heated to various temperatures.

fer's bacillus depends on the existence of two substances, the one related to the blood pigments being the more heat stable. These workers, too, have generally interpreted the reaction as one related to vitamin activity, though Fildes suggests that the phenomenon centers about the reaction between peroxidase and the blood pigment.

I have recently corroborated my work done several years ago, using fresh strains of Pfeiffer's bacillus.[7] Pfeiffer's bacillus isolated from epidemic influenza, from influenza meningitis, from normal throats or from throats the seat of various respiratory diseases behaves

3. Davis, D. J.: Proc. Inst. Med., Chicago **2**: 142, 1919.
4. Fildes: Brit. J. Exper. Path. **2**: 16, 1921.
5. Thjötta and Avery: J. Exper. Med. **34**: 97, 1921.
6. Rivers: Bull. Johns Hopkins Hosp. **32**: 202, 1921.
7. These results will appear in detail in a series of three articles in the forthcoming issue of the Journal of Infectious Diseases.

alike in that there is required for its growth, in addition to plain medium (peptone medium is sufficient) the two substances already referred to. In addition, I have noted certain other observations which deserve mention.

When ordinary unheated blood (defibrinated or whole blood) is added to plain mediums, the growth, though definite, is not abundant. When heated to 55 C. even indefinitely, growth also is slight or at times apparently negative. At 60 C., growth is not profuse unless this temperature is applied from two to five hours. If continued for two to three days, no growth will result. At 80 C., growth is profuse if the blood medium is heated from five to ten minutes. By continuing the exposure for from twenty-four to thirty-six hours, the medium becomes valueless unless reactivated by fresh fluid or tissue. At 100 C., a few moments' exposure, or simply bringing the medium to this temperature, is sufficient to allow profuse growth; but exposure for one or two hours will destroy its growth promoting value. At 120 C. (autoclave), a few minutes' exposure of the blood medium will render it valueless. Thus, with increasing temperature the time necessary to obtain a favorable medium becomes less and less, and also with increasing temperature the time necessary to destroy its growth value becomes gradually less. It should, of course, be understood that the growth promoting value of the heated blood can be restored by adding thereto fresh unheated plant and animal tissue extracts, or bacterial or yeast extracts.

It is readily seen that the heat resistance of this second factor may be represented in the form of a simple curve which would gradually descend to the base line at a temperature of about 120 C. A curve representing the growth of the bacilli on blood medium heated to varying degrees would be more complex because of the several factors involved. Roughly it would be represented as in the accompanying curve. The curve does not take into consideration the time element.

The first factor is apparently hematin or a close derivative. Pure hematin (Merck according to Nencki) medium behaves like the autoclaved blood medium. No growth of Pfeiffer's bacillus appears unless the medium is activated by the addition of

unheated plant, animal or bacterial products. According to Olsen,[8] hemin behaves in the same way as hematin.

The effect of hydrogen peroxid was tested on the activity of the heat labile factor. For this purpose, fresh filtered carrot juice, which I have found to be an excellent activator, was treated with small but varying quantities of hydrogen peroxid for one hour. Medium made by the addition of this treated juice to autoclaved blood yields very scant growth compared with the controls. On some of the tubes a growth was just visible, but in none did a profuse growth appear. In others there was no visible growth. Similar experiments were made by the addition of small amounts of hydrogen peroxid to ordinary blood medium. The medium becomes bleached with the addition with the addition of increasing quantities of the peroxid. On such medium, growth of Pfeiffer's bacillus is nil or very scant.

In seeking some light on the mechanism of the reaction between these two substances, one may suggest the possibility that the second substance may somehow control or make available the iron in the pigmented portion of the hemoglobin molecule. The question naturally arises, Do vitamins or vitamin-like substances influence or to some degree control the metabolism of other elements in the body, such as phosphorus, iodin and calcium? In the cultivation of the gonococcus, phosphate added to tissue fluids makes a most excellent medium. Dorothy Lloyd [9] has interpreted the favoring action of body fluids in the growth of meningococcus and other organisms as one comparable to vitamin activity in animal nutrition. I raise the question as to the possible action of the accessory bodies on phosphorus as one comparable to the action of the labile tissue substance on the iron containing pigment in the nutrition of Pfeiffer's bacillus. Or does the phosphate in the medium function simply as a body favoring growth through its buffer action?

Again, McCollum and Simmonds [10] have pointed out the interesting relation that exists between vitamins and

8. Olsen: Zentralbl. f. Bakteriol. **85**: 12, 1920.
9. Lloyd, Dorothy: J. Path. & Bacteriol. **21**: 113, 1916.
10. McCollum and Simmonds: Bull. Johns Hopkins Hosp. **32**: 160, 1921.

phosphorus in the causation of rickets. From their results with rats it would appear that the phosphate ion plays an important rôle in the causation of this disease and perhaps kindred diseases. They point out that the level of blood phosphate is in all probability determined in part by the amount of the fat soluble A available for the needs of the organism. Thus, an interplay seems to exist between the vitamins and phosphorus in the body, a deficiency of either leading to defective nutrition. I merely mention these processes as being possibly analogous to the interplay between the two substances necessary for the growth of Pfeiffer's bacillus. We may be dealing here with a principle in nutrition of fundamental significance in relation to life processes.

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THE IDENTITY OF AMERICAN AND FRENCH SPOROTRICHOSIS*

David John Davis

Introduction

The reason for presenting this subject at this time will no doubt be deemed adequate by those who have followed the literature on sporotrichosis during the past several years. This disease is known to be relatively common in France, the number of cases observed now running into the hundreds. In America the disease is being commonly reported in both man and horses; the number of human cases now closely approximates a hundred, and several extensive outbreaks in horses in different localities have been observed.

The disease is known under the name of Sporotrichosis in both countries. In France and generally on the continent, also in certain other parts of the world, the cause is given as the *Sporotrichum beurmanni*. In the United States the causal organism is generally but not uniformly recognized as *Sporothrix schenckii*. Certain writers here, now and then, refer to the organism from American cases as *Sporotrichum beurmanni* or as *Sporotrichum schenckii-beurmanni*. The impression is general on the continent and especially in France that the American and French organisms are distinct and that we have to do with two different though closely related diseases. It is my purpose in this paper to analyse the existing data and to present certain new data bearing upon this matter of the identity or non-identity of these two infections.

This discussion does not concern other distinct varieties of Sporotricha either pathogenic or non-pathogenic. The existence of these is recognized. Many saprophytic sporotricha

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grow in the soil, in water, and under other conditions; certain varieties are pathogenic for plants.

Infection with varieties pathogenic for man, excepting the two here under consideration, are apparently very rare. The following are mentioned in this connection.

Sporotrichum dori, an organism isolated from a human case and very imperfectly described by Dor in 1906, was evidently a different organism morphologically, culturally, and in its pathogenicity for animals. The culture has been lost and no similar organism has since been found.

Sporotrichum indicum, an organism described by Castellani in 1908, was isolated from two cases of sporotrichosis in Ceylon. It is impossible now to compare them with other varieties since these cultures have also been lost. From the original description given by Castellani it is clear that the organisms are very similar to, if not identical with, the French and American varieties. He says that "it closely resembles *Sporotrichum beurmanni;* the mycelial threads are somewhat larger, between 2 and 3 microns wide; spores roundish (3 to 5 microns in diameter) or oval (4 to 5 microns long and 3 to 4 microns in breadth). Colonies on maltose agar may be of various colors,—greyish, light brownish, dark brownish, black".[1] There are no differential characteristics here that are important and I am inclined to believe these strains are identical with the American variety of Schenck. De Beurmann and Gougerot provisionally classify it as *Sporotrichum beurmanni var. indicum.*

Sporotrichum gougeroti is an organism isolated from a case in France by Gougerot who thought it different from the Beurmann type in several respects, chiefly in macroscopic growth and pigment production. De Beurmann and Gougerot observed one case only.

The *Sporotrichum jeanselmei* was isolated by Jeanselme and Chevallier in 1910 from a human case and a second case appeared as an experimental infection accidentally obtained in the laboratory from the culture of the first case. According to de Beurmann and Gougerot this organism is very similar

[1] Castellani & Chalmers, *Manual of Tropical Medicine,* London, 1910.

to *Sporotrichum beurmanni*, indeed identical with certain pleomorphic forms of the latter. They make the significant statement in this connection: "Plusieurs de ses pléomorphismes s'identifient au Sporotrichum Schenki et au Sporotrichum Beurmanni, voire même au Sporotrichum Gougeroti. Cette unification dans les pléomorphismes est la meilleure preuve d'une commune origine de ces germes." Their position seems to be that this organism, the American organism *Sporotrichum schenckii*, and the *Sporotrichum beurmanni* were originally one and the same organism, and certain strains later developed pleomorphic characteristics leading to new varieties.

A new pathogenic sporotrichum, *Sporotrichum councilmani*, described very recently by Wolbach, Sisson and Meier[2] of Boston was found in a case of acute arthritis of the knee following injury. From their description it appears quite different from all other strains of Sporotricha. They summarize the distinguishing features as follows: "(1) its pleomorphic growth, characterized by a free aerial growth of hyphae; (2) the abundant spore formation, large size of the spores and absence of lateral spore clusters, and (3) the occurrence in lesions as septate, branching filaments." The last character is especially significant. The clinical history and the character of the lesion in the patient are also of interest and are possibly of importance for differential purposes.

For the purpose of this paper I think we may eliminate *Sporotrichum dori* as being quite different from the other sporotricha; also *Sporotrichum councilmani*. The very rare cases of so called *Sporotrichum indicum*, *Sporotrichum jeanselmei* and *Sporotrichum gougeroti* are much more closely related to *Sporotrichum beurmanni*, the first two indeed being probably identical with it. The existence of these very rare varieties is here recognized but this fact is only indirectly related to the main question at issue here, namely, the possible identity of the organisms causing sporotrichosis as commonly observed in America and in France.

In the further analysis of this question it will be desirable

[2] *Journal of Medical Research*, 36, p. 337, 1917.

to discuss briefly the history of sporotrichosis in America and France and also in the other countries where it has been observed.

Sporotrichosis in North America

The recorded history of this disease in America is brief and simple. In 1898 Schenck[3] reported a case of chronic subcutaneous abscesses from which he isolated in pure culture a fungus which grew readily on artificial media and which was identified by Dr. Erwin F. Smith of the U. S. Dept. of Agriculture as belonging to the genus Sporotricha. The organism was found to be distinctly pathogenic for mice and dogs and from the characteristic lesions the same fungus was recovered pure. Thus, in the first case observed, all of Koch's laws were fulfilled. Illustrations of the human lesions, cultures, and microscopic appearance of the fungus accompany the paper of Schenck.

In 1900 Hektoen and Perkins[4] observed and very carefully described a second case in which the fungus was isolated in pure culture and its pathogenicity for various animals determined. They were able to confirm the results of Schenck and after a careful comparison of the fungi from both cases concluded that they were identical. Schenck also examined their strain and pronounced it identical with his organism. They definitely named this organism *Sporothrix schenckii* at this time. Therefore it is to be noted that in two of the most prominent medical publications of the time, an accurate clinical, pathological, bacteriological, and experimental description of this disease appeared.

A case reported in 1899 by Brayton[5] agreed clinically with the case of Schenck and of Hektoen. The organism was not detected however and cultures were not made. Definite statements as to the nature of the infection cannot be made though it was probably a case of sporotrichosis.

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[3] Johns Hopkins Hospital *Bulletin*, 9, p. 286, 1898.
[4] *Jour. of Exp. Med.*, 5, p. 77, 1900.
[5] *Indianapolis Med. Jour.*, 18, p. 272, 1899.

The next report from America was that of Duque[6] in Cuba in 1908.[7] Duque reported three cases that clinically were disseminated, *gummatous sporotrichosis.* They were treated surgically and in two the amputation of an extremity was resorted to without result. Under treatment of iodide of potassium they all responded promptly and made complete recoveries in periods of from one to two months. Details concerning the diagnosis are not given. Duque states that the diagnosis was made by a careful examination of the pus but does not say whether or not cultures were made. From the clinical and therapeutic data we should judge that these cases were sporotrichosis, but the lack of more complete pathological data prevents further analysis.

Burlew[8] in 1909 observed a case of sporotrichosis of the disseminated gummatous type in a farm laborer in Santa Anna, California. Both legs and the face were involved. The organism was cultured and identified as the *Sporothrix schenckii.*

From this year (1909) to the present time the number of cases recognized and reported has increased rapidly so that

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[6] *American Jour. Dermat. and Gen. Urin. Dis.,* 12, p. 240, 1908.

[7] The period of eight years lapsing between the reports of Hektoen and Perkins and those of Duque, Burlew, Hyde and Davis, etc., in which no cases appeared has been commented upon by de Beurmann and Gougerot. *Bull. et Mem. de la Soc. Med. des Hospit. de Paris,* 35, p. 798, 1910. They infer that sporotrichosis in North America had practically been forgotten and that only after attention had been called to this disease through the later work of the French did Americans begin again to recognize this disease. There is little truth in this statement. Naturally the large amount of work that was being done by the French between 1906 and 1908 did attract attention in America. However, it is surely not correct to state that the disease had been forgotten here when men like Hektoen, Welch, Smith and others, who had recognized or seen the disease and the fungus, continued to engage in active work in pathology. The real reason no doubt was the fact that the disease in the human is restricted as we shall presently see, almost entirely to the valley of the Missouri River. This locality in the central and western portion of the country, especially at that time, was not developed medically and naturally only the cases which drifted out of the region to medical centers would be apt to be detected. Such apparently was true of the cases of Schenck and of Hektoen and Perkins. In later years when men like Sutton and others worked in that locality cases were recognized in much larger numbers.

[8] *South. Calif. Pract.,* 24, p. 1, 1909.

now they can no longer be considered rare. Ruediger[9] gathered together and analysed all the cases in the United States in 1912. He found 57 in which the diagnosis had been made with reasonable certainty. Since then the literature each year has furnished a considerable number of additional reports. Many cases have no doubt not been recorded in the literature. The writer is aware of several in which cultures were obtained and identified as *Sporothrix schenckii* but never reported.

Ruediger called attention to the interesting fact that the disease occurred chiefly in the Missouri River Valley. Five-sixths of the 57 cases were from this locality,—the others being scattered more or less diffusely over the country. North Dakota, which furnished 22 authentic cases, seems to be the chief focus of human infection in this country. Kansas has also furnished a large number of cases. A map showing the location of the cases in the United States accompanies Ruediger's paper and brings out strikingly the geographical distribution. However, K. F. Meyer[10] has more recently analysed the data, especially those dealing with the relation of animal and human sporotrichosis in this country. He shows that as new cases appear it becomes increasingly evident that the disease is widely distributed, though certain localities like the Missouri River valley furnish the great majority of the cases. It has been reported from the following states: Missouri, Kansas, Iowa, Nebraska, Texas, Virginia, West Virginia, Ohio, New Jersey, District of Columbia, South Dakota, North Dakota, California, Illinois, Pennsylvania, New York, Minnesota, Wisconsin, Indiana, Montana and Michigan. Two cases have been reported from Canada.

Sporotrichosis has appeared in horses in several localities in the United States. The disease was recognized clinically in horses some time before it was accurately studied bacteriologically in this animal. Horses were found in 1908 or before in North Dakota suffering from what was then taken to be mycotic lymphangitis. From the description and the

[9] *Jour. of Inf. Dis.*, 12, p. 193, 1912.
[10] *J. A. M. A.*, 65, p. 579, 1915.

illustrations given in the *Second Annual Report* (1908) of the Live Stock Sanitary Board to the Governor of North Dakota, it is evident that clinically these horses were afflicted with sporotrichosis. Furthermore, organisms obtained from these Dakota horses were declared to be identical by the Bureau of Animal Industry in Washington with the organisms from certain horses afflicted with a similar disease in Pennsylvania, from which an organism was isolated and clearly shown to be the *Sporothrix schenckii.* The Dakota organism was at first taken to be *Saccharomyces farciminosus,* the cause of lymphangitis in horses as described by Tokishike and Pallin. Through the comparative studies of Paige, Frothingham and Paige, and also of the writer, of the organisms isolated from the Pennsylvania horses and an organism isolated from a human case from North Dakota by the writer, it was shown that the organisms were without question identical. This established the first clear identity of the organism from lesions in horses and in the human and showed too that it was apparently identical with the *Sporotrichum schenckii* as described by Schenck, Hektoen and others.

K. F. Meyer[11] has also recently studied this disease in horses. He concludes that spontaneous sporotrichosis in this animal is very common, especially in two localities, Pennsylvania and North Dakota. He cites a case of accidental laboratory infection in man as proof of the pathogenicity of equine strains for the human. The evidence collected, however, does not support the theory that sporotrichosis is very frequently transmitted from horse to man in the United States. His opinion is that the *Sporotrichum schenckii, Sporotrichum beurmanni,* the organisms from mules and horses in Madagascar, and the South American strains are all identical. He proposes the use of the term *Sporotrichum schenckii-beurmanni* for all.

[11] *Loc. cit.*

Sporotrichosis in France

In France the history of this disease begins with the report of a case by de Beurmann and Gougerot in 1903. Apparently they completely overlooked the work of the American investigators published several years earlier and thinking they had discovered a new organism they submitted it to Matruchot and Ramond who identified it as a sporothrix and in 1905, in a note to the Biological Society of Paris, named it *Sporotrichum beurmanni.* A second case was observed in France in 1906 by de Beurmann and Gougerot and later they identified other cases and made numerous extensive and admirable studies on all phases of the disease.[12] New cases rapidly accumulated in the French literature and it was soon evident that the disease in that country was not rare. It was observed also in the dog and the horse, the organisms being identical with that from the human. It is to be noted that not until 1906 did the French learn of the American cases and of the *Sporothrix schenckii.* French workers generally contend that the *Sporotrichum beurmanni* is different from the *Sporothrix schenckii.*

Sporotrichosis in South America

In 1907 Lutz and Splendore[13] in San Paulo in Brazil were the first workers to recognize spontaneous sporotrichosis in lower animals. They observed the disease in both gray and white rats. They also reported five human cases from the same locality. They noted that the disease in rats was transmitted through bite wounds usually on the extremities or tail and following an initial lesion a generalized infection would result. Transmission from the rat to man, while probable, was not demonstrated. These studies, it should be noted, were made independently, the work of American and French investigators not being known to them until some time later. The organism as described by them cor-

[12] De Beurmann et Gougerot, *Les Sporotrichoses,* Paris, 1912.
[13] *Cent. für Bact.,* 45, p. 631, 1907.

responds in detail with the North American variety and de Beurmann and Gougerot have examined it and pronounced it identical with the French strains. This identity has been conceded by Lutz and Splendore.

Balino and Marco del Pont[14] in 1907 discovered a case of this disease in Buenos Ayres, and Greco,[15] also in 1907, one from Uruguay. Greco suggested calling his organism *Sporothrichum schenckii-beurmanni.* According to him it agrees with the organisms of both Schenck and de Beurmann. Other cases have since appeared in South America.

Sporotrichosis in Madagascar

On the island of Madagascar Carougeau[16] in 1908 found this infection in mules and in horses. It is a common disease there. Clinically and pathologically it agrees in every way with the disease as it appears in man. It is either a disseminating or an ascending gummatous sporotrichosis and responds promptly to potassium iodide. Carougeau reproduced the disease experimentally in the mule by intravenous injection. He reports a human infection in a veterinarian who punctured himself while operating on a sick mule. He clearly differentiates this infection from the closely related but more serious one of *Saccharomyces farciminosus.* The sporotrichum from the mules was carefully described by Carougeau and agrees with *Sporotrichum schenckii.* De Beurmann and Gougerot have identified it with the French organism and this identity has been acknowledged by Carougeau.

As to distribution, then, sporotrichosis is practically a world-wide disease having now been noted in North America, South America, Europe, Madagascar and probably India. The chief focus in Europe is France but cases have been observed also in Germany, Austria, Switzerland, Italy, England, Belgium and Spain. In North America, as already stated, it is largely confined to the Missouri River Valley.

[14] *Argentina Med.,* 2, p. 23, 1908.
[15] *Argentina Med.,* 45, p. 699, 1907.
[16] *Bull. et. Mem. de la Soc. Med. de Hopit. de Paris,* 34, p. 507, 1909.

Animal susceptibility is rather general, the spontaneous disease having appeared in man, horse, mule, dog and rat. It has been observed as an accidental infection in man. Experimentally it has been produced in a large number of the lower animals, the rat being probably the most susceptible and useful animal for this purpose.

A Statement of the Question

At their request, Hektoen in 1906 sent to de Beurmann and Gougerot at Paris a culture of the American organism which he had isolated seven years before. After studying and comparing this organism with their strains they declared that the American and French strains were different and they continued to retain the name of *Sporotrichum beurmanni* for the French fungus and to use the term *Sporotrichum schenckii* for the North American strains. It is to be noted, too, that de Beurmann and Gougerot and their French colleagues considered the South American strains, the Madagascar strains, the German, Austrian and other strains, all of which were described after their work, as identical with the French organism.

In 1910 the writer took to Gougerot in Paris a strain isolated by himself from a typical human case from North Dakota and reported later by Hyde and Davis.[17] I received from him and also from Sabouraud at that time strains isolated from cases in France and called by them *Sporotrichum beurmanni.* I also obtained from Hektoen a culture of his sporotrichum which he had preserved from his case of 1899, a culture of which, as stated above, he had sent to de Beurmann and Gougerot in 1906. De Beurmann, Gougerot and I, therefore, have French strains, American strains and the original Schenck-Hektoen strain for comparison. In order to simplify and limit the discussion as far as possible I will make the following statement: first, excluding for the time being the Schenck-Hektoen strains, we may consider all the later strains, except *Sporotrichum councilmani,* isolated in

[17] *Jour. of Cut. Dis.,* 28, p. 321, 1910.

the United States from man and horses by numerous workers including the writer identical with each other. My own work as well as the work of K. F. Meyer, Sutton, Ruediger, Page, Frothingham, Paige, and others, all tend to confirm this point. The strains have usually been designated as *Sporotrichum schenckii.* Again excluding *Sp. dori, Sp. jeauselmei* and *Sp. gougeroti* in France, all the French strains of sporothricha are admitted by all, both French and Americans, to be alike. They have been designated *Sporotrichum beurmanni* by the French. Gougerot in a publication[18] made after examining the American cultures which I gave him in 1910 admits that they are identical with the French strains but different from the old Schenck-Hektoen cultures. In another article on this subject he writes as follows: "Hyde & Davis bezeichnen also Sp. Schencki einen Parasiten welchen uns Davis ubermittelt hat und der zweifellos identisch mit dem sp. Beurmanni ist."[19] A further statement in the same paper on this point is as follows:

Die Beantwortung der aufgeworfenen Frage lässt drei Möglichkeiten zu:

(1) Der Stamm Hektoen-Gougerot des Sp. Schencki stellt den Typus des Sp. Schencki dar; das Sp. Beurmanni muss von ihm unterschieden werden, steht ihm jedoch nahe und stammt von demselben Urstamme ab wie er.

(2) Sp. Schencki und Sp. Beurmanni stammen beide von ein und demselben Urstamme ab; sie zeigen zwar gegenwärtig einige Verschiedenheiten, sind aber durch Zwischenglieder, wie z. B. den Stamm von Hyde & Davis, miteinander verbunden.

(3) Der Stamm Hektoen-Gougerot des Sp. Schencki ist ein fest gewordener pleomorpher Vertreter des Sp. Schencki und von den Stammen "Schencki initial" und "Hektoen initial" unterschieden. Es ist jedoch unmöglich, auf Grund unserer Kenntnisse von diesem Stamm das echte Sp. Schencki zu beurteilen, weil letzeres sich vollkommen vom Stamm Hektoen-Gougerot unterscheidet und beispielsweise durch den Stamm Hyde-Davis vertreten ist. Da aber der Stamm Hyde-Davis mit dem Sp. Beurmanni identisch ist, so müssen das Sp. Schencki und das Sp. Beurmanni ebenfalls identisch sein.

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[18] *Sporotrichosis Nord Americanus: Bull. et Mem. de la Soc. Med. de Hop. de Paris,* 35, p. 798, 1910.

[19] Gougerot, Kolle & Wasserman, *Handbuch der Pathogenen Microorganismen,* 2 Auflage, 5, 225, 1913.

Die Frage kann nur entschieden werden durch Vergleich der Original-kulturen von Schenck und Hektoen. Entschliesst man sich, diese dritte Hypothese einer Unität des Sp. Schencki und des Sp. Beurmanni anzunehmen, so wurde trotzdem die Tatsache bestehen bleiben, dass dieser Parasit in zwei Typen sich darstallen kann; als Sp. Schencki, der seltener Typus, welcher einzig von den amerikanischen Forschern im Jahre 1898 and 1900 beschrieben worden und von welchem der Stamm Hektoen-Gougerot eine Abart bildet (Sp. Schencki-Beurmanni var. Schencki), und schliesslich als Sp. Beurmanni, ein sehr verbreiteter Typus, der zum ersten Mal von Matruchot & Ramond im Jahre 1903-1905 und später im Jahre 1906 von Beurmann & Gougerot studiert worden ist (Sp. Schencki-Beurmanni var. Beurmanni.)

From the above statements it is evident that Gougerot himself concedes the identity of the French and the later American strains and I entirely agree with him on this point. I have examined several French strains very carefully and compared them with later American strains from man and horses without noting any essential points of difference. Admitting then that the French strains and the later American strains are identical, the question resolves itself into a comparison of the disease and organisms as observed by Schenck and by Hektoen on the one hand and the French and later American disease and organisms on the other. These latter, it is to be noted, are conceded to be identical with the strains from South America, Madagascar, and the other foci mentioned above. If it is shown that they are identical with the Schenck-Hektoen strain, they should all be called *Sporotrichum schenckii*. If they are different, they would be called *Sporotrichum beurmanni* and the strains of Schenck and Hektoen would remain as isolated and unique organisms differing from all other described sporotricha.

Comparison of the Two Cases of Sporotrichosis Observed by Schenck and by Hektoen and Perkins with the Later American Cases

Clinically these two cases were typical ascending gummatous sporothrichosis identical in every way with the many cases observed later by the French and by many observers

here in the United States. No one, so far as I know, has attempted to differentiate them on clinical grounds.

Therapeutically they responded promptly to potassium iodide as did the later French and American cases.

The morbid anatomy and histology in the human were, so far as studied, the same in the two types, the lesions being those of a chronic abscess. In experimental animals the lesions produced by the two types are identical as admitted by Gougerot.

Bacteriologically there is greater opportunity to detect minute or subtle differences should they be present and these we shall consider in detail. In the cases of Schenck and of Hektoen and Perkins, the organisms were not seen in the human tissues or in the pus. In experimental animals the organisms appeared as oval and elongated forms with occasional round forms. They stained with Gram. In no way did they differ from the forms seen in tissues infected with organisms of the French strains. I have made a special study[20] of the tissue forms in experimental animals using strains from France and from America as well as the Schenck-Hektoen strain. No differences could be detected between them. When these various strains are grown in animal fluids, blood etc., in the test tube, elongated forms similar to those seen in the tissues are produced and here again no differences between the various strains were noted.

The question of the virulence of the various strains may not be of any importance in differentiation since this property is such a variable one. However, it may be stated that the Schenck-Hektoen strain even after years of artificial culture is still about as virulent for rats as are the freshly isolated strains of the French type. According to the paper of Schenck, these organisms were virulent for mice and dogs. Hektoen and Perkins produced lesions in mice, dogs, rats and guinea pigs (slight). As far as these results are comparable with those obtained with the French and later American strains, they agree in all essential points.

We now come to a discussion of the morphological and cultural characteristics, both microscopic and macroscopic, of

[20] Davis, *Jour. Infect. Diseases*, 12, p. 453, 1913.

the organisms and in doing this it becomes necessary to state in detail the differences which the French workers have pointed out between *Sporotrichum schenckii* and *Sporotrichum beurmanni* since they very largely center around these properties. They have been stated succinctly by de Beurmann and Gougerot in their article on North American sporotrichosis[21] and in order to avoid misstatements I quote as follows:

Sporotrichum Beurmanni	*Sporotrichum Schenckii*
Cultures difficiles, mais possibles à 38 degrés. Optimum 22 à 30 degrés: donc developpement plus lent.	Cultures facile à 38 degrés. Optimum 30 à 38 degrés: donc developpement plus rapide.
Aspect macroscopique des cultures sur gelose glycosée peptoneé de Sabouraud (milieu d'épreuve).	
Pigmentation rapide et complète. Colonies toujours très colorées, de teinte chocolat ou noire.	Pigmentation très lent, le plus souvent inconstante ou absente. Colonies peu colorées ou blanches le plus souvent.
Circonvolution à la façon des circonvolutions celebrales.	Sillons presque rectilignes divergeants à partir d'un centre comme des vallées du cone d'un volcan.
Aspect microscopique des cultures sur lames sèches et en gouttes pendantes.	
Filaments myceliens de 2 u de large plus rectilignes, quelquefois agrégés, mais surtout enchevêtres, non paralleles.	Chlamydospores.
Spores de 3 sur 5 a 6 u, très nombreuses, inserées sur de longs filaments ou à l'extrémité de filaments lateraux courts ou longs.	Filaments myceliens de 2 u de large plutôt curvilignes, onduleux, presque toujours agrégés et paralleles en faisceaux, sans enchevetrement habituel.
	Spores très rares, souvent même absentes, inserées le long et surtout à l'extrémité de longs filaments. Peu ou pas de conidiophores courts lateraux.
	Pas de chlamydospores connues.

[21] *Loc. cit.*

Caractères biologiques (Blanchetiere et Gougerot).

Fait fermenter la saccharose.	Fait fermenter la lactose.
Ne semble pas faire fermenter la lactose, etc.	Ne semble pas faire fermenter la saccharose, etc.

Matruchot has stated the differences more in detail but since all the essential points are covered by the above outline it will not be necessary to state them again. I shall take up these points in order and attempt to analyze them in the light of data from both American and French sources.

First, as to the optimum temperature for growth, Schenck states that for his organism it was between 20°C. and 37° C. Hektoen says it would seem to be about 37° C. Growth is much slower at 20° C. My own experiments have not convinced me that there is any appreciable or constant difference in optimum growth temperature between *Sp. schenckii* and *Sp. beurmanni*. Slight differences are often observable between various strains of sporotricha. In growing many cultures side by side, including the original *Sp. schenckii*, growth was most rapid and most abundant at temperatures from 28° C. to 32° C. Variation in optimum growth temperatures is common in fungus organisms of this type. They are not delicate in this respect and small differences should not be unduly emphasized as differentiating features.

In the outline quoted above de Beurmann and Gougerot have next emphasized certain points concerning the macroscopic characters on special media of the cultures which in their opinion are important in differentiation. These points center chiefly round the fact that sporotricha generally are especially prone to change and modify their cultural properties on artificial media, a character referred to as pleomorphism. This is so important and so much has been made of cultural differences in distinguishing *Sp. schenckii* and *Sp. beurmanni* that I must discuss it somewhat in detail.

First, the colonies may in a great variety of ways alter their pigmentation, the tints changing through various shades of brown and black; portions or all of the culture may be pure white. These changes may or may not be permanent. I

have for some time made a study of chromogensis[22] in these cultures and have now some pure white strains which sprang from deeply pigmented cultures. Indeed, from the culture I received from Gougerot of Paris, a white colony appeared which has remained pure white and smooth, and though tested on numerous media of the most favorable sort (carrot, potato, Sabouraud medium), remains pure white. Passage through a rat for six weeks did not alter it. It has now passed through twenty-four generations without change. The black colonies continue to produce pigment as usual. Similar alterations have been observed in other strains. Distinct and similar changes have been noted in the Schenck-Hektoen strain but they are less marked. It is interesting to note Gougerot's statement[23] in this connection: "Par exception nous avons eu des pléomorphismes blancs qui sont trestis irreductibles; ils étaient associés à des pléomorphismes de surface et ces pléomorphismes complexes donnaient un Sporotrichum Beurmanni, identique d'aspect au Sporotrichum Schenckii."

A second pleomorphism relates to form of growth, smoothness, wrinkling, etc. Colonies tend to lose their irregular and corrugated surface and become smooth and leathery in appearance. This is a common change in strains of sporotricha which can be brought about, at least to some extent, in all strains by suitable culture, especially on ill adapted media.

A third is the tendency to form on the surface growth hair-like processes or finely pointed spines. This is seen quite commonly and is a striking feature of many cultures of the original Schenck-Hektoen strain.

A fourth pleomorphic change is the appearance of a powdery growth covering part or all of the media. The color is variable and may range from black through brown to pure white. This alteration is largely dependent on surface deposits of spores.

It is to be emphasized that these pleomorphic changes above noted are common. In some strains they are far more frequent than in others, but probably occur in all strains at

[22] Davis, *J. Inf. Dis.*, 17, p. 174, 1915.
[23] *Les Sporotrichoses*, Paris, p. 91, 1912.

times. They are variable but often fixed and permanent. They are so manifold in character that cultures show a great variety of appearances and two strains identical at first may later through these changes become quite different in appearance. De Beurmann and Gougerot state in their monograph on page 133 (*Les Sporotrichoses*) that they have observed strains of *Sporotrichum beurmanni* (notably of the race *alpha*), through pleomorphic change, become identical with *Sporotrichum schenckii*. Others have become identical with *Sporotrichum jeanselmei* or have even simulated *Sporotrichum gougeroti*. Again certain strains have reverted to short forms comparable to yeast or blastomycetes. My own work also confirms in general the above observations of de Beurmann and Gougerot. I have noted yeast-like forms in certain strains and a great many changes in pigmentation and other morphological appearances, some of which are fluctuating, others are apparently permanent.

In the light, then, of the above facts it seems to me that distinctions of these sporotricha based on pigmentation become valueless because of these easy and striking fluctuations. So, too, surface convolutions and forms simulating the cone of a volcano are factors which change under conditions favoring pleomorphism.

Under microscopic aspect of cultures on slides and in hanging drop in their outline de Beurmann and Gougerot consider especially spore formation. They have repeatedly stated that in cultures of *Sporotrichum schenckii* the spores are rare or even at times absent on the filaments. This is true, at least, of certain cultures that develop little or no pigment. As a differentiating feature, however, this point is not necessarily significant. I have noted other strains, especially the non-pigmented ones, which show this same characteristic. This dearth of spores in the strain of *Sporotrichum schenckii* which they examined was, I think, no doubt due to a pleomorphic change. It is important to note the fact, which they have not referred to in their publications, that in the original articles of both Schenck and Hektoen and Perkins several photographs of unstained organisms show the mycelium with spores attached to the sides and ends in great abundance. I think

these photographs are conclusive on this point and show that no doubt a change occurred later in these cultures resulting in a strain bearing fewer spores. Spore formation is extremely variable as de Beurmann and Gougerot admit. Furthermore in their monograph they state that the white pleomorphic forms—those approaching the type of *Sporotrichum schenckii*—are very poor in spores, certain ones becoming entirely devoid of them. I wish again to emphasize this point as practical proof that changes did result in this strain from the time it was first described and the time, some seven years later, when it was sent to de Beurmann by Hektoen. Furthermore, de Beurmann and Gougerot write [24] that Hektoen stated in his letter when transmitting the culture that it seemed to have lost its power of producing spores as compared with the preceding generations. This, I believe, is definite proof of a change which no doubt occurred on artificial media and which these writers have used to differentiate the *Sporotrichum beurmanni* from *Sporotrichum schenckii*. Original organisms and original descriptions should be compared for this purpose, not organisms changed through growth on artificial media.

As to chlamydospores, de Beurmann and Gougerot state that *Sporotrichum beurmanni* forms them while the *Sporotrichum schenckii* does not. Matruchot also makes this statement. I discussed this matter in a special paper some time ago[25] and showed that, at least under certain conditions, the *Sporotrichum schenckii* readily forms typical chlamydospores. This was especially true on media poor in nutrient material. K. F. Meyer[26] confirmed my results in this respect noting chlamydospore formation not only in the original *Sporotrichum schenckii* but also in the many strains of sporotricha which he isolated from horses in the United States.

With reference to the arrangement of mycelial filaments, it may be stated that this is a property decidedly pleomorphic, and with the appearance of the pleomorphic alterations noted

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[24] *Bull. et. Mem. de la Soc. Med. des Hop.*, Paris, 35, p. 745, 1910.
[25] *Jour. Inf. Dis.*, 15, p. 483, 1914.
[26] *Jour. of Inf. Dis.*, 16, p. 399, 1915.

above on artificial media the mycelial filaments may run in straight or curved bundles with little entangling.

As to biological characters the fermentation reactions of these organisms are apparently not very uniform or definite. Gougerot says that *Sporotrichum beurmanni* ferments saccharose but not lactose, whereas *Sporotrichum schenckii* ferments lactose, but not saccharose. He states, however, that he does not know whether these properties are fixed for all strains and for the pleomorphic forms. Greco noted that his strain from South America failed to ferment lactose, saccharose and mannite. This does not agree with the fermentation reactions for *Sporotrichum beurmanni* as given by de Beurmann and Gougerot but they nevertheless classify the South American strains as *Sporotrichum beurmanni.* At the same time they use this difference in the fermentation of lactose and saccharose to differentiate *Sporotrichum beurmanni* from *Sporotrichum schenckii.*

Meyer and Aird[27] have made a careful study of the fermentation of American strains and of *Sporotrichum beurmanni.* They were not able to confirm the finding of Blanchetière and Gougerot that *Sporotrichum schenckii* fermented lactose. Furthermore, they found the fermentation of saccharose irregular. They state that "in considering these results purely from a differential diagnostic viewpoint it is quite evident that it cannot be used for this purpose and the fermentation of carbohydrates is just as little a criterion of the type of sporotrichum as is the absence of pleomorphism and the chlamydospore formation". Their conclusions are so definite and so relevant that I quote them:

> The differentiation of pathogenic sporotricha into two distinct species by means of the fermentation of carbohydrates, is impossible. The reactions are not fixed and are as inconstant as the many variations noted in the formation of chlamydospores and, frequently, in pleomorphism. There does exist however an apparent relation between the pigmentation of the sporotrichum strains and the ability of these strains to ferment saccharose. The alpha and beta types are the most active fermenters.

[27] *Jour. of Inf. Dis.*, 16, p. 399, 1915.

This and other evidence, which will be presented elsewhere, make it apparent that the American sporotricha—of which we studied thirty-five strains—have, in many respects, type characters in common with Sporothrix beurmanni. In the light of de Beurmann's and Gougerot's work, some of the American strains are doubtless Sporothrix beurmanni, and it is not permissible to call such strains "Sporothrix schenckii" merely for the sake of simplicity. The discussion of de Beurmann and Gougerot (28) on this subject can now also in our opinion, be satisfactorily closed, namely: that Sporothrix schenckii, Hektoen-Gougerot strain, is an absolutely fixed type. The true Sporothrix schenckii is represented however by all of the recently isolated strains. Inasmuch as most of these strains are undoubtedly identical with Sporothrix beurmanni, the Sporothrix schenckii is identical with the Sporothrix beurmanni.

The American strains of pathogenic sporotricha are therefore best classified as one species. Sporothrix schenckii-beurmanni (as suggested by Greco.)

Having now completed the discussion of the several points of differentiation quoted above from de Beurmann and Gougerot, I shall next briefly consider certain other similarities of the French and American strains of sporotricha that deserve mention, it seems to me, in a discussion of their possible identity. Slight and otherwise insignificant differences between organisms may be determined often by differences in serum reactions in varying concentrations. In the study of this group of organisms, Widal and Abrami showed that positive agglutination occurred in patient's serum in dilutions often of 1/400 or 1/500 or even higher. This has been confirmed by several workers. For differential purposes they noted that patients suffering with other mycelial infections like actinomycosis, nocardiosos, etc., give a positive but much lower agglutination. Gougerot and Caraven noted that the serum of a case of hemisporosis agglutinated in dilution of 1/400. This was evidently exceptional.

The writer immunized rabbits with several strains of sporothrix for a period of about 8 months. The strains included *Sporotrichum schenckii* obtained from Hektoen, *Sporo-*

[28] *Bull. et Mem. de la Soc. Med. de Hop. de Paris,* 26, p. 9, 1908.

trichum beurmanni from Gougerot of Paris, a sporotrichum isolated by K. F. Meyer from a horse in the United States, and a strain isolated in 1909 by the writer from typical sporotrichosis in a man from North Dakota. The sera of the various rabbits were tested with the homologous organisms and also with other strains. It was found that agglutination appeared quite uniformily in the dilutions of serum varying from about 1/320 to 1/640. In most instances a slightly higher agglutination appeared in the homologous sera but this was not always the case. The several strains tested could not be differentiated by these interagglutination tests in animals.

Similar tests were made with serum from a human case in which agglutination with the homologous organism occurred at 1/160. Here again the original *Sp. schenckii* and *Sp. beurmanni* were agglutinated at approximately the same dilution, namely, 1/160. The controls were negative.

Wilder and McCullough[29] studied the serum from a case of sporotrichosis of the eye. Tests for agglutinins and opsonins in the serum of the patient against several strains of sporotricha, including the infecting strain, the original Schenck-Hektoen strain, a French strain, an equine strain from Meyer, and two other American strains from typical cases, revealed no specific differences in the antibody content of the serum.

The reaction of complement fixation is positive in cases of sporotrichosis but it seems less reliable than that of agglutination. The studies of Widal and Abrami and other French workers have shown that the results are very definite but that an infection with many other mycoses (actinomycosis, hemisporosis, discomycosis, etc.) will also give a positive test. *A priori,* then, one would not expect this test to be useful in differentiating strains of sporotricha. J. J. Moore[30] in our laboratory has made such studies, finding a definite fixation in human serum from a case of sporotrichosis using the homologous organism. He obtained similar results when antigens made from the various other strains, including *Sporotrichum schenckii, Sporotrichum beurmanni* and an equine strain, were

[29] *J. A. M. A.*, 62, p. 1156, 1914.
[30] *Jour. Inf. Dis.*, 23, p. 252, 1918.

used. He concluded that these organisms are identical so far as this test is concerned.

Bloch[31] in 1909 was the first to obtain a positive skin reaction in a case of sporotrichosis though some work had been done along this line by de Beurmann and Gougerot in 1906 without definite results. Bloch used a "sporotrichosine" extracted from a broth culture. The reaction was very definite. French workers, especially de Beurmann, and Gougerot and Chopin, about the same time took up this work, using intracutaneous injections of extracts of the killed organisms in salt solution and obtained positive results. The reaction, however, was shown by them not to be absolutely specific. Other mycoses (actinomycosis, oosporosis, saccharomycosis, exascosis, at times tuberculosis) responded so that the method was useful according to them only for the differentiation of a rather large group. From their data, one would conclude that this method could have no value for differentiating closely related organisms belonging to the genus sporotricha.

Recently Moore and the writer tested a human case of typical sporotrichosis with a sporotrichosine consisting of killed sporothrix in salt solution. The patient was tested both when receiving and not receiving potassium iodide. A very distinct skin reaction was obtained with the sporotrichosine made from the original *Sporotrichum schenckii* and also from a strain of *Sporotrichum beurmanni* obtained from Gougerot. No differences were noted in these reactions which were very definite and measured 5 to 7 centimeters across. Controls with "blastomycine" made in exactly the same way from a blastomycete isolated from a typical case of blastomycosis did not give a positive reaction in this patient. Sporotrichosine injected into the skin of the patient with blastomycosis gave no reaction; nor did he react to his own blastomycine. Agar alone in ½ per cent suspension injected into persons when taking potassium iodide (t. i. d. 10 grains) gave a definite reaction but was not nearly as pronounced as that given by the "sporotrichosine". Normal persons receiving potassium iodide (t. i. d. 10 grains) reacted no stronger to

[31] *Beihefte zur Med. Klinik*, Basel, 8, p. 179, 1909.

sporotrichosine than before the iodide had been administered to them. In either case the dermal reaction measured from 5 to 15 mm. whereas the sporotrichosine reaction in the patient measured 5 to 7 cm.

While, therefore, a striking reaction may be elicited in these cases with sporotrichosine, it probably cannot be considered sufficiently specific to be of value for differentiating these organisms. But it is to be noted that patients taking or not taking potassium iodide may react intradermally to these various strains of sporotricha and not to strains of blastomyces prepared in exactly the same way. Whether or not all cases will so react we do not know.

Summary and Discussion

It appears that the first case of sporotrichosis was reported by Schenck in 1898. The second case was reported in 1900 by Hektoen and Perkins and the organism definitely identified by comparison with that isolated by Schenck. Hektoen named the organism *Sporothrix schenckii.*

In 1903 de Beurmann and Gougerot reported the first case in France and the organism was named by Matruchot and Ramond *Sporotrichum beurmanni.* The work of the American investigators published several years previously was not known to the French workers.

On comparing the American strain sent to them by Hektoen in 1906, seven years after its isolation, de Beurmann and Gougerot pointed out certain differences between this strain and their recently isolated strain. They noted certain differences also between their own strains (strains a, b, c) but contended that these differences were not sufficient to justify creating a new species. But the differences between their strains and *Sporotrichum schenckii* were sufficient, they contended, to justify a new species. The organisms, isolated later in North America and those found in South America and in Madagascar, they claim are the same as their organism, *Sporotrichum beurmanni.* These were all isolated after they discovered and named the organism in France.

It is pointed out that sporotricha, French, American and other strains, are especially subject to undergo pleomorphic changes, some of which are transient while others are fixed and permanent. De Beurmann and Gougerot themselves have called especial attention to this and admit that some of the pleomorphic alterations in the macroscopic growth of certain strains render them identical with the strain of *Sp. schenckii* as it exists today. Furthermore, Hektoen has stated that the culture as sent to them had changed in the seven year interval on artificial media especially in its ability to produce spores. This change is not an uncommon one in both American and French strains and no doubt was associated with other pleomorphic changes. Yet de Beurmann and Gougerot and also Matruchot used this loss of ability on artificial media to form spores as a differentiating characteristic from their own *Sporotrichum beurmanni* though they observed this same change in strains of the latter. There can be no doubt that pleomorphic changes took place in *Sporotrichum schenckii;* and this is borne out also by the photographs in both Schenck's and Hektoen's papers which clearly show that at first both strains produced spores in large numbers.

Changes in pigmentation are common in all strains of sporotricha; poorly or non-pigmented strains may arise from deeply pigmented strains and remain fixed. One would scarcely classify such an organism as sporotrichum on the basis of such a fluctuating character as pigmentation, though this property is mentioned by them as an important differentiating one.

The statements of de Beurmann, Gougerot and Matruchot that the *Sporotrichum schenckii* (original) does not form chlamydospores must be considered erroneous. Under suitable conditions these structures have been observed by the writer, and these results were confirmed by Meyer, not only in the original Schenck-Hektoen strain but in many other American strains from both man and horses. The attempt to differentiate *Sporotrichum schenckii* and *Sporotrichum beurmanni* on this basis must therefore be given up.

The fermentation of sugar is quite inconstant. It is difficult to understand why de Beurmann and Gougerot would use

the differences in the fermentation of lactose and saccharose as a distinguishing feature between the American and French organisms since they say that not all their strains fermented saccharose. Meyer and Aird have shown conclusively the inconstancy in fermentative powers of not only many American strains but also of different French strains. They conclude it is impossible to differentiate sporotricha into two distinct species by means of the fermentation of carbohydrates. These results have been confirmed by the writer. Greco's observation on a South American strain does not agree with those of de Beurmann and Gougerot on French strains.

Specific serum and dermal tests are probably of limited value in differentiating these closely related organisms though they may furnish important data for the basis of a group relationship. So far as the results indicate they show no differences between the French and American strains.

From the above analysis it would seem that the basis upon which French investigators differentiate the *Sporotrichum schenckii* and *Sporotrichum beurmanni* is, to say the least, very inadequate. On account of the pleomorphism of this organism there is an excellent opportunity to take advantage of slight and unimportant differences in order to create new species. It is of course true that no two strains are *exactly* identical. De Beurmann and Gougerot noted that certain of their strains manifested fixed pleomorphic changes that made them appear identical with the original *Sporotrichum schenckii.* Yet they did not suggest that these strains be called *Sporotrichum schenckii.* Concerning these slight differences, especially in organisms of this type, it would seem that it would be wise to assume a conservative attitude and to refrain as much as possible from the use of new and unnecessary terms.

The question arises in connection with an organism of this kind, as it arises so frequently in biology, what differences are sufficient to warrant the creation of a new variety or species? Where shall the line be drawn between varieties since no two cultures of sporotricha are absolutely identical in every detail and strains are ever prone to these striking

changes? To this the answer must be made that this is largely a conventional matter and often it is impossible to state clearly where the line of demarcation should lie. But this I wish to point out, that it is evidently not proper or scientific to use pleomorphism, or any other character for that matter, as a basis for the classification of an early American strain, and not use it in the classification of French strains or later American strains.

In résumé, I believe we are justified in stating that the differences between the American strains, including the original cultures of Schenck and of Hektoen, and the French strains of de Beurmann, Gougerot and others, are easily explained as pleomorphic variations and therefore are insignificant. Furthermore, the disease, clinically, pathologically, experimentally, and therapeutically, is admitted by all to be identical in France and in America.

The above statements being true, according to the rules of botanical nomenclature the organisms in both countries should be called by the name first given to them in 1900 by Hektoen, namely, *Sporotrichum schenckii.* The fact that de Beurmann rediscovered the organism several years later deserves no consideration so far as determining nomenclature is concerned. As regards the use of the compromise term *Sporotrichum schenckii-beurmanni,* suggested first by Greco of South America and more recently concurred in by Meyer in this country, it may be said that this is objectionable because it not only introduces a long cumbersome term but it is not in accord with the rules of botanical nomenclature. There is obviously therefore but one legitimate term for this organism, namely *Sporotrichum schenckii.*

It should be pointed out that even though one maintains that the small differences noted between the pleomorphic forms of the Schenck-Hektoen strain and the other sporotricha are sufficient to justify a species distinction, the important fact remains that the hundreds of strains of sporotricha found in France and in North America are alike. This is admitted by both sides of the controversy. Therefore, whichever view of the original Schenck-Hektoen strain is taken by

the French, the identity of sporotrichosis, excluding the very rare strains mentioned earlier in the paper, in France and America must be admitted. One is as justified in making this statement as in saying tuberculosis in France and in America is identical.

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THE BACTERIOLOGY OF THE BLOOD OF DOGS WITH ECK FISTULA

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There is an impression, supported by considerable evidence, that organisms from the bowel often enter the portal stream and in the liver are effectively disposed of. In order to test this point it was thought that Eck fistula dogs might furnish proper conditions; at least, it would be of interest to note whether or not bacteria appeared in the general circulation or possibly caused occasional infections in the lungs or elsewhere in such animals.

An Eck fistula is an artificial communication between the portal vein and the vena cava with the portal vein ligated above the anastomosis just at the hilus of the liver. This operation destroys the portal circulation so far as the liver is concerned, in that now the flow of blood from the abdominal viscera (except the kidneys and the suprarenal glands) is returned to the general circulation without passing through the capillaries of the liver; in other words, the liver is shunted out of the portal circulation and receives its sole blood supply by way of the hepatic artery, which carries about one-fifth of the quantity of blood that is carried by the portal vein.

The anatomic situation of the liver naturally suggested to investigators a protective function. Under normal condition all the products of intestinal digestion and other absorbable substances (except the greater part of the fats) must first pass through the liver capillaries before reaching the tissues in general, while under the conditions imposed by an Eck fistula they reach the tissues first. It has been amply proved that the liver exercises a protective function against certain poisons absorbed from the alimentary tract, especially against certain of the products of protein digestion, such as ammonia. In fact, dogs with Eck fistulas will not tolerate a heavy meat diet.

To prove whether or not bacteria actually pass the bowel wall into the circulation and, if so, what influence the liver has on them, we instituted the following experiments on dogs with Eck fistula.

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June 25, an Eck fistula was made in a dog weighing 19,000 gm. Recovery was prompt without infection; some emaciation followed. On July 9, 12, 13 and 16 from 5 to 10 c c of blood were drawn from the femoral vein under sterile precautions and introduced into 3 or 4 culture tubes of broth. Of the tubes inoculated, only one taken on the first day yielded a slight growth of a nonhemolytic small streptococcus.

An identical experiment was made with a second dog with Eck fistula operated on July 4. The cultures were made on July 12, 13 and 16. The various tubes were inoculated with the blood as in the first experiment, and all remained sterile indefinitely. These experiments were more or less in the nature of preliminary tests to determine whether or not bacteria in dogs with Eck fistula passed through the bowel wall and remained in the circulation for any length of time. The results we regard as negative. In only one of the tubes did we obtain a slight growth of a small coccus which we believe to be a contaminator.

In the next experiment the conditions were altered somewhat. On July 10, an Eck fistula was made in 2 normal dogs by one of us (Mathews). No untoward results followed the operation. Seventeen days later when healing was complete blood was drawn, with proper precautions, from the femoral vein. From each dog about 5 c.c. of the fresh blood was introduced into 4 large tubes containing 50 c c of ascites broth; 2 were placed under anaerobic and 2 under aerobic conditions. The tubes were kept under observation for a period of 2 weeks and in none did a growth occur. The dogs in this experiment were bled 2 hours after a rather heavy meal of meat, with the idea that possibly the absorption might favor the passage of bacteria through the bowel wall. Evidently this is not the case.

Again on Aug. 2, 23 days following the operation the 2 dogs were bled from the right side of the heart by needle puncture. Some of the blood thus obtained would come directly from the portal circulation without passage through either the liver or lung capillaries.

This time the dogs were bled 4 hours after eating heavily of meat. Cultures were made in ascites broth as before and observed from time to time. No organisms grew in any of the tubes either under aerobic or anaerobic conditions. The results of both of these experiments, then, were negative for the circulating blood of these animals under the condition stated.

Blood from these dogs was then examined bacteriologically after the introduction of cultures of bacteria into the stomach. The bacteria were introduced by means of the stomach tube and 100 c c each of the 48-hour cultures of B. pyocyaneus and B. subtilis were so given to both dogs on a fasting stomach. After 4½ hours, from the heart of each dog 10 c c of blood were removed by needle puncture and introduced into 4 broth tubes in diminishing quantities. Neither B. subtilis nor B. pyocyaneus was obtained from any of the tubes following incubation. From dog 1 a small coccus grew slowly in 2 tubes which was not identified. From dog 2 a larger coccus grew profusely in one tube in 24 hours. We felt justified in regarding these as contaminations.

A week later the same experiments were repeated and 10 c c of blood were taken from the heart for culture at the end of 1 hour, 2 hours and 5 hours. Four broth tubes were inoculated from each sample of blood and 2 were placed under aerobic and 2 under anaerobic conditions. These cultures all remained without growth of B. pyocyaneous and B. subtilis. In 2 instances a small coccus appeared in few numbers, similar to the one obtained previously and which was regarded as a skin saprophyte.

About one week following this experiment the dogs were killed and examined. The Eck fistulae were in perfect condition. No important change was noted in the organs.

The next experiment was designed to compare the length of time bacteria artificially introduced into the circulation remain in dogs with Eck fistula and in normal dogs. In dog 1, several days following the last experiment 8 c c of a 36-hour broth culture of a staphylococcus albus was injected intravenously. As a control, at the same time a normal dog of approximately the same weight was injected with a similar amount. Blood cultures were made at short intervals from both dogs. The results are presented in table 1.

Between 2 and 3 hours after the injection both dogs became sick and vomited. After 24 hours they appeared quite normal. It will be noted that the staphylococci in 24 hours had disappeared from the blood, and at the end of 5 hours had somewhat diminished. It is possible that had many more cultures been made, slight differences might have been detected. It is apparent, however, that no appreciable differences appeared in the elimination of bacteria by the 2 dogs. A second similar experiment was made. Accidental contamination of some of the plates occurred, but so far as the results could be determined, they correspond with those of the first experiment.

TABLE 1

LENGTH OF TIME BACTERIA, ARTIFICIALLY INTRODUCED, REMAINS IN NORMAL DOGS AND IN DOGS WITH ECK FISTULA

Time After Injection	Growth from Normal Dog	Growth from Dog with Eck Fistula
5 min.	+++	+++
10 min.	+++	+++
15 min	+++	+++
30 min.	+++	+++
1 hour	++	+++
2 hours	+++	+++
3 hours	++	++
5 hours	++	+
24 hours	0	0

In this connection it may be mentioned that dogs with Eck fistula do not appear to be especially susceptible to infection. During a period of 10 years, one of us (Mathews) has had under observation about 100 dogs with Eck fistula, living from a few weeks to 3 years and noticed no more tendency to infection than in normal dogs. One thing noted, however, was that about 10% of these dogs failed to maintain a normal state of nutrition regardless of food or hygienic care. They suffered from diarrhea, and the stools were fatty and similar to the bowel discharges following ligation of the common bile duct. These dogs died in an extreme state of inanition after 2 or 3 months. Ulcers of the duodenum were observed in 5, and perforation was the immediate cause of death in 2. The diarrhea and fatty stools were probably due to a suppression of the formation of bile, which has been observed by Voegtlin and others in dogs with Eck fistula.

The obvious relation of the entrance of bacteria to the problem of so-called focal infection need only be mentioned. In spite of the negative character of our data, the suggestion is here offered that in a given case in seeking the source of the infection we should not overlook the possibility that the bacteria entered the body along a route long since closed with no evidence now of its past existence. Or they may have passed through the intact membrane without causing any local alterations, as tubercle bacilli may do for example, in passing through the intestinal wall.

Our results in general are in accord with observations made by many on the relatively rapid disappearance of bacteria when introduced into the circulation. Bull [1] has called attention to the intravascular agglutination of bacteria and their subsequent accumulation in the lungs, liver, and spleen. The endothelium, especially in certain organs, is most active in the destruction of the organisms.

Bartlett and Osaka,[2] investigating the fate of micrococcus aureus in normal dogs, came to the conclusion that they are rapidly stored up in large numbers in the lungs following injection into the left ventricle. A little later they disappear in the lung and appear in considerable numbers in the spleen and liver but again disappear from here in from 48 to 72 hours. In blood, bone marrow, lymph nodes, muscles, etc., they are found in small numbers or not at all. Their method of detection was by means of sections, and they reported no cultural results.

In dogs with Eck fistula in all probability the bacteria in the circulation, however they may have entered, are disposed of by the various mechanisms referred to. It seems that no appreciable differences occur in this respect between normal dogs and those with Eck fistula. Though the amount of blood that flows through the liver is markedly diminished in the latter, apparently this does not appreciably alter the general rate of destruction of the bacteria, since so many cells and organs other than the liver are concerned in this process.

SUMMARY

Experiments do not indicate that in dogs with Eck fistula bacteria in any appreciable numbers appear in the circulation.

Dogs with Eck fistula are no more susceptible to infection of the lungs or of other organs than are normal dogs.

Bacteria disappear from the circulation as rapidly in dogs with Eck fistula as in normal dogs.

[1] Jour. Exper. Med., 1915, 22, p. 475.
[2] Jour. of Med. Res., 1916, 35, p. 465.

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THE FATE OF STREPTOCOCCUS HEMOLYTICUS IN THE GASTRO-INTESTINAL CANAL

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Dangerous streptococci, especially Str. hemolyticus, are found very commonly in the throat. Our observations[1] show that in the crypts of the faucial tonsils hemolytic streptococci occur in nearly 100%. Often they occur in large numbers on the pharyngeal mucosa in normal persons, and especially in persons suffering with various respiratory infections, in which these organisms so often play an important rôle. Large numbers of streptococci are therefore constantly passing down the esophagus into the stomach in both normal and diseased persons. Furthermore, during milk-borne epidemics of streptococcus sore throat, milk from infected cows containing large numbers of streptococci is swallowed. The fate of these virulent hemolytic streptococci in the gastro-intestinal canal is the subject of this paper.

Three questions arise: First, the possibility of secondarily infecting the intestinal canal; second, the invasion of the body through the wall and third the possible danger of transmission of dangerous streptococci through sewage, etc.

As is well known, the gastric contents exercise a strong bactericidal effect on bacteria entering the stomach. Certain acidophils are able to live and develop in the stomach; spores and certain fungi, on account of their resisting properties, can do likewise. But the great mass of bacteria that enter the stomach through the act of swallowing are killed, and therefore the food passing into the duodenum contains relatively few organisms.

While these statements are generally true, there are exceptions due to several reasons; bacteria become enmeshed in particles of food and are thereby protected from acid until they enter the duodenum. This is especially true in cases of hypermotility. In achylia the bacteria pass through the stomach little affected by the feebly acid gastric contents.

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[1] Pilot and Davis: Jour. Infect. Dis., 1919, 24, p. 386.

Consequently, under certain conditions many bacteria from the throat may pass through the stomach unharmed and invade the intestinal canal.

Little work has been done to determine specifically the effect of the intestinal juices on bacteria, and apparently nothing has been done to determine their effect on virulent streptococci.

The rabbit is especially susceptible to experimental infections with Str. hemolyticus; for this reason it was selected for certain experiments designed to test the action of the gastro-intestinal contents on this organism.

In the examination of material for hemolytic streptococci the following routine method was employed: Plain blood agar plates were made, using 0.5 c c of human blood to 5 c c of medium. This medium was inoculated usually with a small quantity of intestinal contents, which, whenever necessary, was properly macerated in sterile fluid. Several plates were made in order to obtain proper dilutions. The hemolyzing colonies were isolated and, when necessary, confirmatory tests were made. By hemolytic streptococci I mean streptococci having a wide, clear zone of complete hemolysis (type B or Theobald Smith) and not the imperfect hemolyzers (type A).

The intestinal contents from three normal rabbits living on a routine diet of carrots, hay and water were examined for hemolytic streptococci in the following manner: The animals were killed, the abdomen at once carefully opened, and several specimens of the contents were obtained from stomach, duodenum, ileum and colon, and plated on blood agar. In none of the specimens were hemolytic streptococci isolated; green streptococci of the fecalis type were common. Numerous other organisms, including hemolytic colon bacilli, appeared on the plates. Another normal rabbit was placed under observation for a period of nearly 3 weeks and each day one or more specimens of feces were cultured. Nineteen specimens thus collected were examined and all yielded negative results as to hemolytic streptococci. Many green producing streptococci appeared. This preliminary experiment was made primarily to determine the incidence of hemolytic streptococci in the intestinal canal of normal rabbits.

The following experiment was carried out to determine whether or not hemolytic streptococci fed to rabbits would live in the gastro-intestinal canal and cause symptoms: It was found that feeding was

unsatisfactory and the streptococci were therefore introduced by means of a small catheter which serves as a satisfactory stomach tube for this animal.

Three healthy rabbits were carefully selected and their stools examined for hemolytic streptococci, with negative results. They were then given, by means of the rubber catheter, suspensions of streptococci. Three strains were given, one an old laboratory strain that appeared to be somewhat hardy and had lost most of its virulence (3 c c of a broth culture intravenously did not cause lesions) and two virulent strains recently isolated, one from a tonsillar abscess which killed a rabbit in doses of 1 c c given intravenously, and another isolated from the spinal fluid of a case of meningitis. Each day for ten days, from 10 to 20 c c of a 24-hour milk and broth culture of the relatively avirulent strain were given to the three rabbits, and each day the feces were collected and examined. In rabbit 1 on the ninth day a very considerable number of streptococcus colonies appeared on the plates which, on examination, were shown to be identical with those injected into the stomach. These cocci appeared suddenly, not being noted before in the feces. On the following day also a smaller number of the same streptococci were found, but on the third day none appeared. At the end of fourteen days, and for six days thereafter, the strain of streptococci from the tonsillar abscess was fed to the rabbit. No hemolytic streptococci were found in the feces in this time. Then for the next twelve days the freshly isolated strain of hemolytic streptococci from a fatal case of meningitis was given by mouth. During this time no hemolytic streptococci appeared in the feces. At no time did this rabbit show any evidence of infection or appear abnormal in any way. It was noted that on the two days when the hemolytic streptococci were found in the feces the latter were slightly more moist and softer than usual. Otherwise they remained throughout normally firm and well formed.

In rabbit 2 the streptococci were given as in 1. In this animal, on the fifth day after giving the meningitis streptococcus, there appeared in the feces numerous typical hemolytic streptococci like those given by stomach. They appeared suddenly, and the feces were slightly moist. On the following day they disappeared and were not recovered thereafter.

In rabbit 3 on the sixth day after feeding the avirulent streptococcus, streptococci appeared suddenly in the stools in large numbers. Two days later they were not found. Again six days after beginning

the administration of the meningitis streptococcus about 25 colonies appeared on the plates, which proved to be identical with the original meningitis streptococcus. They did not reappear.

At the end of 31 days, during which these observations were being conducted, the three animals were killed and specimens for examination were obtained from various levels of the gastro-intestinal tract: 2 from the stomach, 8 from the small intestine and 4 from the cecum and large intestine. Cultures made from this material in the 3 rabbits in every case yielded no hemolytic streptococci. The stomach and intestinal mucosa appeared normal, as did all the other organs in the animals.

It appears from these experiments that when large numbers of streptococci are introduced they are usually killed promptly, presumably in the stomach, but that occasionally, as in each of the three animals, they may appear for a short interval in considerable numbers in the feces. They do not, however, permanently seed the intestinal canal with streptococci as evidenced by their absence in the examination made at necropsy. Nor is it possible, judging from these experiments, in this way to infect the rabbits or cause lesions of the intestinal tract.

In the light of these experiments it is difficult for me to understand the results of certain workers who report finding intestinal lesions and generalized infections followed by death after the administration by mouth of even small quantities of streptococci. Bail[2] states that he was able to so infect rabbits from 10 to 12 weeks old by injecting into the empty stomach far smaller quantities of streptococci than were used in my experiments. He believes that virulent pyogenic organisms may pass through the uninjured intestinal mucosa and cause a general infection. Holst[3] reports similar results with animals and with himself after feeding with cultures of streptococci. These results are not in accord with my own, nor are they in harmony with the general fact that in streptococcus pneumonia and other respiratory infections large numbers of streptococci are swallowed, but are not found, at least in large numbers, in the feces. The organisms may not have been hemolytic streptococci.

It was thought that perhaps the intestinal canal might become infected during a generalized infection with streptococci. Three rabbits were therefore inoculated with a virulent hemolytic strepto-

[2] Arch. f. klin. Chir., 1900, 62, p. 369.
[3] Baumgartens Jahresbericht, 1895, 11, p. 52.

coccus intravenously. Each developed severe multiple arthritis and had fever, loss of weight and other evidence of a generalized infection. Twelve days later the three animals were killed and cultures made from stomach, duodenum, ileum and cecum, as well as from heart blood and joint fluid. The various involved joints yielded streptococci in large numbers as did also the heart blood in one rabbit; but no hemolytic streptococci were found in the intestinal contents. In the duodenum very few bacteria of any kind were found in the intestinal contents; lower down the numbers were greatly increased. It appears therefore, that though the streptococci are in the blood and other tissues, the intestinal canal may remain free from these organisms.

HEMOLYTIC STREPTOCOCCI IN HUMAN FECES

There is little in the literature concerning the occurrence of hemolytic streptococci in human feces. Broadhurst[4] isolated strains from human feces, dog intestines and stomach, and various other sources, but does not clearly state whether they are the wide hemolyzers of the beta type.

A routine examination of 53 specimens of human feces was made. They were obtained from a variety of persons many of whom were patients in a large hospital and suffering with a variety of diseases, few of which were intestinal, however. Since we have shown that practically all tonsils contain hemolytic streptococci,[1] and since a large proportion of persons harbor many of these organisms at all times in their throats, we may be sure that many, if not all, of these patients were swallowing large numbers of them constantly in their foods. The feces were collected and cultured usually within a few hours after evacuation. Small particles were fished and stirred in a small quantity of broth or salt solution from which several blood-agar plate cultures were made. Suspicious colonies were fished and subjected to proper tests. In none of the 53 specimens were hemolytic streptococci demonstrated. In a number of the specimens broth was inoculated with a small particle of feces and incubated for from 12 to 18 hours, and from this material plates were made. These examinations also failed to reveal this organism in the specimens examined.

Since scarlet fever patients practically always have hemolytic streptococci in their throats and usually in very large numbers,[5] the feces from four cases were examined to determine if in this disease these

[4] Jour. Infect. Dis., 1915, 17, p. 277.
[5] Ruediger: Jour. Infect. Dis., 1906, 3, p. 755.

cocci passed through the canal. Examinations made in the usual way failed to reveal the hemolytic variety; many green streptococci were always present. Small particles of three of the specimens were suspended in salt solution and inoculated into the peritoneal cavity of three white mice. One white mouse was sick for two days, but recovered. On the fourth day examination revealed no streptococci in the peritoneal cavity. A second mouse died two days later. Small green streptococci were in the heart blood, but no hemolytic streptococci were found in the blood or peritoneal cavity. The third mouse died after four days. A few B. coli were in the peritoneal cavity and the heart blood was sterile; no hemolytic streptococci.

Thus, in none of the feces from normal or from the scarlet fever patients examined were hemolytic streptococci found. In this connection Kraft[6] working in this laboratory has examined 125 normal and pathologic appendices, cultivating the contents and also the scrapings of the mucosa. In 48 normal appendices he found only 2 in which hemolyzing streptococci occurred, and in these there were only a few. In 77 pathologic appendices examined he found 4 containing this organism and in decidedly larger numbers than in the normal ones.

The following experiment was made to determine how long hemolytic streptococci will live in feces: A specimen of fresh human feces was examined and no hemolytic streptococci found. A virulent freshly isolated throat hemolytic streptococcus was mixed with the feces, placed in the icebox, and at daily intervals cultures were made. At the end of seven days under these conditions the hemolytic streptococci seemed as numerous as in the first control. In a similar way, feces were mixed with hemolytic streptococci and kept at incubator temperature and cultivated from time to time. The results were different. While at the end of two hours numerous hemolytic streptococci were present, at the end of 24, 48 and 72 hours none were obtained in the feces. In the incubator the streptococci appear to be rapidly overgrown and destroyed by the normal fecal organisms. It should be pointed out that conditions in the test tube are not directly comparable to those in the intestinal canal where fresh alkaline intestinal secretions are being constantly poured, the reaction being thereby maintained normally not far from the neutral point. In the intestine also the mucosal surface might under certain conditions, especially pathologic, favor the development of hemolytic streptococci.

[6] Unpublished observations.

ACTION OF GASTRIC JUICE ON HEMOLYTIC STREPTOCOCCI

Strauss and Wurtz[7] showed that the destructive effect of stomach contents on bacteria, in vitro, runs parallel with the HCl content. Others have obtained evidence that in addition to the acid effect the pepsin may exert some germicidal action. This is probably slight, if it exists at all. The concentration of HCl in gastric contents varies according to Clark and Lubs,[8] from P_H 0.9 to P_H 1.6. This is far below the limiting H-ion concentration (5.2) at which hemolytic streptococci will develop, as determined by Jones[9] and others.

With concentrations of HCl in the stomach in ranges approaching normal we should, therefore, expect the hemolytic streptococci to be rapidly destroyed and the contents to become free from these organisms in a short time. This does occur.

In an examination of 15 stomach contents no hemolytic streptococci were found. In these the total acidity ranged from 14 to 85. In four no free acid was present when tested with the dimethyl test. In these cases of low acidity certain varieties of bacteria were far more numerous than in the other specimens, but hemolytic streptococci were not found.

In order to test the direct bactericidal action of the gastric juice on hemolytic streptococci pure suspensions of this organism were added to 5 c c of the stomach contents. Of 15 samples thus tested, 11 had free acid with total acidities ranging from 28 to 85, and in all the hemolytic streptococci were dead in five minutes, many of them in two minutes. Of the four samples with no free acid and with total acidities of from 14 to 19, the hemolytic streptococci lived from one to three hours, but were dead in twenty-four hours. Presumably in the stomach the action would go on somewhat more effectively than in the test tube owing to more favorable conditions. These results strikingly illustrate the effectiveness of proper acidity in the stomach in determining the character of the gastric flora.

In the older literature there are many references to work on streptococcal enteritis which seems to center largely around that of Escherich[10] and his school. The examinations made at that time do not permit one to classify the streptococci accurately, but there are reasons to believe that many, if not all, belong to the streptococcus fecalis

[7] Quoted from Kolle and Wassermann, 1914, 1, p. 993.
[8] Jour. Bacteriol., 1917, 2, p. 218.
[9] Jour. Infect. Dis., 1919, 24, p. 386.
[10] Die Darmbacterien des Säuglings, 1886.

group. Baermann and Eckersdorf[11] describe a series of cases of croupous enteritis simulating dysentery in which they say streptococci were found and cultured in large numbers from the stools and the intestinal exudate, and were strongly hemolytic on blood plates. Holst[12] describes a family epidemic of enteritis arising from the use of milk from a sick cow. These observations, together with several others of a similar character, suggest that streptococci of the hemolytic variety may at times play a rôle in gastro-intestinal infections, even as primary invaders. However, much of the literature is old and consequently the data are difficult to analyze in the light of more modern methods and conceptions. The problems involved, therefore, in the possible relation of hemolyzing streptococci to pathologic lesions (enteritis, diarrheas, dysenteries, etc.) in the intestinal canal require reinvestigation, and certain work along these lines has already been undertaken and will be reported shortly.

SUMMARY

Hemolytic streptococci do not occur normally in any appreciable numbers in the gastro-intestinal canal of rabbits. When introduced into the stomach of rabbits in large numbers, virulent hemolytic streptococci may occasionally pass through the canal and appear in the feces. Examination of the gastro-intestinal canal at various levels in rabbits thus fed indicates that the hemolytic streptococci do not develop appreciably in the intestines, nor do they readily gain a permanent foothold there. Rabbits with generalized streptococcus infection in joints, blood, etc., showed no hemolytic streptococci in the intestinal contents.

Gastric juice of normal acidity from man and from rabbits kills hemolytic streptococci in from two to five minutes. Gastric juice in achylia may not kill them in several hours.

In normal human feces hemolytic streptococci were not found in 53 cases.

Hemolytic streptococci when mixed with normal human feces will live in the icebox for at least several days. In the incubator they tend to die out rapidly.

[11] München. med. Wchnschr., 1909, 56, p. 1169.
[12] Baumgartens Jahresbericht, 1895, 11, p. 52.

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THE OCCURRENCE OF HEMOLYTIC STREPTOCOCCI IN THE NORMAL THROAT

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The incidence of hemolytic streptococci in the throat has been studied by many workers. In normal persons the percentages as given range from 10 to 60 per cent. or higher. From the mass of literature on this point I think the impression may be gained, indeed the conclusion has been drawn by some, that this organism does not occur in the throats of some persons. Others have the impression that certain persons are definite carriers in that they constantly harbor large numbers of the organisms in the throat.

The data have been obtained in most instances by making ordinary throat swabs and then plating either by the poured plate method or by surface plate smears. These methods will determine with reasonable accuracy the incidence of hemolytic streptococci on the surface of the mucosa of the throat. It is now known, however, that hemolytic streptococci prefer to inhabit the crypts and grooves of tissues about the throat. In the crypts of the tonsils Pilot and Davis [1] have shown that they are found in nearly 100% of cases, and others have obtained substantially the same results. Pilot,[2] recently has also shown that in the folds of the adenoids these organisms are found in about 60% of cases. The surface flora and the crypt flora, therefore, are by no means alike.

The fact that in the crypts of nearly 100% of tonsils these cocci are found would clearly suggest that probably every one would have them on the mucosa at times; but I know of no definite data on this point. In order to determine whether or not this is true the following experiments were made:

Throat cultures from groups of normal adult persons were obtained. Swabs were made by firmly pressing a cotton applicator against the pharyngeal mucosa and also over the surface of the palatine tonsils when present. They were then

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[1] Jour. Infect. Dis., 1919, 24, p. 386.
[2] Ibid., 1921, 29, p. 62.

immersed in melted blood agar and poured plates made, using proper dilutions. After 24 and 48 hours the plates were examined and suggestive colonies picked and submitted to confirmatory tests.

First the throats of a group of 15 normal persons (medical students) were examined as described; 5 or 33⅓%, gave positive results. Two weeks later a second examination was made of the same persons; 10, or 66⅔%, yielded positive cultures. Three of those yielding negative cultures were negative at the first examination of the group. Two weeks later a third examination of the same group was made and 9, or 60%, were positive. The three negatives mentioned were now positive. In the period of one month, therefore, at one time or another each of the group had hemolytic streptococci in his throat. Four gave positive results on all three examinations.

In a second group of 15 students, all different from the first group, similar tests were made but at shorter intervals. In the first test 7, or 46.6% gave positive results. After 5 days cultures were again taken from the 8 who gave negative cultures, and 3 of these were now positive and 5 still negative. One week later, cultures were again taken from the 5 who had yielded negative cultures, and 4 were positive. A culture was taken 2 weeks later; from the one remaining, who had given 3 successive negative cultures a positive culture resulted. As in the preceding series, all the persons therefore had hemolytic streptococci in their throats at some time during a relatively brief interval. Both of these series of examinations were made during Dec. and Jan., 1920-21.

Cultures were taken from a third group of 15 students in May, 1921. In the first examination 8, or 53.3%, of the 15 gave cultures of hemolytic streptococci. Ten days later 8 of the 15 gave positive cultures, but the persons were different from the first 8. Three of these who gave cultures were negative at the first examination. Eight days later these 3 negatives yielded a positive result in a third examination.

In summing up the results of these 45 examinations made 3 or more times at varying intervals, it will be seen that all at some time during an interval of about 1 month showed the presence of hemolytic streptococci in the throat. Probably they might all have been positive in a shorter time had more cultures been taken at shorter intervals. The percentages positive of the different groups varied from 33 to 66.

Nine of the 45 persons examined had had their tonsils removed months or years previously. In the first examination 3 of the 9 gave positive cultures of hemolytic streptococci; in the second examination 4 of the 9 yielded positive cultures. Of the 6 negatives in the first series, 3 were positive in the second examination. A third examination yielded 5 positives. Only one person gave negative cultures in the 3 tests. It was not possible to make further examinations on this person.

In these tests the number of streptococci in the plates were noted in relation to the other organisms. On the whole, they were few, and at times only one or two colonies appeared. Roughly, they comprised

from 1 to 10% of all the colonies that grew. One or two interesting exceptions appeared which were easily explained. One young man at work in the laboratory was feeling quite normal when the culture was taken. A cursory examination of his throat did not reveal an abnormal condition. The culture yielded an abundant almost pure growth of highly hemolytic streptococci. In 24 hours he was quite ill with fever, headache, malaise, and a red throat with fine white spots on his tonsils —a typical streptococcus sore throat. In another person the plate culture of the throat yielded between 60 and 70% of widely hemolytic streptococcus colonies. Inquiry revealed the fact that a few days before he had been ill with a cold and sore throat. The throat was fairly normal, but the tonsils were somewhat inflamed.

On the whole, the cultures from the tonsillectomized persons contained fewer hemolytic streptococci than those from persons with tonsils. There were exceptions to this, however; and in relation to incidence in this series there was little difference in the percentages in the two groups. According to previous observations of Pilot and Davis [1] and of others, the incidence of hemolytic streptococci in persons without tonsils is decidedly less than in those with them.

The streptococci isolated appeared to be the ordinary hemolytic variety of the human type. They were gram-positive, spherical or slightly oval cocci, some growing in short chains, others in moderately long ones. They were not encapsulated. On blood they caused a wide, clear and complete zone of hemolysis from 2 to 4 cm. wide. They grew practically not at all at room temperature, and best at 37 C. In plain broth they developed poorly but more profusely when dextrose or body fluids were added. They were no doubt the ordinary hemolytic Streptococcus pyogenes of the human type.

The streptococci obtained in ordinary throat swabs would appear to come from two sources. No doubt some arise from the relatively large numbers of these cocci that constantly inhabit the crypts and folds of the tonsillar and other tissues of the throat. The grooves about the teeth also not uncommonly harbor moderate numbers as Kordenat has shown. Then again a certain number appear to grow on or in the mucosa of the throat in the normal as well as in the pathologic state, as indicated by the positive cultures in the tonsillectomized persons. Presumably the latter are few, but it is difficult to

estimate exactly how numerous they may be because of the possible discharge of the same cocci from the grooves and pockets first mentioned.

SUMMARY

Cultures taken at short intervals sooner or later reveal the presence of hemolytic streptococci in the throats of practically all normal adult persons.

The cocci as revealed by throat swabs are not numerous; far less according to our experience than in the crypts of tonsils or adenoids.

THE TONSIL IN RELATION TO INFECTIOUS PROCESSES

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THE TONSIL IN RELATION TO INFECTIOUS PROCESSES *

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Some years ago when I became engaged in the study of focal infections, interest centered largely round joint and various rheumatic lesions, and the tonsil was receiving first consideration as the probable responsible focus and portal of entry. It early became clear that in order to justify many of the charges made against the tonsil, and to solve even some of the numerous problems arising in connection therewith, we were in great need of intensive studies on certain phases of the bacteriology and pathology of these organs. Even more, perhaps, than studies designed to relate infectious lesions in various localities to the tonsil as a focus, we needed fundamental studies on the bacteriology and pathology of these organs in normal persons. I wish here to present briefly certain studies along these lines.

DISTRIBUTION OF LYMPHOID TISSUE

An interesting point appears in connection with the distribution of lymphoid tissue in the throat and gastro-intestinal canal in relation to the bacterial flora. It is well known that lymphatic nodes are so distributed as to protect the body against the absorption of dangerous matter from certain well-recognized sources. Indeed, lymphoid tissue occurs, generally speaking, only in those localities where such absorption is occurring. So we have the clusters of glands at the hilum of the lungs, in the mesentery, in the axillary and inguinal regions, etc.

In the alimentary tract from the lips to the rectum there are two localities where striking accumulations of lymphoid tissue appear, namely, in the region of the throat, and in the lower small intestine, especially about

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the ileocecal valve and appendix. The intervening localities, like the stomach, duodenum, etc., have lymphoid tissue, but it is irregularly distributed and far less in quantity. *A priori,* this would indicate excessive absorption of dangerous matter in the localities where lymphoid tissue is abundant, and as a matter of fact this appears to be true; for in the throat and in the region just above and below the ileocecal valve we find normally the greatest number and variety of bacteria. This can readily be shown by making smear and culture preparations at intervals along the alimentary canal. If one should represent the amount of lymphoid tissue along the canal by one curve, and the number of bacteria normally present by another, the two curves would in general parallel each other. Beginning at the mouth, the curves would rise rapidly, attaining a maximum in the pharynx; they would then descend in the region of the esophagus and stomach, only to rise again in the small intestine, gradually approaching another maximum about the ileocecal valve, and then decending somewhat toward the rectum, where many of the bacteria die.

PROTECTIVE MECHANISM OF LYMPHOID TISSUE

In the throat, the palatine tonsils represent the greatest single accumulation of lymphoid tissue, while in the intestine, the agminated follicles of Peyer and the appendix represent the same. The significance of these accumulations appears to be that of a protective mechanism against various products of absorption, bacterial and other.

In these two localities, not only is the normal bacterial flora more highly developed, but here occurs the greatest number of infections: in the throat streptococcus, pneumococcus, meningococcus, staphylococcus infections, the viruses of numerous exanthems and other diseases; in the lower intestine, typhoid, paratyphoid, dysenteries, tuberculosis, appendicitis, etc. In the intervening localities, relatively few infections occur. The pathogenic organisms attack primarily the lymphoid structures or, at any rate, the parts rich in lymphoid tissue. It would appear that in many instances these organisms become adapted to grow in lymphoid tissue—in other words, to attack the very mechanism which the body has apparently designed for protection against bacteria. Striking examples of

this are the hemolytic streptococcus infections in the tonsil and typhoid infection of Peyer's patches. Lymphoid tissue thus may not be equally protective against all bacteria. In certain infections this mechanism breaks down entirely, and instead of being protective it furnishes a fertile soil for invasion. It is on account of the prevalence of certain infections in this tissue that it may be of advantage to remove this mechanism, or a part of it, as is done in tonsillectomy and in appendectomy.

SURFACE AREA OF THE TONSILS

Another point of importance in connection with tonsil infections is the surface area involved, since this is one factor in determining absorption of bacteria and their products. The epithelial surface of the tonsils is many times increased on account of the branching crypts penetrating deeply into the organ. We have attempted to measure the total surface, and find that roughly in an average tonsil of 2 by 1.8 by 1 cm., the entire epithelial surface would amount to about 25 sq. cm. This is only an approximation. Tonsils vary markedly in size, as also in the number and size of crypts; in hypertrophied tonsils, the surface would be far greater than this. Furthermore, the surface epithelium is loose and spongy, the round cells penetrating the layers even to the surface, giving rise to the well known epithelial structure of the crypts, interpreted and spoken of as a physiologic wound. While the extent and nature of the surface are important factors in any infectious process, it is not usual for all parts of the tonsils to be involved equally. When making smears and cultures from the individual crypts in a given tonsil, one is struck by the variation in the number and the kinds of bacteria from them. Some swabs may be sterile, others may contain many bacteria; in microscopic sections of diseased tonsils certain parts of the organ, or more often certain crypts, may show marked exudation and change, other parts or crypts revealing little or no significant alterations.

PLASMA CELLS

The distribution of plasma cells in the body is suggestive in connection with infections of lymphoid tissue. Generally speaking, these cells in the body are indicative of chronic inflammation or irritation, and

most writers regard them as pathologic cells, at least when found in appreciable numbers. They accumulate in masses about centers of chronic inflammation, and in general are characteristic of granulation tissue. They appear in many low-grade inflammations of the skin and mucous membranes.

The tonsils and crypts become infected at birth or within a few hours thereafter. Even pathogenic organisms very early appear, *Streptococcus pyogenes* having been noted as early as ten hours after birth. The flora of the infant mouth is largely streptococcal.

Using the local accumulation of plasma cells as a possible criterion of the absorption of bacteria or their products, I studied the time of appearance and the distribution of plasma cells in tonsils. About 240 pairs were examined for these cells. As a routine, the methyl green-pyronin stain of Pappenheim was employed. One hundred and eighty pairs had been extirpated from children and adults, and about sixty pairs came from necropsies on subjects of various ages ranging from fetuses to the very aged; seventeen were from infants less than 3 months old.[1]

The results briefly were as follows: These cells are not found in tonsils of the fetus or of the new-born. They make their appearance regularly about the second or third week, and are always found thereafter. In children several months old they are constantly found, usually in abundance. They are present throughout life and even to very old age (88 years) regardless of the anatomic condition of the tonsil. In pathologic tonsils, and especially in hypertrophy, they are very numerous. They occur under the epithelium of the crypts along the strands of connective tissue, and clustered about small blood vessels.

In view of the rôle that these cells play in general pathologic processes, and since they occur so regularly in tonsils a short time after the entrance of bacteria, one is led to suggest that their presence here indicates a chronic infection focus where absorption of irritating products is constantly occurring. Aschoff has noted the same facts in connection with the appendix. Along the entire gastro-intestinal canal, too, one observes large numbers of plasma cells under the mucosa and especially in the region of lymphoid follicles. These

1. Further details are given by the author in the Journal of Infectious Diseases **10**: 142, 1912.

facts are quite in harmony with the observations made by Adami and others on the more or less constant penetration of the mucosa by organisms and termed "subinfection." No doubt many bacteria are constantly passing through the alimentary wall into the lymphatics and blood stream, there to be disposed of in different ways. To these bacteria and their products after penetrating the epithelium, the plasma cells probably offer the first barrier or line of defense. In the sense, therefore, that the term subinfection has been used in connection with the condition of the so-called normal tonsil, or in the sense in which Aschoff uses the term "chronic inflammation in the appendix," we may regard all tonsils as chronically inflamed a short time after birth. One should, however, interpret rationally such findings in tonsils; and when the terms are used as above they should not necessarily convey the idea of a dangerous or serious pathologic state requiring surgical intervention. Nor should they be interpreted as a focus of infection in the sense in which that term is now commonly used.

BACTERIAL FLORA OF TONSILLAR CRYPTS

The statement is often made that the flora of the tonsils and the crypts is abundant and varied. This does not appear to be true. By no means will any or every germ that enters the tonsil live and develop there. Recently, in our laboratory, Miss Sexsmith tested the viability of a number of organisms in the crypts. After careful cultures of a crypt for control purposes had been made, a few drops of a live bacterial suspension were injected into it by means of a curved blunt needle. Daily cultures then were taken of the crypt. *Bacillus prodigiosus* after injection gradually became less numerous, and at the end of the fourth day had completely died out. Injection of *B. pyocyaneus,* a pathogenic chromogen, caused a slight reaction in the throat lasting a day or two. The organisms gradually diminished in number, and by the fifth day had disappeared. *B. coli* will likewise disappear in the course of a few days. It is evident from these data that certain bacteria, even those well adapted to grow in certain parts of the body, will not flourish in the tonsillar crypts. In other words, it is not proper, as has been done, to look on the tonsil crypts as a cluster of culture tubes set in the upper part of the alimentary canal, growing

numerous varieties of bacteria and discharging them into the lumen. As we shall see, the flora of the tonsils is a highly specialized one, restricted quite definitely to a few varieties.

From the anatomic structure of the tonsils, one might expect organisms requiring varying degrees of oxygen tension to thrive here. A few years ago I reported some work on the so-called actinomyces-like bodies often found in the crypts.[2] Since then, further work has confirmed the observations made at that time. These bodies are found in 30 per cent. or more of tonsils, and are composed of three kinds of organisms evidently growing together in symbiosis: fusiform bacilli, spirochetes and streptococci. The fusiform bacilli grow under anaerobic conditions, and in the crypts develop into a cluster of filaments, forming a central stalk about which the fusiform rods are arranged perpendicularly, closely resembling the structure of a test tube brush. Scattered throughout this growth are very large numbers of spirochetes, actively motile. The streptococcus forms in these masses have recently been studied in detail by Pilot and myself. There are hemolytic and nonhemolytic varieties. The hemolytic are aerobic and quite like the varieties that occur commonly in the throat. Many of the nonhemolytic streptococci from these granules are distinctly anaerobic when first cultivated. They exhibit a green halo on blood agar plates, and if the initial cultures are not made anaerobically they will not appear. After a few transplants under aerobic conditions, however, they will adapt themselves to grow equally well in the presence of oxygen. This anaerobic property of the green strains is very definite and is readily discernible in the first series of cultures. According to Holman's classification they belong to *Streptococcus mitis* and *Streptococcus salivarius* varieties. They are not highly virulent for rabbits, being comparable in this respect to the ordinary *Streptococcus viridans* of the buccal mucosa.

Fusiform bacilli.—B. fusiformis occurs in the crypts either in the granules as above described, singly or in small, loose, irregular clusters. In either form they are probably found in all tonsils at some time or other. They appear quite like the bacilli occurring about the

2. Davis, D. J.: J. Infect. Dis. **14**: 144, 1914.

teeth. I am inclined to the view that the crypts are the normal and usual habitat of these bacilli. From here they readily infect the mouth, especially the teeth, when these are not properly cleansed or are decayed or pyorrheic.

The possible relation of these bacilli to the infections included under the term Vincent's angina is interesting. A preparation from a Vincent lesion is indistinguishable from one made from a tonsil granule. On the tonsil, Vincent's angina often begins about the mouth of the crypts, and may involve the sides. The question arises whether or not the fusiform bacilli of the tonsil crypts are a common source of the organisms in Vincent's angina, the crypts serving as the primary breeding grounds for them. These bacilli, together with streptococci, are a common cause of brain abscess resulting from bronchiectatic cavities in lungs, where these bacilli find favorable conditions for development. Presumably they pass down to the bronchi from the tonsils or teeth. Brain abscesses have followed tonsillectomy.

Streptococci.—Some years ago when studying the bacteriology of extirpated tonsils from certain cases of chronic infection, I noted a striking difference between the surface flora and the crypt flora of tonsils.[3] On the surface the predominant organisms were of the *Streptococcus viridans* type, whereas the predominant organisms in the crypts of the same tonsil were as a rule hemolytic streptococci. The exceptions were few. The difference was so striking that at first I attributed great significance to this point, since the hemolytic varieties are so much more virulent as a rule than the green varieties. Later I found that most tonsils, regardless of the associated condition, contained a similar flora. Hypertrophied tonsils especially, but also others that show no noteworthy pathologic condition, reveal the same distribution of the varieties of streptococci on the surface and in the crypts. Pilot in our laboratory recently also examined 100 tonsils, extirpated chiefly for hypertrophy, though many were normal in size. Hemolytic streptococci were found on the surface in 61 per cent.; they comprised usually less than 10 per cent. of the total number of bacteria. In

3. Davis, D. J.: The Pathology and Bacteriology of the Faucial Tonsils, etc., J. Infect. Dis. **10**: 148, 1912.

the same throats from which these tonsils were removed, cultures taken just before extirpation yielded 43 per cent. positives. Crypt cultures yielded 97 per cent. positives, and in almost all the hemolytic variety was greatly predominant. Furthermore, in another series of twenty-four normal persons, cultures from the throat and pharynx yielded hemolytic streptococci in 58 per cent.; in nineteen persons without tonsils, cultures similarly made gave positive results in 15 per cent., and in these persons were found either bad teeth or tonsil remnants.

It appears from these results that the crypts are an almost constant source of hemolytic streptococci, and this location may be considered in a way their normal habitat. We have not been able to find that any other part of the body so constantly harbors them. The throat, as we have known for a long time, is their chief source and habitat in the body, and it would now appear that the crypts of the tonsils usually supply the throat with these organisms. From the throat they may be distributed to various parts of the body by contact and otherwise. Or they may be transferred to other persons through the usual channels by which respiratory diseases are transmitted.

In the isolation of streptocicci from crypts of tonsils, certain technical points should be kept in mind. In extirpated tonsils the organ should be cut lengthwise with a sterile, sharp knife; and then with a sterile forceps the crypts may be opened, more incisions being made if necessary, and cultures taken with a platinum loop from the depths of several crypts. It is necessary to do this for the reason that one crypt may be practically sterile and an adjacent one may contain many streptococci. This is not the rule, however; hemolytic streptococci when present at all are usually found in all or nearly all the crypts. Cultures of both tonsils should be made, since the organisms may be found on one side and not on the other. In tonsils from fresh postmortems the technic is essentially the same, and one finds the organisms commonly in such material.

In making cultures from tonsils in situ, certain difficulties are encountered. A small elongated loop of heavy wire should be inserted deep into several of the crypts, turned from side to side when in position, then carefully removed without touching other structures,

and at once plated on blood agar. The green streptococcal colonies are usually present with the hemolyzers under these conditions, often in large numbers, since they are carried away on the wire from about the mouths of the crypts, where they are found abundantly. Excepting in acute infections of the tonsils, the cheesy or purulentlike material that exudes from the tonsillar crypts on pressure is no more, indeed is less, apt to contain hemolytic streptococci than the empty crypts. Much of the fluid material expressed from tonsils, enlarged or otherwise and commonly called pus, is not purulent when examined microscopically.

In conclusion, this point deserves emphasis: Nearly every one is harboring typical hemolytic streptococci in his tonsils which have not been differentiated from strains that cause serious infections, pneumonias, etc. Presumably such infections may or may not cause arthritis, iritis and other so-called focal infections; but finding them in the tonsil may mean nothing in relation to a possible systemic disease. Should one find abscesses or other definite pathologic lesions in the tonsils, a bacteriologic examination may be of value in determining the cause of an associated condition.

SUMMARY

In order to understand clearly the genesis of certain diseases, it is necessary to study intensively a suspected focus of infection, like the tonsil, in both normal and infected persons.

Lymphoid structures attain two maxima of distribution: one in the throat and another in the region of the ileocecal valve and appendix; these maxima correspond in general to the normal distribution of bacteria in the alimentary canal. At these points also the greatest number of pathogenic micro-organisms attack the body.

Plasma cells appear shortly after birth (therefore after infection) under the mucosa, and their presence probably indicates chronic absorption of infectious and other material.

Certain organisms injected into the crypts of the tonsils disappear in a few days. The flora normally found in the tonsils is a restricted one.

Actinomyces-like granules, composed of fusiform bacilli, streptococci and spirochetes growing together, appear as more or less normal inhabitants of the crypts.

Here may be an important source of *B. fusiformis* in certain infections about the mouth caused by this organism.

In the tonsil crypts, *Streptococcus hemolyticus* is almost constantly found. This focus is one source of these organisms in the throat and adjacent structures. This fact must be considered in making throat cultures and in a study of the problem of hemolytic streptococcus carriers.

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Certain Epidemic Micro-Organisms of the Respiratory Tract

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CERTAIN EPIDEMIC MICRO-ORGANISMS OF THE RESPIRATORY TRACT *

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In view of the fact that in respiratory epidemics the problem often arises as to whether or not many of our common organisms play a primary or a secondary rôle, this brief statement concerning certain epidemic strains of bacteria is made.

In the normal throat are several varieties of bacteria prone to become dangerous under certain conditions by either causing or complicating epidemic infections. These bacteria have been called opportunists. The most important are streptococci, pneumococci, Pfeiffer's bacilli (hemophils), staphylococci, meningococci, diphtheria and diphtheroids. Possibly the viruses, natural or modified, of measles, scarlet fever, anterior poliomyelitis and lethargic encephalitis might also be here included, but our knowledge of them in the normal respiratory passages is too meager and uncertain for discussion here.

In the streptococcus group are certain strains constantly found in the tonsils or throat, and ordinarily doing no harm there. At times, however, they take advantage of conditions in the host and invade the tissues, causing local or general infection (secondary infection). Ordinarily such infections are not contagious, even though they are highly virulent and fatal to the individual. The altered conditions making infection possible appear to be chiefly and primarily in the host.

At other times, streptococci may behave in another way. Not only do they occasionally become virulent for individuals, but the infection becomes highly con-

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tagious or epidemic. Whether the contagiousness is always necessarily dependent on the virulence is open to doubt. It may depend on some other property, such as the ability of the organism to implant itself on the respiratory mucosa, or on the manner of implantation. It has been shown that bacteria vary in their ability to gain a foothold on mucous membranes.

Examples of the appearance of such epidemic strains of hemolytic streptococci are given here. Many epidemics of sore throat caused by streptococci distributed through milk are now on record. These epidemics are very definite, and the cases are confined largely to those drinking milk. They spread little by contact or in ways other than through the contaminated milk. Therefore, the epidemics are local and restricted to very definite regions, and promptly subside when the contaminated milk is withdrawn. They are all caused by hemolyzing streptococci. The evidence in many epidemics indicates that the streptococci come from the udders of cows infected with strains virulent for the human being. In some of the epidemics the cocci may have entered the milk from a human carrier.

Now, studies made on these streptococci show that they have certain properties that differentiate them from other strains of hemolytic streptococci. They tend to arrange themselves in pairs even in the chain, are somewhat lanceolate, are encapsulated, grow moist and slimy on enriched mediums, form a clear and complete though narrow zone of hemolysis on blood agar, and may cause some green discoloration of the mediums. They are insoluble in bile and do not ferment inulin. For animals they are more virulent, on the whole, than most streptococci, but the lesions in general are the same. All the foregoing properties may vary somewhat, and when the strains are grown on artificial mediums for a time the properties become more like ordinary streptococci. Moreover, specific serum tests made by a number of observers on these strains show that from a given outbreak the organisms have common agglutinating properties by which they may be distinguished from other hemolytic streptococci. Here, then, are definite virulent and epidemic strains of streptococci distinguishable from other related strains and responsible for primary infections in normal persons. The term *Streptococcus epidemicus* has been applied to them by some workers.

Closely related to these milk epidemics are the streptococcus epidemics that appeared in military camps secondary to measles in the contagious wards of the camp hospitals. Here the streptococci appeared first as virulent secondary invaders in measles and quickly spread, involving a large percentage of those sick. Apparently so virulent and aggressive did these cocci ultimately become that they were able to attack normal persons, causing pneumonia, empyema, etc. Keegan [1] described an epidemic of streptococcus throat infections beginning in the throat ward at the Chelsea Hospital and spreading through several surgical wards, causing wound infections generally in the patients attacked. These streptococci had properties similar to the milk epidemic strains described above.

Streptococcus sore throat outbreaks, especially in the winter months, are fairly common, often attacking several members of a family or making rapid headway in an institution. Such outbreaks assume more or less epidemic proportions, and many become quite general in a community or a considerable section of country. They are not as clear cut as are the milk epidemics, and the cases are more scattered. Their epidemiology is not well known, but apparently the infection is spread by contact, being influenced, probably, by atmospheric or other undetermined factors. While it appears that streptococci are the primary cause of at least some of these outbreaks, it is probably true that in many of them this organism merely plays a secondary rôle, the infection being due to some unknown virus. More knowledge is greatly needed on the etiology of these common throat infections, and little or no study has been made with reference to special characteristics of individual or epidemic strains.

In pneumococcus infections, to some extent, at least, the same general rules seem to apply. Many of them are caused by a variety of strains which occur commonly in the mouth (Group IV). But at times certain strains (Type I, Type II, etc.) appear to acquire epidemic properties, and then the pneumococcus outbreak occurs on a more or less extensive scale. Other factors no doubt enter here to complicate the epidemiology. In epidemics of pneumonia, several strains or types

1. Keegan, J. J.: A Hospital Epidemic of Streptococcic Sore Throat with Surgical Complications, J. A. M. A. 72: 1434 (May 17) 1919.

of the pneumococcus are encountered even in the earlier stages of the outbreaks. We are led, therefore, to the assumption that environment and conditions surrounding the host are the important determining factors in the spread of the epidemic. Either this must be true or we must postulate that rapid changes occur in a given epidemic strain, resulting in the development of the several different types found in the epidemics. Possibly both assumptions are true in varying degrees at different times and under different conditions. Usually pneumococcus epidemics are less clearly defined than streptococcus milk epidemics, probably because of the difficulty pneumococci have in getting into lung tissue, their seat of predilection, as compared with infections of the throat by streptococci.

In this connection, studies of the epidemic of influenza are suggestive. Both in the 1889-1890 epidemic and in the 1918 epidemic, nearly every one who worked with the disease described the diplostreptococcus. There is little doubt that all were dealing with the same organism. This is what has been called the Mather's coccus and has been especially emphasized by Rosenow, Tunnicliff and many others. It was commonly found in the blood, sputum, lung exudates, pleural-cavities, etc. According to most workers, this organism forms large, moist, watery, green colonies, is encapsulated, and even approaches the *Pneumococcus mucosus* type; they are very virulent for certain animals. Some of their properties, however, are variable; for example, inulin fermentation, bile solubility and specific agglutination. Though found widely both here and in Europe, still it would seem that in certain localities and especially where the epidemic was most fatal, these cocci appeared more commonly and seem to be responsible for many deaths. It may therefore be possible that these diplococci at certain times and places take on highly virulent properties in a way quite comparable to the strains of hemolyzing streptococci mentioned above. The fact that hemolytic streptococci so commonly complicate measles and scarlet fever and the diplostreptococci influenza, probably depends on the part of the respiratory tract primarily attacked—the throat by the measles and scarlet fever virus, the tracheobronchial apparatus by the influenza virus.

It would appear, then, that this coccus, which at present we might hesitate to call either a streptococcus or a pneumococcus, is an organism prone to cause serious infections in the respiratory tract. There is no clear evidence that it is ever a primary invader, but as with hemolytic streptococci, at times it may acquire aggressive properties enabling it to implant itself on a normal respiratory tract by contact or in some other way. As noted above, it has certain properties which, according to some workers, give to it quite definite distinguishing characteristics.

Hemophils (Pfeiffer's bacilli) have a distribution quite like the streptococci. They are widely found in normal and diseased throats, and at times are very virulent for man. The evidence indicates that they rarely, if ever, acquire epidemic properties. Perhaps the nearest approach to this is the occurrence of outbreaks of so-called influenza meningitis. In 1910, in Chicago, in a little more than a year, I encountered seven cases; since then, apparently, they have been rare, one case a year or thereabouts being reported. In New York, too, about that time, these cases were common, but have been far rarer since. Cohen [2] encountered it in Paris about the same time (1909) and called attention to the high virulence of the strain for rabbits. He noted a specific agglutination for these strains by immune rabbit serum, and therefore considered them in a group by themselves and distinct from the Pfeiffer organism. These outbreaks all occurred when ordinary influenza was not prevalent.

Observations by many observers indicate that while the bacilli of this group (hemophils) have many characteristics in common, still in their agglutination and other reactions they may vary decidedly. In my own work, several years ago,[3] I found that immune rabbit serum agglutinated the homologous strains to 1:800 or 1:1,000, whereas all other strains from the same disease or from other disease agglutinated 1:200 or thereabouts. At that time I was inclined to interpret the results on the basis that the homologous strain was agglutinated in higher dilutions than the heterologous strain, a phenomenon known in other well defined

2. Cohen: Ann. de l'Inst. Pasteur **23**: 273, 1909.
3. Davis, D. J.: Bacteriology of the Respiratory Tract, J. A. M. A. **48**: 1563 (May 11) 1907.

varieties of bacteria. However, the interpretation may be made that the individual strains vary and comprise a heterogeneous group, which is quite in accord with the results of Park and Williams [4] and of other observers working with strains of Pfeiffer's bacilli from the recent influenza epidemics. Small and Dickson [5] found that ten strains fell into four groups, and Fleming, Huntoon, Jordan and others, on the basis of specific and cultural reactions, have obtained evidence that the group is a heterogeneous one. I have elsewhere [6] discussed this question, calling especial attention to the several varieties of hemoglobinophilic bacteria. In all probability, as this group is further studied, it will be further differentiated in accordance with general principles of bacterial variation.

An analysis of the evidence thus far available from studies of the recent influenza epidemic does not indicate that a single epidemic strain of *B. influenzae* appeared in the influenza patients. This fact has been interpreted by Park and others to mean that this organism was not the etiologic agent, for the reason that it is difficult to think of an explosive epidemic like influenza as having been caused in a given locality by more than one strain of Pfeiffer's bacillus.

Staphylococci rarely, if ever, cause epidemics of any magnitude. Small localized outbreaks of furuncles, impetigo contagiosa, etc., may be mentioned, but there appears no clear evidence that staphylococci ever cause respiratory epidemics. As secondary invaders they may play a rôle, but in this regard they appear to be of decidedly less importance than the streptococci or pneumococci. Little or nothing has been done in identifying strains having individual or epidemic properties.

The meningococcus is found commonly in the normal throat, and is prone to cause epidemics either localized or widespread. The epidemics are quite definite and sharp, though not a large percentage of persons exposed are attacked. Organisms found in such outbreaks usually belong to two or more groups so far

4. Park, W. H.: Bacteriology of Recent Pandemic of Influenza and Complicating Infections, J. A. M. A. **73**: 318 (Aug. 2) 1919.
5. Small, J. C., and Dickson, G. K.: Grouping of Bacillus Influenzae by Specific Agglutination, J. Infect. Dis. **26**: 230 (March) 1920.
6. Davis, D. J.: Infections with Hemoglobinophilic Bacteria, J. A. M. A. **64**: 1814 (May 29) 1915.

as their agglutinating properties are concerned. It is generally conceded that meningitis is a disease dependent in large measure on factors other than the mere presence of the organism in the respiratory tract. It would appear that the various strains or groups are normally fairly widely distributed, and under suitable conditions the several groups are enabled to invade their hosts and cause the disease. Another possibility is that one given strain may readily and rapidly alter its characteristics in passing from person to person, so that even in a given outbreak members of different groups may appear.

Diphtheria bacilli are present in throats of a certain percentage of normal persons. They cause sporadic cases and under certain conditions and especially during certain seasons may give rise to serious outbreaks or epidemics. Recent work [7] tends to show that there are at least two groups of diphtheria bacilli differing in their agglutinating properties. They differ also, but to a less degree, in the character of the toxin produced, a point of importance, if verified, in the production of the specific serum. The relation of the groups to each other is not known; and whether or not outbreaks of diphtheria may be due to a specific strain or to several strains belonging to different groups still remains to be worked out.

Other organisms found at times in the respiratory tract are *B. fusiformis,* which may be responsible for very definite outbreaks; but little or nothing is known concerning the characteristics of the epidemic strains. The terrible outbreaks of plague pneumonia are clear-cut epidemics spread by direct contact and, so far as available data indicate, are due to a strain of *B. pestis* of high virulence. Various strains of plague bacilli even from remote regions show little or no difference in their agglutination reactions. They may vary markedly, however, in their virulence for animals.

From an analysis of epidemics and outbreaks caused by bacteria, such as I have considered above, it appears that there is found no example comparable in extent and in epidemiology to the influenza epidemic. This might be interpreted as an argument against the view

7. Havens, L. C.: J. Infect. Dis. **26**: 388 (May) 1920.

that this epidemic was caused by strains of any of the ordinary bacteria of the respiratory tract.

A number of points arise here for discussion, one being the value of specific serum tests for identification; in particular, agglutination tests in revealing relationships of strains of bacteria. Considerable data [8] now exist pointing to the ease with which agglutination and other properties may be made to vary under experimental conditions. It is quite reasonable to assume that under the more complex conditions of natural infection, specific properties of bacteria vary even more rapidly than under experimental conditions. Differences in the agglutination titer as manifested by different strains may mean simply that the organisms have been perhaps transferred a number of times or grown in different mediums. The assumption would appear reasonable that in a sharp, sudden epidemic, differences between individual strains of organisms responsible for the epidemic would be slight or nil; whereas, in more diffuse and less explosive outbreaks, wider differences in the organisms, especially in their finer and more delicate properties, might be expected to appear. Greater differences would be anticipated in organisms obtained from widely separated localities. This may be a partial explanation of the higher incidence of typhoid fever in our soldiers when in France than when in this country.

The relation between capsule development and virulence is a question of interest here also. No doubt a strict parallelism between these characters does not exist, for many bacteria have an abundant capsular substance and are entirely without virulence. However, there is evidence that capsular material may vary in composition; the profuse mucinous envelop about some saprophytes my be a very different substance and have a different function from the capsule seen about certain pathogens like the pneumococcus. At any rate, in the streptopneumococcus group, and apparently in other groups, the more virulent and aggressive the organism the more apparent is the capsule; and, vice versa, after cultivation on artificial mediums the capsule tends to disappear. This was especially

8. Dawson, A. I.: J. Bacteriol. **4**: 133 (March) 1919. Rosenow: J. Infect. Dis. **14**: 1, 1914. Jordan: Ibid. **26**: 427 (May) 1920.

noticeable with the milk epidemic strains of hemolytic streptococci.

In concluding this brief statement of a subject which deserves a far more detailed analysis, I will emphasize the fact that just as there are opportunists about the body ready to invade the individual, so there are opportunists at times able not only to invade the individual but also to originate an epidemic. We have, then, individual opportunists and epidemic opportunists, the latter differing from the former not so much in virulence, perhaps, as in their aggressive power and ability to gain a foothold under adverse conditions on the mucous membranes or in the tissues of the body. Some of the distinguishing characteristics of the latter are a moist, slimy, watery growth, formation of a capsule or a capsule-like substance, and specific agglutination reactions.

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DUCREY BACILLUS INFECTION (CHANCROID) OF THE FACE

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DUCREY BACILLUS INFECTION (CHANCROID) OF THE FACE

D. J. DAVIS AND H. W. POTTS

A negro 23 years of age came to the Cook County Hospital complaining chiefly of a painful swelling beneath his chin which began five days ago. It was marble sized when he first noticed it, gradually growing larger and more painful. It was because of this painful lump under his chin that he came to the hospital for treatment.

In addition to the above, he gave the following history. Two years ago he had a chancre and had had several attacks of gonorrhea at various times. Two weeks ago he developed a sore on his genitals and stated that after handling this lesion he remembered having scratched a small pimple on his chin.

When he entered the hospital genital examination revealed a typical chancroid of the penis of two weeks' standing and a definite inguinal lymphadenitis.

Upon his chin to the left of the median line was a small raised lesion which when the crust was removed revealed a ragged punched out ulcer ½ cm. in diameter whose floor was covered with a dirty grey exudate. He said it had existed for four or five days and was growing larger. Its base was slightly infiltrated and not very painful when compressed.

In the submental region, well forward, was a very painful, firm, movable tumor whose general contour was round and was about 5 to 6 cm. in diameter.

The scalp, ears, nose, eyes, tongue and throat were negative. His mouth was unclean and the lower left bicuspids were pulpless and decayed.

During the ensuing five days the lesion on the chin enlarged to 1 cm. in diameter and had ragged edges with some exudation which tended to form abundant crusts. The submental tumor increased in size and became soft and very tender.

Blood examination gave 5,280,000 reds, 11,000 leucocytes and 95 per cent. hemoglobin. The highest temperature recorded was 100 F.

With reference to the submental tumor, at first it was thought that a lymph adenitis might have developed from the decayed bicuspids as foci. This would have been unusual, however, since the lymphatics which drain the bicus-

pid region course farther back beneath the angle of the jaw. The presence of a genital chancroid, a lesion on the chin quite like the genital one, and the painful and progressive regional lympadenitis therefore seemed to warrant a tentative diagnosis of chancroid of the chin with submental bubo.

Crystals of argyrol were applied to the lesion on the chin, which promptly healed. The submental bubo softened and in a few days developed into a large abscess which was incised and the cavity packed with iodoform gauze. Recovery was uneventful.

The content of the abscess was examined bacteriologically. It contained many pus cells, necrotic tissue and much blood. In stained smear preparations no bacteria were found even on prolonged search.

Suspecting the presence of B. ducreyi, the material was cultured in blood. For this purpose six small tubes containing about 2 c.c. of fresh human defibrinated blood were prepared and each inoculated with diminishing amounts of the material. Heavy blood agar tubes also were inoculated. After incubation the tubes were examined and in three of the six, growths of B. ducreyi appeared. A few staphylococci albus grew in some of the tubes but not in all. It was impossible to state whether or not they were contaminators or secondary invaders; probably the former, since they were not found in all the tubes.

The bacilli after twenty-four to forty-eight hours' growth appeared in typical tangled clusters of streptobacilli. Many also appeared in short and long chains. On superficial examination morphologically they resembled growths of streptococcus viridans. They are short oval bacilli, stained with difficulty by methylene blue and were gram negative. On solid media they form small grey, opaque colonies ½ mm. across, which appear in twenty-four hours and grow to maturity in two days. On plain media they gave no growth.

That the Ducrey bacillus is the specific cause of chancroid there can be no doubt. Ducrey in 1889 made fifteen successive human inoculations with material from the lesion, finally obtaining a chancroid in which practically only the streptobacilli were seen. He did not then know how to grow it. This was done by Lenglet in 1898, who cultivated it on human skin agar covered with blood. Heavy blood media or pure blood, defibrinated or not, have now come into general use in growing it.

At least three workers, Fischer, Lipschütz and Tomasczewski have inoculated themselves with pure cul-

tures and caused typical chancroids. Monkeys are susceptible; also the cornea of the rabbit.

Extragenital chancroids are not uncommon on the lower part of the body, but rare about the head and face. In Kolle-Wassermann's *Handbuch,* Stein quotes Petersen as having observed twenty-seven extragenital lesions in a series of 9,000 chancroids. Ullmann collected from the literature and his own experience sixty-four extragenital chancroids. Of these, forty-nine had both genital and extragenital lesions (as in the case here reported); twenty-two were on the fingers. Of chancroids in other localities, one has been reported on the gum of the upper jaw, two on the conjunctiva, one on the tonsil, one on the tongue, one on the soft palate and wall of the pharynx and one in the external auditory canal. None on the chin or face was found reported.

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BACTERIOLOGIC STUDIES OF THE UPPER RESPIRATORY PASSAGES

I. HEMOLYTIC STREPTOCOCCI OF THE ADENOIDS

I. PILOT AND S. J. PEARLMAN

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Numerous bacteriologic investigations of the throat and nasopharynx have been carried out to determine the incidence of dangerous bacteria in the normal as well as in diseased conditions. Most of the previous observations were made from swab cultures of the throat and pharynx, and as it is impossible for this method to reach the recesses of the tonsillar crypts and folds of the adenoids, it is obvious that the results obtained are inaccurate and not a true index of the flora of these regions. In a previous work by Pilot and Davis [1] it was noted that the crypts of 100 pairs of extirpated tonsils contained hemolytic streptococci often in large numbers in 97%, whereas from the swab cultures of the same persons before tonsillectomy the same organisms were recovered in fewer numbers in only 61%. In order to ascertain the extent to which the other lymphoid structures harbor the streptococci a study of the flora of the extirpated adenoids of a similar group of persons was undertaken, together with a smaller series of extirpated tonsils for comparison.

In the present work cultures were made from the adenoids of 103 children. In 25 instances swabs of the nasopharynx were obtained by means of the West tube before adenoidectomy and in the same group both the extirpated tonsils and adenoids were studied. In the remaining 78 cultures were made from the adenoids. The patients were children varying from 5 to 16 years of age who presented adenoids and tonsils of varying degrees of hyperplasia with no evidence of any recent acute inflammation, fever or subjective symptoms of sore throat. The adenoids in most instances were removed by the La Force adenectome and the tonsils by the Beck tonsillectome under general anesthesia at the Cook County Hospital during the months of April, May and June, 1920.

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[1] Jour. Infect. Dis., 1919, 24, p. 386.

The adenoids consisted of hyperplastic lymphoid tissue from 1 to 1.5 cm. square and about 1 cm. thick. Structurally many presented from 3 to 6 deep folds; in others fibrous union had taken place between the folds leaving pits not unlike the tonsillar crypts but not quite as deep. Most of the specimens had both folds and crypt-like depressions. Fatty débris and cholesterol crystals were encountered in a few instances, but in none was a purulent exudate seen. The tonsils of the same persons, like the adenoids, were of lymphoid tissue of varying degrees of hyperplasia. Occasionally the crypts contained fatty débris and hard yellow actinomyces-like granules. Such granules were not grossly visible in the adenoids.

THE PERCENTAGE OF HEMOLYTIC STREPTOCOCCI IN CULTURES OF THE NASOPHARYNGEAL SWABS AND ADENOIDS OF THE SAME PERSONS

	Number of Persons	Percentage Positive
Series i.		
Swab cultures	25	40
Cultures from depths of adenoids	25	60
Series ii.		
Cultures from the surface of the adenoids	78	58
Cultures from the depths of the adenoids	78	62
Total number of swab and adenoid surfaces from which cultures were taken	103	55
Total number of cultures from the adenoid depths	103	61

All the material was collected separately in sterile gauze and cultures made in 1 to 4 hours after removal. Material for culture was obtained by streaking the epithelial surface of the vegetations with a wire loop and then carefully separating the folds and mouths of the pits with sterile forceps another culture was obtained from the depths. The tonsils were inverted with the capsule outside and incised transversely at right angles to the crypts with a sterile knife and cultures made from the bottom of the crypts. The wire loops were streaked on the surface of blood-agar plates made up of infusion agar titrated to a hydrogen-ion concentration of 7.6 to which human blood was added in proportion of one part of blood to 10 to 15 parts of agar. In addition the same medium in the melted state of 45 C. was inoculated from the same sources and poured into plates. The plates were incubated at 37.5 C. and examined at the end of 24 and 48 hours for hemolytic colonies.

Small, discrete, biconvex, gray-white colonies forming zones of complete hemolysis measuring from 1 to 4 mm. across were noted and isolated in pure culture on blood-agar slants for further study and confirmation.

In the cultures of the nasopharygeal swabs hemolytic streptococci were present in 10 of 25 instances, or in 40% of the cases. From the same persons the streptococci were present in the depths of the adenoid vegetations in 15, or 60%, demonstrating the inaccuracy of the swab culture. In the remaining 78 cultures made from the surface of the adenoids they occurred in 45, or 58%, as compared with 48, or 62%, positive in the adenoids depths. In both the swab and surface cultures they were relatively few in number, seldom predominant and numerous in only 9 instances. In the depths, however, they were present in decidedly larger numbers, being quite numerous in 18 instances and in pure culture in 3. The foregoing figures were obtained by examination of the poured plates. It is interesting to note that from the streaked blood-agar plates the hemolytic streptococci were observed in only 26% of the cultures of the adenoid surface and in 37% of those of the depths, demonstrating definitely the superiority of the poured plate in the detection of these organisms.

Of the 21 pairs 20, or 95%, revealed hemolytic streptococci in either both or one tonsil. In the adenoids of the same persons these organisms occurred in 15, giving 5 instances in which streptococci were recovered from the tonsils and not from the adenoids. Furthermore, the tonsillar crypts harbor these organisms in greater numbers as compared witih the adenoids. The crypt-like structures of the nasopharyngeal vegetations, like the crypts of the tonsils, frequently contain these streptococci in strikingly large numbers, showing the strong tendency of the hemolytic streptococcus to flourish in the deep depressions of the lymphoid tissue of the oro- and naso-pharynx.

The streptococci corresponded to the beta type of Smith and Brown. The narrow, indefinitely hemolytic colonies of the alpha type were present in 20% and were included in the nonhemolytic streptococcus group.

Sixty-five strains of hemolytic streptococci, of which 60 were isolated from the adenoids, were studied in pure culture. All were bile insoluble and hemolytic in the subcultures. In infusion carbohydrate broth they formed a flocculent sediment, while the supernatant fluid usually remained clear. Smears revealed gram-positive cocci in moderately long or often very long chains. Four differential sugars were inoculated—lactose, salicin, mannite and inulin. The medium consisted of infusion broth with 1% carbohydrate and Andrade indicator. Readings were made at the end of 4 and 10 days. All fermented lactose,

all but 3 salicin; 3 fermented mannite, and 2 inulin. Inulin from another source was not fermented by the 2 strains. According to Holman's classification, 59 were streptococcus pyogenes, 2 strep. infrequens, 2 strep. anginosus, and 1 strep. hemolyticus 3. Litmus milk was acidified by all in 7 days, 10 coagulating spontaneously and the remainder on gentle heating.

Eight strains were selected at random and injected intravenously into rabbits. These strains were isolated from the adenoids or the nasopharynx. In 2 instances 2 c c of a serum-broth culture incubated 48 hours were employed and one rabbit died in 24 hours with evidences of a septicemia and beginning arthritis. The second rabbit died in 72 hours and at necropsy had a moderately purulent polyarthritis and vegetations on the aortic valves. Five other rabbits, weighing from 800 to 2,000 gm., were inoculated with a blood-agar slant (incubated 48 hours) suspended in salt solution. One died in 48 hours and revealed petechial hemorrhages on the serous surfaces and many streptococci in the heart blood. Another succumbed on the 13th day from a marked purulent polyarthritis and periarthritis, and periostitis of two adjacent ribs. The remaining 3, killed on the 11th day, showed polyarthritis of varying severity. One rabbit which received one-half of blood-agar slant also developed purulent arthritis. The joints involved were chiefly those of the wrists, ankles, knees and phalanges. Five other strains were introduced into white mice in doses of 0.5 c c of a 24-hour serum-broth culture intraperitoneally. Four died within 18 hours and the fifth in 36 hours, and from all the streptococci were recovered from the peritoneal exudate and the heart blood.

SUMMARY

Hemolytic streptococci are common in the nasopharynx and nasopharyngeal vegetations. From nasopharyngeal swabs and the surface of the adenoids hemolytic streptococci were recovered in 55%; from the depths between the folds and of the crypt-like depressions of the adenoids of the same persons, in 61% in larger numbers. The excised tonsils of the same patients revealed hemolytic streptococci in still larger numbers in 95%.

These streptococci agree in their morphology, cultural characteristics, fermentation reactions and pathogenicity, and are practically identical with hemolytic streptococci from various human sources.

The adenoids, like the tonsils, are to be considered as common foci harboring hemolytic streptococci.

BACTERIOLOGIC STUDIES OF THE UPPER RESPIRATORY PASSAGES

II. THE PNEUMOCOCCI AND NONHEMOLYTIC STREPTOCOCCI OF THE ADENOIDS AND TONSILS

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In the study of the incidence of the pneumococcus in the mouths of healthy persons it has been found that this organism is common in the saliva and throat. Park and Williams [1] identified typical pneumococci in about 50%; Longcope and Fox [2] and Buerger [3] gave similar percentages. Dochez and Avery [4] found pneumococci in 58.4% and Stillman [5] in about 45%, the great majority of which were types 4 and 3. The investigations were made on the saliva and less often on swabs of the throat and pharynx of normal people. In a study of the hemolytic streptococci of the adenoids and tonsils it was noted that streptococci were present in larger numbers in the crypts and folds than in the swab cultures of the same structures before their removal. To determine more accurately the true incidence of the pneumococci in the oro- and naso-pharynx cultures were made of the extirpated adenoids and tonsils.

The same material from which the hemolytic streptococci were isolated was cultivated on blood-agar plates, both streaked and poured, and studied for the pneumococci and nonhemolytic streptococci Nasopharyneal swabs, the excised adenoids and tonsils from 21 persons were first cultivated; the extirpated adenoids of 82 persons were also investigated as regards their flora, on the surface as well as the depths, between the vegetations. The patients were children from 5 to 16 years of age, who presented tonsils and adenoids of varying degrees of hypertrophy with no other marked gross changes.

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[1] Jour. Exper. Med., 1905, 7, p. 403.
[2] Ibid., p. 430.
[3] Ibid., p. 497.
[4] Ibid., 1915, 22, p. 105.
[5] Ibid., 1916, 24, p. 651.

This material approaches the normal as nearly as possible. The specimens were obtained during the months of April, May and June, 1920.

Green-producing cocci of three types were encountered; small round, gray, discrete, biconvex, glistening colonies surrounded by a grayish-green zone corresponding to the viridans type of streptococcus; flatter, often checker-like or umbilicated colonies revealing usually lancet-shaped diplococci surrounded by a similar zone of methemoglobin, corresponding to the pneumococcus group; lastly, somewhat larger green moist colonies, often mucoid, showing a marked tendency to become confluent, some corresponding in their appearance to the pneumococcus mucosus, others to the so-called "Mathers" type of streptococcus. Confirmatory studies by means of bile solubility and inulin fermentation were carried out on the isolated pure cultures. Most of the flat checker-like colonies proved to be bile-soluble and to ferment inulin, while the convex type were bile-insoluble and noninulin fermenting; but some exceptions were noted in both. The bile soluble, inulin fermenting strains were classified as pneumococci, and the insoluble, nonfermenting strains as Streptococci viridans.

THE INCIDENCE OF STREPTOCOCCUS VIRIDANS AND PNEUMOCOCCUS IN CULTURES OF NASOPHARYNGEAL SWABS, ADENOIDS AND TONSILS

	Number of Persons	Percentage Positive	
		Pneumococcus	Streptococcus viridans
Series 1. Swabs	21	71.4	90.5
Adenoids	21	71.4	90.5
Tonsils	21	66.6	81.0
Series 2. Adenoids	82	62.3	88.0
Total number adenoids examined	103	65.0	89.0

In the first series of 21, pneumococci occurred in 16 of the swab cultures, in 16 of the adenoids cultures and in 14 of the cultures of the tonsillar crypts. Streptococcus viridans was found in 19 of both swab and adenoid cultures, and in 17 of the tonsil cultures. The Mathers type of streptococcus was encountered once in all. The results are indicated in the table. In the second series of 82 adenoids pneumococci were encountered in 51, streptococcus viridans in 72. It is interesting to observe that in the nasopharyngeal swabs the pneumococci seldom were predominant, whereas in the adenoids from the same persons they were quite numerous in 4 instances and in almost pure culture in 2. Occasionally they were also numerous in the tonsillar crypts.

The crypt-like depressions of the adenoids seemed to harbor larger numbers. In two instances in which the pneumococcus and streptococcus viridans were absent in the tonsils although present in the adenoids, hemolytic streptococci were recovered in pure culture from the crypts. No great variations were noted in the numbers of streptococcus viridans excepting that in the crypts of the tonsils they were often fewer than in the nasopharynx.

In 12 instances typical streptococcus colonies revealing gram-positive cocci in chains were encountered, causing neither hemolysis nor methemoglobin formation.

Forty strains of Streptococcus viridans were inoculated in litmus milk and carbohydrate broth. All acidified and coagulated milk in from 7 to 10 days. The sugar medium contained 1% carbohydrate in infusion broth with Andrade's indicator. Lactose was fermented by all, mannite by 3, salicin by 14. According to Holman's classification, 6% are Strep. fecalis, 30% Strep. motis and 64% Strep. salavarius.

The moist green-forming colonies were somewhat variable in their appearance. Altogether they were encountered in the adenoids of 26 persons. Nine were distinctly moist mucoid colonies like the Pneumococcus mucosus. The other 17, though quite moist, showed a tendency toward flattening and conformed to the description of the so-called Mathers streptococcus. Of the former 9 strains, 6 were bile soluble, 3 insoluble. The 6 strains conform to the true Pneumococcus mucosus; the other 3 might possibly be termed Streptococcus mucosus. Of the 17 Mathers type, 9 were bile soluble, 8 insoluble, 6 fermented inulin and 11 did not. Morphologically, most of these organisms revealed gram-positive capsulated diplococci often lanceolate shaped; the Mathers coccus frequently appeared in chains of variable length.

Agglutination with specific types 1, 2, and 3 serums from the Rockefeller Institute and the New York State Board of Health was carried out on the bile-soluble organisms. Of the total 67 strains, none was agglutinated by type 1, 2 by type 2 and 10 by type 3. Two strains clumped by type 3 serum were not typical mucoid like the Pneumococcus mucosus. Two others were of the Mathers type.

Of the 14 strains from the tonsil, 2 were type 3, 12 were type 4.

SUMMARY

To ascertain more accurately the incidence of pneumococci in the throat and nasopharynx cultures were made from the extirpated adenoids and tonsils.

In a series of 103 adenoids, pneumococcus occurred in 65%, 2% of which were type 2, 13% type 3 and 85% type 4. In the nasopharyngeal swabs of 21 persons the pneumococcus was recovered in 71.4%; from the tonsils of the same persons in 66.6%, and the adenoids in 71.4%. It was observed that in the depths of the folds and the crypt-like depressions of the nasopharyngeal vegetations and from the tonsillar crypts the pneumococci were decidedly more numerous than in the swabs. In 4 instances the pneumococci occurred practically in pure culture from the adenoids.

Streptococcus viridans was found in 89% of the adenoids and 81% of the tonsils. Streptococcus mucosus was encountered in 3% of the adenoids, and indifferent streptococci in 12%. The Mathers coccus was noted in 17% of the adenoids, once in pure culture.

The adenoids and tonsils are foci in which pneumococci and nonhemolytic streptococci commonly flourish.

BACTERIOLOGIC STUDIES OF THE UPPER RESPIRATORY PASSAGES

III. THE INFLUENZA BACILLI (PFEIFFER) OF THE ADENOIDS AND TONSILS

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Influenza bacilli have frequently been cultivated from the throat and sputum both from normal persons and from patients affected with various respiratory diseases. The incidence in normal persons varies somewhat according to different observers. Thus, in 1907 Davis [1] noted the organisms in 2 of 20 normal throats (10%). More recently Pritchett and Stillman [2] found the bacilli in 42% of 177 normal persons, and in school girls and boys of an orphan asylum, Winchell and Stillman [3] noted them in 25% to 39%. Opie and associates [4] give the incidence of Pfeiffer bacilli in from 24% to 35.1% of healthy soldiers. Lord [6] obtained the organism in as high as 76% of the swab cultures of 34 normal young men, while Jordon [6] recovered them in 40% of normal throats. From well children most of whom were under 2 years of age Wollstein and Spence [7] state that the bacilli occurred in 10% of 266. All of these figures were based on studies of the saliva or swab cultures of the pharynx and occasionally of the nasopharynx.

In previous studies of the extirpated tonsils and adenoids it was indicated that occasionally direct cultures of these structures would reveal hemolytic streptococci which were apparently too few on the surface to be recovered in the swab cultures of the same persons. To determine more accurately the frequency of the influenza bacillus in the oro- and naso-pharynx the excised tonsils and adenoids were cultivated with special reference to this organism. The material consisted of adenoids and tonsils consisting of lymphoid tissue revealing varying

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[1] Jour. Am. Med. Assn., 1907, 48, p. 1563.
[2] Jour. Exper. Med., 1919, 29, p. 259.
[3] Ibid., 1919, 30, p. 497.
[4] Jour. Am. Med. Assn., 1919, 72, p. 108.
[5] Ibid., p. 188.
[6] Ibid., p. 1542.
[7] Am. Jour. Dis. Child., 1920, 19, p. 459.

degrees of hyperplasia but no other marked pathologic change. The patients were children who presented no acute evidences of sore throat or any elevation of normal temperature. The age varied from 5 to 16 years, and the patients had had their tonsils and adenoids removed during the months of April, May, June and July, 1920. In 25 instances naso-pharyngeal swabs were made by means of the West tube immediately before the operation. Altogether cultures were made from the adenoids and tonsils from each of 115 persons.

The material was collected in sterile gauze and cultures were made within 4 hours after removal. Cultures were made from the surface of the adenoids and from depths between the folds and cryptlike slits which frequently occur in their structure. The tonsils were incised with a sterile knife transversely to the long axis of the crypts and cultures made from the bottom of the crypts. Ten per cent. blood agar for pouring into Petri dishes were inoculated, as well as the surface of blood-agar plates. Additional cultures were made on the so-called chocolate medium consisting of infusion agar to which defibrinated human blood was added in 5% proportion and the whole heated at 90 C. for 5 minutes.

On the chocolate plates the influenza bacilli occurred in the cultures of the adenoids in 47 instances or 40.9% and of the tonsils in 62 or 53.9%. In the nasopharyngeal swabs they were found in 10 or 40%. In each instance in which the bacilli appeared in the adenoids they were also recovered from the tonsils. From the depths of the adenoid folds the organism was isolated in one instance but did not grow out on the cultures of the surface or the nasopharyngeal swab. The brown plates frequently revealed these organisms in large numbers growing as gray, often flat, colonies of variable size, not infrequently being the predominating bacterium on this special medium. On the streaked and poured blood-agar plates the bacilli grew as small translucent dewdrop-like colonies occasionally showing strikingly the tendency to grow in clusters immediately around other colonies observed most readily in the zones of hemolysis of the Streptococcus hemolyticus. This symbiotic tendency was seen only when the influenza bacilli occurred in relatively large numbers, for in many instances a few transparent colonies revealing gram-negative pleomorphic bacilli were observed without this tendency. In several instances the bacilli were apparently absent on the fresh blood agar, while the were present in few or even moderate numbers on the heated blood medium.

The bacilli appeared as gram-negative small rods, some strains showing a uniform size, and a few exhibited an extreme tendency toward larger and thread-like forms, while several displayed coccoid forms. Pure cultures, isolated on chocolate-agar slants, revealed apparently more marked pleomorphism. Each strain was inoculated on a plain infusion-agar slant, and in no instance did growth occur. They were further studied by inoculating the entire surface of a fresh unheated blood-agar slant on which another organism like the staphylococcus albus was subsequently streaked linearly. In each instance the influenza bacilli grew as small dew-drop colonies which were largest and most numerous immediately adjacent to the linear streak of the foreign organism demonstrating the property of symbiosis so characteristic of the Pfeiffer bacillus.

THE INCIDENCE OF INFLUENZA BACILLUS (PFEIFFER) IN THE CULTURES OF NASOPHARYNGEAL SWABS, ADENOIDS AND TONSILS

	Number of Persons	Percentage Positive
Series I. Swabs	25	40.0
Adenoids	25	44.4
Tonsils	25	48.0
Series II. Adenoids	90	38.8
Tonsils	90	55.5
Total number of adenoids from which cultures were made	115	40.9
Total number of tonsils from which cultures were made	115	53.9

It is interesting to observe that the influenza bacilli occurred more often in the crypts of the tonsils and the crypt-like structures of the adenoids than in the nasopharyngeal swabs or cultures from the surface or folds of the adenoids. The organisms were also inclined to be decidedly more numerous in the depths of these structures than on the surface. In these respects the Pfeiffer bacillus resembles the streptococcus hemolyticus, illustrating the rôle played by the lymphoid structures of the oro- and naso-pharynx in furnishing foci in which dangerous bacteria flourish.

SUMMARY

Gram-negative, pleomorphic, hemoglobinophilic bacilli, showing a preference for heated blood agar and revealing the characteristic prop-

erty of symbiosis, were isolated and identified in 40.9% of extirpated adenoids and in 53.9% of the excised tonsils from 115 persons. In the nasopharynx they were present in 40% of 25 persons and in fewer numbers.

The tonsils and adenoids therefore are foci in which influenza bacilli (Pfeiffer) commonly flourish.

BACTERIOLOGIC STUDIES OF THE UPPER RESPIRATORY PASSAGES

IV. THE INCIDENCE OF PNEUMOCOCCI, HEMOLYTIC STREPTOCOCCI AND INFLUENZA BACILLI (PFEIFFER) IN THE NASOPHARYNX OF TONSILLECTOMIZED AND NONTONSILLECTOMIZED CHILDREN

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It was noted previously by Pilot and Davis [1] that the incidence of hemolytic streptococci was less in the oropharynx of tonsillectomized that in the nontonsillectomized, the organisms occurring in 15.8% in few numbers in the former group as compared with 58% in larger numbers in the latter group. Nichols and Bryan [2] reported the disappearance of these organisms from the throat in 27 of 31 patients 11 days after extirpation of diseased tonsils. Simmons and Taylor [3] noted the streptococci in fewer numbers in a somewhat larger percentage (23%) after tonsillectomy. Tongs [4] found these streptococci in 5% of tonsillectomized persons as compared with 60% of the cultures of the surface of the tonsils of the nontonsillectomized. Van Dyke [5] obtained positive cultures in 16.4% of tonsillectomized persons, mostly adults. In all of these investigations cultures were made of the pharynx or the region of the tonsils, and studied with special reference to the incidence and numbers of hemolytic streptococci. In all it is quite evident that tonsillectomy reduces considerably the frequency of Streptococcus hemolyticus. As no nasopharyngeal cultures were made, a study was undertaken of the flora of the nasopharynx with reference to the pneumococcus and the influenza bacillus, as well as the hemolytic streptococcus in children whose tonsils and adenoids had been removed, and a comparison made with a similar group in which tonsils and adenoids were present. The investigation was carried out from September to December, 1920.

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[1] J. Infect. Dis., 1919, 24, p. 386.
[2] Jour. Am. Med. Assn., 1918, 71, p. 1872.
[3] Ibid., 1919, 72 p. 1885.
[4] Ibid., 1919, 73, p. 1050.
[5] Ibid., 1920, 74, p. 448.

The children studied were normal girls and boys from 5 to 15 years of age of the Marks Nathan Orphan Home. In some the tonsils and adenoids had been removed from 2 months to 5 years previously. All the children had normal temperature and no subjective or objective evidences of acute inflammation of the throat or respiratory passages. The nasopharynx was swabbed with a curved wire swab which was spread on 10% human blood-agar and 5% heated blood-agar plates, and then placed in infusion broth and incubated for 24 hours. The infusion broth cultures were then inoculated into melted blood agar and poured plates made. The blood-agar plates were examined particularly for the pneumococcus, the heated blood-agar plates for the influenza bacillus and the poured plates for the hemolytic streptococcus (Table 1).

TABLE 1

THE INCIDENCE OF PNEUMOCOCCI, HEMOLYTIC STREPTOCOCCI AND INFLUENZA BACILLI IN TONSILLECTOMIZED AND NONTONSILLECTOMIZED CHILDREN

Organisms	Tonsillectomized Number of Persons	Tonsillectomized Percentage Positive	Nontonsillectomized Number of Persons	Nontonsillectomized Percentage Positive
Pneumococcus	49	32.5	68	32.3
Streptococcus hemolyticus	27	40.8	40	60
B. influenzae	29	26.5	35	37.1

Pneumococcus.—Small flat checkered colonies were isolated and inoculated into plain broth. They were then studied as to morphology, bile solubility, inulin fermentation and specific agglutination with types 1, 2 and 3 serums. In 49 children without tonsils and adenoids pneumococci were identified in 15 (32.5%). Ten of these strains were type 4, 2 type 3, 1 type 2a and 2 type 1. In one instance a green streptococcus insoluble in bile, but which fermented inulin, was found. In the 68 with tonsils and adenoids the pneumococcus occurred in 21 (32.3%), of which 20 were type 4 and one type 2a. In two instances a green streptococcus bile-insoluble but fermenting inulin was encountered. In 8 children whose throats revealed remnants of tonsils the pneumococcus was present in 3; two of the strains were of type 4, the other type 3. It was noted throughout that while the percentage of pneumococci did not differ in the 2 groups, the number of colonies of pneumococci when present was fewer in the cultures from the tonsillectomized than those from the nontonsillectomized.

Streptococcus hemolyticus.—Small gray colonies with zones of complete hemolysis from 1 to 4 mm. wide corresponding to the beta type of the streptococcus hemolyticus were isolated. These organisms fer-

mented lactose and salicin, but did not ferment inulin or mannite. They were pathogenic for rabbits, one blood-agar slant usually causing arthritis and often death in a young rabbit. They resembled in their properties the hemolytic streptococci of the crypts of the tonsils and adenoids. In 27 children whose tonsils and adenoids were absent the beta type of streptococcus was found in 11 (40.8%); the alpha type occurred in one instance. In 40 children with tonsils the beta type was present in 24 (60%) and in 2 the alpha was encountered. Of the 8 with remnants beta type was present in 3 and the alpha in 2. It is interesting to note that in the cases with tonsils the hemolytic streptococci were often present in moderate numbers only occasionally exceeding 10% of the total colonies on the plate. In the cultures from the children without tonsils the number of colonies was decidedly less, in none exceeding 10% of the total number and in 3 instances only a single colony was present.

B. Influenzae.—On the heated blood-agar plates typical gray, often flat, colonies were studied and subcultivated. The organisms were small gram-negative bacilli often pleomorphic, particularly on the subcultures. Transfers were made on infusion agar where no growth occurred when the organisms were true influenza bacilli. They were further subcultivated on unheated blood-agar slants and another organism (staphylococcus) streaked linearly on the same slant. The influenza colonies showed the property of symbiosis growing in larger size and numbers about the foreign organism. Of 29 persons whose tonsils were absent, 8 (26.5%) gave positive cultures for B. influenzae. Of 35 whose tonsils were present, 13 (37.1%) were positive. In the 8 with remnants, 4 gave positive results. Here also it is noteworthy that the number of colonies were more numerous in the cultures of the nontonsillectomized group.

SUMMARY

Pneumococci, hemolytic streptococci and B. influenzae were often found in the nasopharynx of normal children.

The incidence and numbers of hemolytic streptococci and influenza bacilli in the nasopharynx is decidedly less in the children whose adenoids and tonsils had been removed. In case of the pneumococcus the numbers are less in the same children than in those whose tonsils were present.

The removal of tonsils and adenoids reduces the number of certain bacteria in the oro-pharynx and naso-pharynx, but does not cause their disappearance.

BACTERIOLOGIC STUDIES OF THE UPPER RESPIRATORY PASSAGES

V. THE DIPHTHERIA BACILLI AND DIPHTHEROIDS OF THE ADENOIDS AND TONSILS

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The tonsils are the foci that usually harbor diphtheria bacilli in carriers, and their removal frequently terminates the carrier state.

Pegler[1] was perhaps the first to observe this effect, which has been described also by Friedberg[2] who reported 6 carriers that gave negative cultures for B. diphtheriae after extirpation of the tonsils and adenoids. Ruh, Miller and Perkins[3] obtained similar results in 19 cases and Rabinoff[4] had the same experience in 10 instances. In his study of diphtheria carriers Weaver[5] reports that cultures became negative in 40 persistent carriers within 18 days after the removal of the tonsils and adenoids. Keefer, Friedberg and Aronson[6] state that in 77.2% of the carriers they studied the organisms were in the tonsils, often in pure culture, and that 91.3% became negative within 2 weeks after tonsillectomy.

The diphtheria bacilli apparently are in the crypts of the tonsils as well as on the surface. Ruh, Miller and Perkins[8] call attention to 5 carriers who gave positive cultures of the crypts while the surface cultures were negative. Dwyer and Gignoux[7] obtained 5 positive reactions from direct cultures of the crypts of 72 persons. Brown[8] demonstrated in microscopic sections gram-positive bacilli morphologically like the diphtheria bacillus in the crypts and in the tissues beneath the thin epithelium of the tonsils of 7 carriers. Ballantyne and Cornell[9] found diphtheria bacilli in the tonsillar crypts of 6 carriers and once in the adenoids. Hartley and Martin[10] found the bacilli in sections of the tonsils, in the crypts but not in the tissues.

In all of these observations it is noteworthy that cultures were taken from the tonsils only occasionally, from the adenoids practically never, and apparently none of the bacilli identified was tested for

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1 Brit. Med. Jour., 1905, 2, p. 621.
2 Jour. Am. Med. Assn., 1916, 66, p. 810.
3 Ibid., p. 941.
4 Ibid., 67, p. 1722.
5 Ibid., 1921, 76, p. 831.
6 Ibid., 1918, 71, p. 1206.
7 Laryngoscope, 1910, 20, p. 1042.
8 Jour. Infect. Dis., 1916, 19, p. 565.
9 Brit. Med. Jour., 1917, 2, p. 686.
10 Quoted by Weaver.[5]

virulence. To determine the frequency in the naso- and oro-pharynx of virulent and avirulent forms of diphtheria bacilli and of related organisms, a bacteriologic study with special reference to these points was made of the extirpated adenoids and tonsils of the same patients.

The material was obtained from 100 children who entered the Cook County Hospital to have their tonsils and adenoids removed chiefly because of hyperplasia. They were afebrile and had no evidences of acute inflammation of the throat or respiratory passages. They varied from 5 to 15 years of age, and were boys and girls living in scattered parts of Chicago. A reliable history of an attack of diphtheria was not to be obtained in any case. The adenoids and tonsils were removed during the months of October and November, 1920.

The material was selected and cultures were taken only from adenoids which were not macerated. Cultures were obtained from the tonsils of the same persons. Altogether 100 adenoids and 100 pairs of tonsils were studied. Cultures were made in from 1 to 3 hours after the operation.

The adenoids revealed no marked pathologic change other than hyperplasia. There were no gross evidences of necrosis or of a purulent exudate. They consisted of lymphoid tissue from 0.5 to 1.5 cm. in all dimensions, presenting 3 to 6 folds and often cryptlike depressions not unlike the crypts of the tonsils. The tonsils, like the adenoids, often showed marked hyperplasia, some however, being of normal size without any gross change. Occasionally fatty débris and cholesterol crystals, rarely pus, were observed in the crypts. In none was necrosis seen.

Cultures were taken from the surface of the epithelial lining of the adenoids and the crypts of the tonsils. From the adenoids cultures were obtained by passing a wire loop over the surface, and then after carefully separating the folds or orifices of the depressions another culture was taken of the sides and bottoms of these structures. The tonsils were incised transversely and cultures made with a loop from the bottoms of crypts. Slants of Loeffler serum medium with a broad surface were used and examinations made at the end of 18 to 24 hours

The diphtheria bacilli and diphtheria-like organisms grew as small gray opaque colonies and were identified with the methylene blue and gram stains. Bacilli with typical polar granules corresponding to the C and D and occasionally A types of Wesbrook were indicated as diphtheria bacilli. In several instances the barred types C′ and D′ were encountered, but granular types were also present. The remaining

bacilli, all gram-positive, occurring often in palisades, were termed diphtheroids. Pure cultures were obtained from subcultures on blood-agar plates.

Diphtheria bacilli were recovered in 12 instances, both from the adenoids and tonsils (table 1). In one case the bacilli were found on the surface of the adenoids and not in the depths. In 5 instances the bacilli occurred in one tonsil and not in the other. In all instances when the bacilli were present they appeared in the adenoids as well as in one or both tonsils. The organisms on Loeffler's medium appeared frequently in pure culture or in predominating numbers (table 2). In the crypts of the tonsils they generally occurred in larger numbers than in the adenoids. It is of interest to note that two strains which proved virulent occurred in practically pure culture in both the adenoids and tonsils.

TABLE 1

THE INCIDENCE OF THE B. DIPHTHERIAE AND DIPHTHEROIDS IN THE EXCISED ADENOIDS AND TONSILS OF 100 CHILDREN

	B. diphtheriae, Percentage Positive	Diphtheroids, Percentage Positive
Surface of the adenoids	12	25
Depths of the adenoids	11	24
Surface and depths of the adenoids	12	30
Right tonsil	12	14
Left tonsil	7	11
One or both tonsils	12	17

TABLE 2

RELATIVE NUMBERS OF B. DIPHTHERIAE IN THE ADENOIDS AND TONSILS OF 100 CHILDREN

	Surface of Adenoids, Percentage	Depths of Adenoids, Percentage	Right Tonsil, Percentage	Left Tonsil, Percentage
Pure culture	3	2	5	2
Predominating numbers	3	2	2	2
Moderate numbers	1	3	3	2
Few numbers	5	4	2	1
None	88	89	88	93

On blood agar 4 strains caused hemolysis; only one proved virulent. In 1% carbohydrate broth with Andrade indicator all 12 strains produced acid in dextrose and dextrin, 10 in maltose and lactose and none in saccharose and mannite.

Two strains were repeatedly virulent in doses of 1, 2 and 3 c c of 48-hour cultures of infusion broth for 300 to 400 gm. guinea-pigs, causing death within 24 hours with typical local edema and hemorrhage,

fluid in pleural cavities and hemorrhagic adrenals. The antitoxin controls inoculated with 250 units survived in all instances. A third strain showed low virulence, killing in doses of 3 and 5 c c serum broth, while the antitoxin controls survived. Three other strains in large doses (5 c c) were pathogenic, producing local abscesses, hemorrhages in mucous membranes and muscles with congestion of the kidneys and adrenals, less marked, however, than in the lesions of the more virulent strains. Subsequent cultures were totally avirulent. The remaining 6 strains were avirulent. The 3 strains of low pathogenicity correspond closely to the bacilli described by Hamilton [11] in otitis media complicating scarlatina.

In this connection it is worthy to compare the incidence of virulent and avirulent diphtheria bacilli in swab cultures of the throats of normal persons determined by several investigators with the incidence in the adenoids and tonsils. It should be remembered that a surface swab culture has been repeatedly shown not to be a true index of the flora in the crypts of the tonsils in which the organisms are present in larger numbers free from contamination of the bacteria of the saliva and sputum. It is also important to note that most of the statistics available are based on a single swab culture and that the percentage undoubtedly would be higher if cultures of the nasopharynx, each tonsillar surface and the pharynx were taken separately. The report of the Massachusetts Association of Boards of Health [12] indicates a percentage of 1 to 2% of the urban population and 5 to 8% in institutions, and of the strains tried 17% were found virulent. Pennington [13] found that 9.3% of 375 school children carried the bacilli, of which 14% were virulent and 30% of attenuated virulence. Von Sholly [14] obtained in the cultures of 1,000 normal throats virulent bacilli in 1.8% and nonvirulent in 3.8%. Goldberger, Williams and Hachtel [15] report B. diphtheriae in 0.928% of 4,093 healthy persons, of which of 19 strains tested 10.5% were virulent. Guthrie, Gelien and Moss [16] encountered the organisms in 3.55% of 2,507 children and adults, of which 18.18% of 33 strains were virulent. In general, the bacilli recovered from normal persons not convalescent, or contacts from 10.5 to 18.18%, were virulent comparing closely to the strains of the adenoids and tonsils, of which 16.66% were virulent.

[11] Jour. Infect. Dis., 1907, 4, p. 316.
[12] Jour. Mass. Assn. of Boards of Health, 1902, p. 1202.
[13] Jour. Infect. Dis., 1907, 4, p. 36.
[14] Ibid., 1907, 4, p. 337.
[15] Bull. 101, Hyg. Lab., 1915, p. 29.
[16] Bull. Johns Hopkins Hosp., 1920, 357, p. 388.

The diphtheroid bacilli appeared in the smears as nongranular, shorter and stouter than the Klebs-Loeffler organism. In the adenoids they occurred in 30% and in the tonsils in 17% (table 1); 9 of the 30 and 4 of the 17 resembled the D2 type of Westbrook or the Hoffman bacillus. In the adenoids the diphtheroids were usually encountered in larger numbers on the surface than in the depths. In the crypts of the tonsils these organisms were decidedly less common and numerous (table 3). In the 13 instances in which the diphtheroids were absent in the crypts, although present in the adenoids, the predominating growth of the crypt cultures were coccus forms chiefly streptococci. The greater incidence of the diphtheroid group in the nasopharynx than in the tonsils is of interest for it has often been observed that these organisms are more common in the nose and nasal passages than in the oropharynx (Gorham [17]). Twenty strains isolated in pure culture did not ferment dextrose, lactose, maltose, saccharose or dextrin resembling the strains from the tonsils studied by Eberson.[18] None of the strains were hemolytic.

TABLE 3

RELATIVE NUMBERS OF DIPHTHEROIDS IN THE ADENOIDS AND TONSILS OF 100 CHILDREN

	Surface of Adenoids, Percentage	Depths of Adenoids, Percentage	Right Tonsil, Percentage	Left Tonsil, Percentage
Pure culture	3	3	0	0
Predominating numbers	2	2	0	0
Few or moderate numbers	20	19	14	11
None	75	76	86	89

SUMMARY

Cultures made of the excised adenoids of 100 children revealed B. diphtheriae in 12.

The crypts of the extirpated faucial tonsils of the same persons harbored the bacilli in 12. When present in the tonsils the bacilli also occurred in the adenoids of the same person.

In the tonsillar crypts the diphtheria bacilli were usually more numerous than in the adenoids.

Two of the 12 strains were virulent; one showed attenuated virulence; three were pathogenic in large doses of the first culture while subsequent cultures were without virulence; the remainder were totally avirulent.

Diphtheroids occurred in 30 of the adenoids and in 17 of the tonsils; when present in both they were decidedly more numerous in the nasopharyngeal vegetations than in the tonsillar crypts.

[17] J. Med. Res., 1901, 6, p. 201.

[18] Jour. Infect. Dis., 1918, 23, p. 14.

THE BACTERIOLOGY OF THE ADENOIDS

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February 25, 1921

The bacterial flora of the nasopharyx has frequently been investigated with reference to the detection of carriers, particularly of the meningococcus. In such examinations cultures were obtained by the assistance of the West tube or a curved wire swab. The results of the various surveys made would indicate that the nasopharynx harbors many dangerous bacteria. It has been generally assumed that these organisms flourish among the vegetations or adenoid tissue of this region. It is, however, surprising to find that no studies have been made with the aid of improved bacteriologic methods to determine the flora of these lymphoid structures.

Pilot and Davis [1] demonstrated that swab cultures of the oropharynx were inaccurate and not a true index of the actual flora in the faucial tonsils, particularly with reference to the hemolytic streptococcus which occurred in 61% of the swab cultures of the surface of the tonsils as compared with 97% from the crypts of the excised tonsils. In order to establish definitely the incidence and number of the common pathogenic bacteria in the nasopharynx and the nasopharyngeal vegetations, a bacteriologic study of the extirpated adenoids was undertaken.

METHOD

The adenoids studied were those removed at the ear, nose and throat ward of the Cook County Hospital from April to November, 1920. The patients were children from 5 to 16 years of age without fever or acute inflammations of the respiratory passages. The adenoids selected consisted of lymphoid tissue revealing varying degrees of hyperplasia. They maesured from 0.5 to 1.5 cm. in all diameters, and usually presented from 3 to 6 or more folds. In many union had taken place between the free margins of the folds, leaving slits in the tissue not unlike the crypts of the tonsils. In a few instances fatty débris and cholesterol crystals were found in such depressions, but in

[1] Jour. Infect. Dis., 1919, 24, p. 386.

none was any purulent exudate grossly visible. Macerated adenoids were discarded. These adenoids approached the normal as closely as can possibly be obtained. Cultures were made from the epithelial surface and from the depths of the vegetations between the folds and from the bottoms of the cryptlike depressions. Blood-agar plates, heated blood agar for the isolation of the influenza bacillus and Loeffler's medium for the diphtheria bacillus were employed. In 25 instances the nasopharynx was swabbed through a West tube and cultivated on fresh and heated blood agar.

RESULTS

The common pathogenic organisms were identified and studied; the results are indicated in table 1.

TABLE 1

THE INCIDENCE OF PATHOGENIC BACTERIA IN THE ADENOIDS, NASOPHARYNGEAL SWABS AND TONSILS

Organism	Number of Patients	Adenoids % Positive	Nasopharyngeal Swab % Positive	Tonsils % Positive
Streptococcus hemolyticus	103	61	..	..
	25	..	40	..
	21	..	..	95
Streptococcus viridans	103	89	..	..
	21	..	90.5	81
Pneumococcus	103	65	..	..
Type 2	...	2	..	..
Type 3	...	10.3	..	..
Type 4	21	52.7	71.4	66.6
Mathers coccus	103	17	..	..
Staphylococcus	103	60	..	..
Gram negative cocci	103	79	..	..
B. influenzae (Pfeiffer)	115	40.9	..	53.9
	25	..	40	..
B. diphtheriae	100	12	..	12
Diphtheroids	100	30	..	17
B. mucosus capsulatus	103	14	..	..
B. fusiformis	10	20	..	..

Streptococcus Hemolyticus.—Hemolytic streptococci occurred in 61% in cultures taken from the depths between the folds and of the crypts. In 18 these organisms were quite numerous and in 3 in pure cultures. In their hemolytic properties, morphology and carbohydrate fermentation they conformed to the beta type mostly of the Streptococcus pyogenes group. They were bile insoluble and pathogenic for rabbits in doses of 2 c.c. and for mice in 0.5 c.c. of broth culture. The alpha type was encountered in 20%.

Streptococcus Viridans.—Streptococcus colonies causing methemoglobin formation on blood agar occurred in 89%. They were bile insoluble and did not ferment inulin. On the basis of carbohydrate

fermentation 30% of the strains were of the Streptococcus mitis group, 64% of the Strep. salivarious and 6% of the Strep. fecalis. Streptococci causing neither hemolysis nor green formation were noted in 12%; streptococcus mucosus in 3%.

Pneumococcus.—Bile soluble, inulin fermenting strains were found in 65%. Of these, on the basis of agglutination with specific serums, 85% were type 4, 13% type 3, and 2% type 2. With the exception of 2 strains, the type 3 were culturally like Pneumococcus mucosus.

Mathers Coccus.—Large, moist, often flat, green producing colonies variable in bile solubility and inulin fermentation conforming to the streptococcus described by Mathers [2] in the past influenza epidemic were found in 17%.

Bacillus influenzae (Pfeiffer).—On heated blood-agar mediums influenza bacilli were recovered in 40.9%, often in large numbers. They were identified as gram-negative, pleomorphic bacilli failing to grow on infusion agar, and showing the characteristic property of symbiosis.

Bacillus Diphtheriae.—From Loeffler's medium granular types morphologically identical with the Klebs-Loeffler bacillus were isolated in 12%, often predominating on this medium, and in pure culture in 2 instances. Two strains were toxin-producing killing guinea-pigs in doses of 1 c c of broth culture, while the antitoxin controls survived. The remainder were relatively avirulent. In this connection it should be noted that the patients whose adenoids gave positive cultures did not have diphtheria.

Diphtheroids.—Organisms resembling closely in their cultural characteristics the diphtheria bacillus, but morphologically different in that they did not show metachromatic granules, occurred in 30%. They were further differentiated by their inability to ferment the ordinary sugars.

Other Organisms.—Straphylococci were noted in 60%, and most of them were of the albus and only a few the aureus type. Gram-negative diplococci were encountered in 79%, appearing chiefly as Micrococcus catarrhalis, Micrococcus pharyngis siccus and a few resembling the meningococcus. A study is now being made for their further differen-

[2] Tunnicliff, R.: Jour. Infect. Dis., 1920, 26, p. 405.

tiation. The Bacillus mucosus capsulatus, growing as large mucoid colonies, was found in 14%. Anaerobic cultures were made of 10 adenoids and the B. fusiformis occurred in 2 (20%).

CULTURES OF THE NASOPHARYNGEAL SWABS OF THE SAME PERSONS

Nasopharyngeal swab cultures were taken from 25 persons before operation. Few hemolytic streptococci were found in 10 persons (40%) as compared with those found in the depths of the adenoids in 15 persons (60%). Influenza bacilli occurred once in the adenoids of a person in this series (44.4%) when it was not recovered from the swab (40%). Pneumococci and streptococcus viridans appeared in the same percentages. It was notable, however, that all of these organisms were decidedly more numerous in the depths between the folds and in the cryptlike depressions than on the epithelial surface and the nasopharyngeal swab, demonstrating the tendency of these organisms, especially the hemolytic streptococcus, to flourish in the recesses of the nasopharynx.

CULTURES OF THE CRYPTS OF THE TONSILS FROM THE SAME PERSONS

The extirpated tonsils of 21 persons were cultivated and revealed hemolytic streptococci in 20 (95%), frequently in predominating numbers, while the adenoids of 15 (71.4%) of these patients contained these organisms in less numbers. The pneumococci were present in 66.6% and the Streptococcus viridans in 81% as compared with 71.4% and 90.5%, respectively, in the adenoids. In those instances in which the pneumococci and green streptococci were absent in the tonsils and present in the adenoids the tonsillar crypts harbored hemolytic streptococci in pure culture. The tonsils of 115 patients were found to have influenza bacilli in 53.9%, whereas the adenoids of the same persons gave positive results in 40.9%. Diphtheria bacilli occurred in 12% of the tonsils and adenoids of 100 children, appearing more often in predominating numbers in the tonsils.

THE INCIDENCE OF HEMOLYTIC STREPTOCOCCI, PNEUMOCOCCI AND B. INFLUENZAE IN THE NASOPHARYNX OF TONSILLECTOMIZED AND NONTONSILLECTOMIZED CHILDREN

Normal children from 5 to 15 years of age, inmates of an orphan asylum, were swabbed in the nasopharynx. After the bacteriologic

work was completed, they were divided in two groups, those who had had their tonsils and adenoids removed from 2 months to 5 years previously and those who did not have either excised. A few children having remnants of tonsils were classified as nontonsillectomized. In 48 with tonsils hemolytic streptococci were recovered in 27 (56.3%) and in 27 without tonsils in 11 (40.8%); influenza bacilli occurred in 17 (39.3%) of 43 persons with tonsils and in 8 of 29 (26.5%) persons without tonsils. J. Meyer found pneumococci in about the same percentage (32.5%) in both groups. It is noteworthy that all of these organisms when present were decidedly fewer in tonsillectomized than in the nontonsillectomized persons.

While the removal of the tonsils and adenoids reduces the incidence and numbers of bacteria in the throat and nasopharynx it is quite evident that it does not cause their disappearance, and that the other lymphatic structure of the oro- and nasopharynx continue to furnish foci for these organisms.

The Flora of the Nasopharynx of Normal Persons.—Every culture taken from the nasopharynx and adenoids showed various organisms. Regardless of the size of the adenoids, or whether or not the vegetations were to be considered normal or markedly hypertrophied, microorganisms, particularly the streptococci, pneumococci and B. influenzae were present, occasionally one predominating over the other, seldom, however, in pure culture. Usually several different organisms were found in the same person in a considerable percentage. It would therefore seem that for children, at least, the organisms commonly encountered should be regarded as part of the normal flora of the nasopharynx, just as we regard certain bacteria as part of the normal flora of the mouth or intestine.

Significance with Special Reference to the Carrier Problem.—On the basis of these results, it is quite apparent that many healthy persons harbor pathogenic bacteria in the nasopharynx, organisms which do not differ from the strains isolated from various pathologic processes of the human body. Can all such persons be termed carriers in the sense that they are sources from which others may become infected? Under exceptional circumstances transmission of hemolytic streptococci from one person to another directly by droplet infection or indirectly through milk, for example, occurs as illustrated in the epidemic streptococcal complications following measles and the milk epi-

demics of streptococcus sore throat. In such instances an epidemic strain of enhanced virulence exists in the throats of the affected persons and those who come in contact with them. Under ordinary circumstances, however, these organisms apparently lie dormant in the tonsils and nasopharynx without harm to the healthy person or to the healthy contact. The carrier state may possibly be limited to persons harboring epidemic strains and to the convalescents and contacts giving positive cultures of the organisms responsible for cases of diphtheria, meningococci meningitis, and pneumonias due to type 1 and 2 pneumococci. The adenoids, like the tonsils, should be considered as foci where such dangerous organisms may persist.

SIGNIFICANCE OF THE BACTERIA OF THE ADENOIDS AS SECONDARY INVADERS

In the healthy person the micro-organisms apparently exist without doing harm. When, however, the resistance is lowered, either locally by acute inflammations of the respiratory tract as in the exanthemata, pneumonia and influenza or generally by an acute or chronic toxemia or a chronic debilitating disease, these organisms may become a great source of danger. Hemolytic streptococci are commonly secondary invaders in measles, scarlet fever, pneumonia and other primary infections. The pneumococcus, particularly types 3 and 4 and the influenza bacillus are the predominating secondary invaders in influenza. In chronic conditions, such as tuberculosis, nephritis, malignancy, leukemia and other diseases, the streptococcus and pneumococcus are the chief causes of the terminal infection. It seems unlikely that the organisms causing secondary infection in such a variety of conditions are all introduced from without. They may sometimes be acquired from other persons, but it is more logical to assume that many patients succumb to the secondary invasion of the organisms of their own oro- and nasopharynx. It should also be pointed out that from the same sources the complications of otitis media and inflammations of the accessory sinuses of the respiratory tract undoubtedly originate.

SUMMARY

Cultures were taken from the excised adenoids to determine more definitely the flora of the nasopharynx. Pathogenic bacteria were found in every specimen. Of the more important organisms, hemolytic strep

tococci occurred in 61%, pneumococci in 65%, B. influenzae in 40.9%, B. diphtheriae in 12%. Other streptococci, diphtheroids, staphylococci, gram-negative cocci, B. mucosus capsulatus and B. fusiformis were encountered.

The depths between the folds and bottoms of the crypt-like structures of the adenoids harbored hemolytic streptococci, pneumococci and B. influenzae in larger numbers than the epithelial surface or the nasopharyngeal swabs.

A series of nasopharyngeal swabs of normal children revealed the same organisms in a considerable percentage; it is therefore evident that these organisms are a part of the normal flora of the nasopharynx.

The removal of tonsils and adenoids reduces the bacteria of the nasopharynx, but considerable numbers still persist.

The adenoids, like the tonsils, are to be considered common foci where dangerous bacteria flourish and sources from which secondary infection may arise in the course of primary acute respiratory diseases and of chronic debilitating diseases.

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THE FOCAL REACTION

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During the course of recent medical history our store of knowledge concerning resistance to infections has been greatly advanced, not only by new methods of research, but by new instruments of precision and by innumerable new discoveries in the collateral fields of science. Immunology in particular gave brilliant aid to both the theory and practice of medicine. The more recent results with chemotherapy, too, are of great value.

There remains withal a definite number of acute diseases that, once established, are still beyond our therapeutic influence, even though our preventive immunization against some of them is effective. When we examine the more chronic infections—granulomatoma, for instance—we must frankly admit that our chief reliance is still the intangible "general resistance," that elusive something which so far has escaped our efforts of exact definition.

Perhaps we have erred to a degree in neglecting to study the reaction of the body to disease—the inflammatory reaction—on a sufficiently broad basis. We have studied separate diseases and local inflammation and partial phenomena, but when one surveys modern medical literature it becomes apparent that the large share that the body as a whole plays in resistance to a pathological alteration, even though seemingly a localized one, is either wholly ignored or our deficiencies in knowledge glossed over under the convenient term "antibodies." "Antibodies," in the strict immunological sense, are by no means the sole factors in resistance, perhaps not even the chief factors in the mechanism.

In the study of inflammation, phagocytosis, the rôle of fibrin, the diluent effect of the fluid exudate, alterations in nerves and blood vessels, local changes in the hydroxyl-hydrogen balance, antibody response (both local and general) and numerous other factors have been considered; but, among the reactions, two that are seemingly of fundamental importance seem so far to have received little attention. These concern (1) the possible activation or stimulation (*Plasmaaktivation* of

Weichardt) of remote cells and organs by products absorbed from the inflammatory focus and (2) the effect which alterations in the permeability of the cell membrane have on increasing or diminishing susceptibility to intoxication.

Recent studies in this direction seem of considerable interest in clarifying our conception of certain phenomena observed in connection with inflammatory reactions; and the studies dealing in particular with the focal reaction might warrant a more detailed discussion, the more so since a number of European papers dealing with the subject have not been generally accessible.

That a pathological lesion, localized in some remote portion of the body, may unfavorably influence a pathological process elsewhere seems logical enough; that a pathological alteration (and this includes induced irritations as well) might favorably influence a coexisting process we cannot so readily accept, yet it is just this fact that we must recognize, study and, if possible, utilize therapeutically. The practice of irritating chronic inflammatory foci in order to bring about healing has been in use since time immemorial. A variety of procedures were devised that ranged from the actual cautery, acupuncture, the seton and the fontanelle, to the milder forms of counterirritation still found in the household even if no longer in the clinic. Curiously enough, measures such as these were in use among all primitive races. Common usage, as well as perhaps occasional successful application, led early to speculation concerning the mechanism of its therapeutic action and a number of fallacious theories were evolved, the scientific refutation of which led finally to complete scepticism concerning the whole subject. But in some form or other we find it cropping out again and again—in the fixation abscess of Fochier and in the autoserotherapy of Gilbert, and clearly recognized in the Bier treatment by hyperaemia.

In the more modern methods of nonspecific therapy we find a more extended effort to make use of the same general phenomenon. But here, just as in former immunological studies, we have given perhaps too much attention to the resistance of the diseased organism to infection, and not enough to the forces that prevent the body from becoming diseased. This Much (1) would designate as nonspecific immunity (*unabgestimmte Immunität*). Morgenroth, Biberstein and Schnitzer (2) have already approached the problem experimentally, in that they have demonstrated that a coincident avirulent streptococcus infection (in mice) will protect from the immediately fatal effect of a peritoneal injec-

tion of highly virulent streptococci. Citron and his associates (3) have added their observations with chicken cholera in guinea pigs, in which a subcutaneous injection given just preceding an intraperitoneal injection (normally rapidly fatal) alters the course of the infection, so that merely the chronic type of infection results. I cite these experiments in acute infections in experimental animals merely to illustrate the broad character of the field that is open for study, a field in which the local reaction occupies but a small part.

Ever since the tuberculins were introduced early in the nineties the concept of the focal reaction, the *Herdreaktion*, at the site of the inflammatory lesion has been so closely associated with the diagnostic and the therapeutic principles of tuberculin as hypothecated by Koch that the field has, to a great extent, been limited largely to a consideration of this particular disease. This view of the focal reaction, exemplified, let us say, in a local disease such as lupus or an apical involvement, needs no further elucidation. By some the term, local reaction, is however used synonymously; it should of course be reserved for those reactions that occur at the site of the injection of the tuberculins.

By common consent we may assume that positive local and general reactions are regarded as corroborative evidence that at some time the organism has been infected with tubercle bacilli. To the focal reaction we generally attach greater significance, in so far that the observation of focal disturbance following tuberculin injection is regarded as proof not only of infection but of activity as well. It is regarded as strictly specific in the sense that only tuberculous processes respond to tuberculin injections. On this assumption the immunologists have elaborated theories to picture the processes going on at the site of the reaction. According to Wassermann and Bruck the reaction takes place when the injected tuberculin joins with an "antituberculin" at the focus and complement is bound by these two reacting bodies. The fixed complement is then able to "digest" the focal material and so cause the well known lytic phenomena that we associate with focal activation. Wolff-Eisner and others have expressed the idea that all the tuberculin manifestations are due to preformed specific "lysins." These break up the nontoxic tuberculin; and only the tuberculin so altered can initiate the various reactions. The entire mechanism, according to the humoral views, depends on the presence of lysins, that is, "much lysin—much reaction."

As a matter of fact, little or no evidence has been put forward to support this humoral theory; but, on the contrary, much convincing evidence discredits it. No antibodies of the kind hypotheticated have been demonstrated. Nor does a parallelism exist between the local, focal and general reactions, such as would be predicated if the reaction were a humoral one. On the contrary, while a general reaction follows in the wake of a focal reaction, the local reaction is under these circumstances suppressed. But practically every clinical phenomenon has, nevertheless, been interpreted according to these theories, with resulting confusion. One illustration will suffice. Menzer (4) noted that tuberculous foci would respond with a typical *Herdreaktion* after the injection of a streptococcus vaccine and that tuberculous patients would very frequently have a general reaction. From this he drew the conclusion that such reactions merely indicated that a secondary infection with streptococci had been imposed on the focus and that the evidences of reaction which he observed merely confirmed the specific concept.

It was largely through the demonstration of the focal or *Herdreaktion* that the theory of specificity for the tuberculins gained wide credence. We fitted a theory to the observation, and then proceeded to interpret every clinical observation in this or the related fields to conform to our theory. Reasoning in a vicious circle retarded the study of actual clinical conditions; and particularly held back the proper recognition of certain factors in cellular resistance which are of great importance not only in tuberculosis but in inflammation in general.

Inasmuch as experience with tuberculosis is common to every physician, the consideration of the focal reaction in the tuberculous is perhaps of greatest interest and importance; but the problem is so closely bound up with inflammatory reactions in general that it may be permissible to include certain references to conditions of nontuberculous origin in this discussion.

CONCERNING THE SPECIFICITY OF FOCAL REACTIONS

Perhaps the fact that the focal reaction is not a specific reaction must first be emphasized. Practically every inflammatory focus, irrespective of its etiology, will react (focal activation) to tuberculin, as well as to a great variety of other agents, chemical or biological in character. Workers in tuberculosis have long recognized the fact that the tuberculous lesion responds with a focal reaction to a variety of substances.

Baldwin mentions nucleoprotein, nuclein, albumoses, cinnamic acid, cantharidin, pilocarpin. Fishberg adds potassium iodide and creosote. But the recognition that nontuberculous inflammatory foci will likewise respond to such agents has only been discussed in recent years. Perhaps the paper of Schmidt (5) is of greatest value in this connection in so far as the theoretical consideration of the problem is concerned: his practical deductions are however open to serious criticism, as will be pointed out later.

Schmidt began his observations on the Poncet type of arthritis. This tuberculotoxic form of disease exhibits a well marked focal reaction following the injection of tuberculin; that is, the joint becomes more painful and swollen, there is an increased limitation of motion and the tissues become hyperaemic. This stage is later followed by improvement in the clinical picture (the negative stage is followed by a positive one), the end results usually being an improvement over that obtaining before the tuberculin injection.

But Schmidt found that if, instead of injecting tuberculin, milk was used, he obtained exactly the same reaction at the site of the lesion, during the same time period, and with the same positive phase following in the wake of the reaction (that is, a therapeutic effect). Further, when he turned to nontuberculous joint lesions and injected minute doses (0.001 gram) of old tuberculin (relatively rich in nonspecific proteins) he obtained a similar focal reaction.

CLASSIFICATION OF FOCAL REACTIONS

The German clinicians have gathered considerable data concerning the reactions that follow milk injections (used merely as a convenient nonspecific protein) and Schmidt has arranged the following groups in which there is a response with a typical focal reaction following milk (and other) injections:

1. Inflammatory foci of infectious origin
2. Localized inflammatory processes endogenous or traumatic in origin
3. Diatheses

1. Classified under the first group we include pulmonary foci of tuberculous origin. Schmidt and Kraus (6), Petersen (7), Holler (8), Dollken (9) and others have called attention in recent papers to this phenomenon. Proteoses, iodides, milk, nucleins, among other agents,

will bring about a focal reaction and a sharp general systemic reaction. *Tuberculous foci in lymph glands, kidney, genitourinary tract* and elsewhere respond in a similar manner. Closely related we find the effect on *leprous lesions*. Josephson (10) describes the activation of a case of macular leprosy following the accidental injection of a relatively large intravenous dose of vaccine.

Among the nontuberculous inflammatory foci can be included the *tonsils* which at times flare up after a nonspecific injection. Schmidt describes such a case in which a milk injection activated a latent angina with a coincident appearance of mild joint pains. *Furuncles;* inflammatory activation can be observed following milk injections in cases of furunculosis and in some instances more than the usual amount of constitutional reaction. Thus the normal diabetic does not react with a temperature rise to milk injections, but if suffering from furuncles will frequently do so. *Arthropathies;* the negative phase with its increased pain, swelling and limitation of motion is not an uncommon clinical observation and has been fully discussed by a number of observers. *Buboes and adnexal inflammations.* Inflammatory foci in the *appendix and the gall bladder. Erysipelas.*

The activation of quiescent *malarial foci* has assumed diagnostic importance and will be more fully discussed. *Papules of syphilitic origin* react with an increased hyperaemia just as do other inflammatory foci.

In a general way, one can make the statement that any circumscribed inflammatory process, irrespective of its bacterial etiology or its location, will frequently light up with a typical focal reaction after a nonspecific injection, and that usually within twenty-four hours after the injection. Of these various processes tuberculous lesions are perhaps more sensitive because of a more profound sensitization of the tissue cells of the host *against protein in general*, as suggested by a recent discussion by Wolff-Eisner (11).

2. Localized inflammatory processes endogenous or traumatic in origin. In this category must be placed certain of the *toxic forms of arthritis*, such as the Grocco-Poncet type; *inflammatory lesions of the kidney;* inflammatory lesions of the eye, including iritis, albuminuric retinitis, etc. Healing fractures, such as Dollken has described, respond with a typical focal reaction.

3. Localized lesions on a basis of a diathesis. Using this term in the broader significance as defined by Pfaundler, the following conditions might be included:

The *lancinating pains of tabes* which at times follow nonspecific injections, as well as an occasional gastric crisis.

In *general paresis* the psychic state may be decidedly disturbed and the disease manifestations become more apparent following nonspecific injections. This is a not uncommon experience in the treatment of paresis, as outlined by v. Jauregg. In *epilepsy* an attack may be inaugurated if a large dose of a nonspecific agent is administered, just as in chronic alcoholics an attack of *delirium tremens* may follow such an injection.

In this sense we may consider that a latent symptomatology may abruptly unfold its various manifestations following injections.

ACTIVATING AGENTS

We must for a moment stop to consider the agents that are involved in eliciting this nonspecific reaction. Because of the early work in this field we commonly regard the nonspecific reaction such a one as follows the intravenous injection of a vaccine, for instance, typhoid vaccine, or such protein derivatives as proteoses, or, more recently, the use of milk or casein given intramuscularly. Not only do such injections bring about the reaction and focal activation, but certain general biological alterations, such as coincident but remote disease processes. Metabolic alterations and alterations of endocrine origin, fatigue, intestinal intoxication, blood-letting, X-ray exposures, trauma, alterations in the skin (counterirritation), as well as a number of drugs bring about exactly the same focal alteration. The activation of a tuberculosis by an angina, a remote trauma, the menstrual cycle, by influenza or measles; the provocation of a malarial paroxysm (the result of a splenic focal activation) by an intercurrent disease, by prophylactic vaccination, by severe fatigue, long railroad journeys, overheating, exposure to intense light, chilling or drenching, dietary faults, alcoholic excess and the various other factors that the experience of the war has brought to light; the activation and focal reaction about latent arthritic lesions such as Pemberton has pointed out (by raying, radium, thyroid extract, excitement, etc.); the classical activation of gonorrhoeal processes by a variety of seemingly remote causes; the precipitation of lancinating pains or a tabetic crisis following a "cold;" the origin of a delirium tremens following a trauma; these are but a few examples of a universal phenomenon heretofore commonly observed but not recognized as having a common background.

A BROADER CONCEPTION OF THE FOCAL REACTION

It is very probable that many puzzling clinical manifestations and unusual features of certain diseases may readily be explained and will appear in a very simple light if we keep in mind the basic idea that the particular symptom complex under study may be due solely to the activation of an inflammatory focus of exogenous or endogenous origin in the manner of the *Herdreaktion.* Many of the curious metabolic disturbances at times associated with diabetes and nephritis can readily be accounted for on such a basis. Even pharmacological study reveals evidence of this same nonspecific effect on inflammatory lesions. Thus, the commonly observed Jarisch-Herxheimer reaction (the flaring up of syphilitic skin lesions under specific treatment) is an example, while the activation of a tuberculous lesion after iodide medication is an even older observation. So too, we may get an ulcerative catarrhal condition when an uraemic colitis is carelessly treated with calomel; while Königer (12), in a recent paper, has even been able to demonstrate that the antipyretics given in proper interval doses, all bring about a nonspecific *Plasmaaktivation* and must therefore be included among those potentially capable of bringing about focal reactions.

This widening of the concept of the focal reaction makes it of decided importance in the special pathology of internal diseases. Acute conditions may often be nothing more than the exacerbation of heretofore latent processes of definite bacterial etiology or perhaps of a diathesis. On this basis can be explained the fact that children often respond with a severe angina to a fault in the diet; that an appendicitis will become acute following an angina, a remote trauma or an injection of a prophylactic dose of vaccine; or that a gastric crisis or lancinating pains will commence after some remote exciting cause or an asthmatic attack occur under the influence of some meteorological or climatic alteration.

In many ways this basis for the immediate etiology of an appendiceal inflammation or the flare up of a gall bladder infection seems more rational than the suggestion that we deal with a specific localization of bacteria, so altered in their metabolic demands and peculiarities that they will grow only in certain tissues. This latter hypothesis, which Rosenow (13) has developed, ignores the fact that in the history of acute inflammatory processes one may note a preceding history of remote trauma or systemic shock of some kind or a metabolic disturbance of nonbacterial origin, just as often if not more frequently than a history of a preceding infection.

THE DIPHASIC CHARACTER OF THE FOCAL REACTION

While in the preceding pages attention has been centered on the fact that a variety of agents may be used to elicit the tuberculin reaction (focal), one can also determine that nontuberculous processes respond with a focal reaction to tuberculin. Schmidt illustrates this with two convalescent cases of polyarthritis which had been free from fever and local symptoms, pain, etc., for over two weeks. Both cases on receiving 0.001 gram, O. T., subcutaneously, responded with severe pain in the joints previously involved, slight periarticular swelling and some limitation of motion; the duration of the reaction was of course transient. I have had occasion to repeat these observations of Schmidt's and in a considerable percentage of the cases studied I have found that the observation holds true. In processes that can be observed at the external surfaces of the body, particularly in the violent activation of chronic inflammatory lesions, one can observe the dualistic nature of the reaction—and this is of paramount importance in its therapeutic application—a negative phase in the sense that the tissue is altered more from the normal than heretofore, that is, the evidences of inflammation are increased, pain and swelling augmented, function impaired, etc.; then a positive phase during which there is a progressive diminution of the inflammation until the preinjection status is again reached or passed, the balance swinging in this direction until practically normal conditions are restored. Augmentation of the inflammation is followed by a diminution until healing is accomplished—the pendulum swings from one side to the other in the wave like curve that we find expressed so commonly in many biological processes—in the opsonin curve, the antiferment curve, in the leucocytic response, in the coagulation mechanism, in cell permeability, all indicative of the exquisite lability of the balance that exists in living protoplasm.

This diphasic alteration of the pathological picture comes to notice particularly in the arthritic focal reactions where soon after the provocative injection (typhoid bacilli, milk, tuberculin) there can be observed increased pain, redness, and swelling and limitation of motion, followed by an analgesia, a lessening of the swelling and restitution *ad integrum*. By way of the circuit of an increase in inflammation we reach the therapeutic goal of a restoration to normal.

The focal reaction has its counterpart in the general reaction of the patient that usually accompanies the reaction, occasionally in the

tuberculous, more often in acute infections. Let us examine the reaction that follows when a typhoid patient is injected with vaccine or milk or proteoses or other nonspecific agents which in the normal individual cause little or no reaction. Following the injection there is first an intensification of symptoms, subjective as well as objective. A chill, a rise in temperature, an increase in pulse rate, gastrointestinal engorgement with occasional nausea and vomiting, increased tendency to hemorrhage, occasionally increased mental impairment, delirium, etc. Subjectively the patient complains of headache, chilliness, lassitude, general muscular and joint pain. The course now changes and the positive phase sets in. When the headache ceases the patient usually falls into a profound sleep, the temperature comes to normal either temporarily or permanently, the pulse rate is lowered and the quality becomes better, the sensorium is clear, the patient feels well. During the course of the next day or two the rose spots fade, the splenic tumor recedes, the diazo reaction becomes negative. The blood culture may or may not become negative, shortly after the injection. This period corresponds obviously with Weichardt's period of *Plasmaaktivation*. This duality observed in both the focal reaction and the general reaction is of utmost therapeutic interest.

It is reasonable that we would seek to make the negative phase, representing an augmentation of the inflammatory process, as short as possible and the positive phase relatively intense and protracted. But the possibility arises, based on clinical observation, that the degree and extent of the positive phase (curative) is closely dependent upon and correlated with the negative phase, that is, that their relative intensity is proportional. We may conceive the negative phase, which in point of time always precedes the positive one, as an exogenously produced irritative process; the positive one, on the other hand, as an endogenously prepared, more or less physiological process of vital repair. Clinically, as far as focal reactions are concerned (as for instance in tuberculosis), it is chiefly the negative phase that comes to our attention, in the form of the activation phenomena with which we are familiar. But from the analogous studies in focal reactions elsewhere it must be assumed that with proper dosage, the positive phase and the increased tendency to healing follows about the lung focus just as it does about a joint lesion which can be objectively studied. In this duality of the reaction lies the usefulness of the focal reaction as a therapeutic measure.

THE MECHANISM OF THE FOCAL REACTION (VASCULARIZED INFLAMMATORY FOCI)

During the course of the past five years we have become familiar with the biological alterations that take place in the organism after tuberculin injections, as well as after so called nonspecific agents. These changes are numerous and complex but the more important can be placed in two groups, (1) those that deal with cellular stimulation and those (2) that result primarily from alterations in the permeability of the cells.

The former have been broadly included by Weichardt under the term *Plasmaaktivation.* Under the stimulus of moderate doses of nonspecific agents cellular activity is markedly increased. This finds its expression in increased secretory activity of gland cells, increased activity of muscle cells (myocardium), increased activity of leucocytes (phagocytosis), etc. The changes that take place in the permeability of the cell membrane have been studied by Luithlen (14), by Starkenstein (15), and others, and represents a decidedly diphasic phenomenon. The permeability of the capillaries is first increased as evidenced in the great increase in the lymph flow and in the concentration of the blood; the permeability of the tissue cells is increased—with a resulting outpouring of enzymes, of fibrinogen and prothrombin, of immune bodies, etc.; the increased permeability of the nerve cells is associated with a lowering of the threshold for nervous impulses and becomes manifest clinically in increased susceptibility to pain, general irritability, headache, etc. When this first phase has passed, compensation takes place in a lessened permeability of the cells, with effects that are to be anticipated—lessened susceptibility to intoxication, lessened nervous irritability, lessened exudation, a lowering of enzyme concentration, etc.

Numerous other observations have been made that are, in my opinion, subordinate in interest to these two fundamental alterations in the permeability of the cell membranes and the general stimulation of protoplasm.

With these considerations in mind we can approach the study of the mechanism possibly involved in the focal reaction about inflammatory tissues from a relatively simple point of view.

In an inflammatory focus, supplied with highly vascularized granulation tissue, the systemic effects of a tuberculin injection or the injection of a nonspecific agent will bring about (1) an increase in the exudation

of fluids, with increased redness and swelling, because of the transient increase in the permeability. With this there is associated (2) an increase in pain and tenderness both because of the increased pressure and the lowering of the threshold of nerve stimuli. There will be (3) increased digestion at the focus of inflammation; if there is no necrotic material present in the focus there may be no evidence of increased systemic intoxication; if the amount of necrotic material is large there will first result an increase in systemic intoxication when the material split down is absorbed, and with more complete digestion at the focus complete detoxication may result.

All these changes we associate with the focal activation that follows nonspecific injections. To these must be added another factor and one more complex. It concerns the observation that any cell previously involved in an inflammatory reaction responds to stimuli of all kinds more readily than a normal cell. Objectively we can observe this in involuting skin lesions.

From the immunological point of view the work of Conradi (and Bieling (16) and Bieling (17) is of interest in this connection. They have found, for instance, that if a rabbit is first immunized with typhoid bacilli, the dosage of bacteria needed to bring about a high degree of immunization against some other organism—cholera, for instance—is only a fractional part of that required to immunize a normal rabbit. This alteration in immunity response, which is, of course, omnicellular, merely illustrates the alteration in reactivity that we find in the epidermal tissues, and very likely in inflammatory tissues in general.

The augmentation in the inflammatory reaction which we have induced brings with it as we have seen an increased lymph flow. Coincident with it there has been a relative increase in enzymes,—proteases, ereptase (peptidase), lipase, etc.—an increase in the antibodies (if the patient has been previously immunized or if the infection has existed for some time) and an increase in the leucocytes (after the initial leucopenia), together with an increase in their phagocytic activity and an increased coagulability of the blood. The antibody, the leucocytic and the enzyme alterations must exert a considerable effect on an infecting agent as well as on the removal of necrotic material; the tendency toward restitution to the normal would be enhanced. It is this phase that we see in the so called second or positive phase. Its coincident constitutional effect that we witness in the euphoria, the lowering of the temperature, the improvement of the circulation, etc., is due to at

least three factors: (1) the destruction of toxic material at the focus after the primary increase in digestive activity, (2) lessened susceptibility of the cells of the body to intoxication (due to the lessened permeability), and (3) actual protoplasmic stimulation (from the nonspecific or specific agent injected, partly from the toxic material liberated from the inflammatory focus). This latter factor varies greatly and the clinical estimation of the possible degree of this variation requires experience and care.

TUBERCULOUS FOCI

If we turn now from the vascularized inflammatory focus to the tubercle, other conditions confront us. In Schmidt's paper we find the view emphasized that in the general tuberculin reaction we are most likely dealing with both specific and nonspecific factors, an opinion similar to that which we expressed in a previous paper (7). Schmidt had stated this concept as follows:

> But it is probable that in the question of specificity *or* nonspecificity the placing of the one versus the other is a mistake—that it should rather be the examination of specificity *and* nonspecificity, that is, that both factors enter into the reaction and it should be determined how far each factor is involved.

Our concept has been that while the systemic reaction was largely nonspecific in that the means used to elicit it need not be specific, the focal reaction itself, once initiated, brings in its wake a truly specific stimulation because the inflammatory reaction may lead to the liberation of disintegrating bacterial material and possibly even living bacteria. These substances would secondarily lead to a specific response on the part of the body.[1]

[1] It is this factor that Klemperer (18) in his recent criticism of Schmidt's claims has ignored. Klemperer found that following milk injections in tuberculous patients they did not become resistant to following injections of tuberculin and vice versa. Injections of milk bring about a febrile reaction in a large percentage of individuals, and a focal reaction in only a limited number of tuberculous patients, just as tuberculin injections are followed by focal reactions in an irregular number. If the injection of milk brings about (in the tuberculous individual) a systemic reaction without focal activation, a following injection of tuberculin may still give rise to a typical general reaction. If, on the other hand, a focal reaction results, either by specific or nonspecific means, local tuberculin reactions are suppressed for some time following the general reaction. Klemperer is however quite justified, both in his criticism of the local reactions reported by Schmidt following milk injections in tuberculous patients and in his views concerning the possible harm from activation of tuberculous foci following milk injections.

At least three factors must be considered in the mechanism of the focal reaction in tuberculosis, apart from the anatomical peculiarities of the tubercle as contrasted with other inflammatory processes.

Specific. These concern primarily a tissue sensitization against tuberculoprotein, strictly specific in character, cellular in its localization and not necessarily associated with the older conception that was built up about the humoral antibodies. Indeed, I am of the opinion that the latter may very well be relegated to a subordinate position in the field of tuberculosis. Inasmuch as this subject of tissue sensitization has been extensively discussed by a number of workers, particularly by Krause in this country, it will be unnecessary to enter into this phase here.

General hypersensitiveness of the tuberculous. Granted that the tuberculous focus responds to smaller doses of tuberculin than does a focus of nontuberculous origin, how are we to account for the fact in view of the practical avascularity of the tubercle? That the specificity concept of the immunologist will no longer explain the accumulated evidence is to-day acknowledged and Wolff-Eisner accepts the change in our viewpoint in a recent paper (11). I can but very briefly enter into the more lengthy theoretical discussion that he presents. He first emphasizes the relation that exists between the diet and exudative diathesis, defining this latter condition as due to the absorption of proteins and protein fragments insufficiently degraded in the intestinal tract, that is, a protein sensitization. He then develops the more or less definite association of the exudative diathesis and spasmophilia with scrofula. While he does not regard the scrofula as the cause of the diathesis, he inclines to the definition of Feer that "scrofula is tuberculosis on the basis of exudative diathesis (19)." Wolff-Eisner is inclined to the view that in tuberculosis there is evidence of an exudative diathesis with sensitization against tuberculin and also *against proteins in general.* This latter, which is nonspecific and general in character, accounts, in his estimation, for many of the evidences of similarity in the clinical course of tuberculosis and those observed in an exudative diathesis. Not only is scrofula "tuberculosis on the basis of a diathesis" but the tuberculous lesion itself, involving as it does the prolonged absorption of partially split proteins from the necrotic foci, may ultimately bring in its train symptoms that are commonly regarded as due to a diathesis. As such, he regards the changes observed in the cornea, skin lesions such as the tuberculids, and the decided alterations in the reactivity

of the sympathetic and central nervous systems to which Moro, Pottenger and Ferranini have called attention. While we have been familiar with the increased nervous lability of the tuberculous individual for a long time, we have failed to grasp the dependence of the increased irritability on the general hypersensitiveness to proteins. Not only does this nervous irritability indicate the close parallelism to the diathesis of the child, but the tendency to effusion is also evident in the tuberculous patient: one has but to call to mind the common appearance of pleural, peritoneal and joint effusions. The alteration in the vasomotor stability also finds its expression in the frequent appearance of urticarial eruptions after tuberculin injections.

It will be recalled from the previous discussion of the effects of nonspecific injections on the permeability of vascular endothelium that, depending on the dosage or the degree of irritation (or stimulation if we wish to use that term), there may result either an increase or a decrease in permeability. We may expect that the effect of the tuberculin (or the living virus) will also find some expression in changes in permeability in one direction or the other. According to Wolff-Eisner we find the clinical demonstration of this experimental observation in the effect of the tuberculous invasion of lung tissue, where in one instance we find an exudative change, in another an indurative process.

Plasmaäctivation. The third factor involves the consideration of the effects which any nonspecific provocative agent would have on an inflammatory lesion such as the tubercle. The tubercle would react as any seminecrotic focus of other etiology would react, were it not for the fact that the tubercle is practically avascular. Tubercles react to nonspecific injections (or to nonspecific stimuli of other origin) only when they are of the exudative type or when the connective tissue delimination of the tubercle is either incomplete or exceedingly labile. It is to be recalled that, as one of the results of plasmaäctivation, proteolytic enzymes appear in the serum and in inflammatory foci and that the polymorphonuclear leucocytes are increased in number and in activity. The augmented digestive activity results in a loosening of the connective tissue defense of the tubercle. If sufficiently intense a typical focal reaction (activation, absorption of necrotic material, and systemic reaction) can result in this way, just as after stimulation due to specific tuberculins.

THE THERAPEUTIC APPLICATION OF THE FOCAL REACTION

Schmidt is of the opinion that the focal reaction, elicited by means of specific or nonspecific agents (milk), is of definite value in tuberculosis and together with Krauss cites some twenty odd cases to support his view. While it is of course not to be denied that in a very limited number of cases this may be true, we are of the opinion that the tubercle offers a decided exception to the general rule that the active stimulation of a chronic inflammatory focus is of therapeutic value. We have pointed out that for vascularized inflammatory lesions such stimulation affords a rational method of therapy. In the tubercle we deal with the constant danger that the limitation of the lesions, by means of the connective tissue encapsulation, may be sufficiently disorganized, so that an extension of the process and irreparable injury may result. Irrespective, therefore, of the theoretical probabilities that therapeutic focal activation may be beneficial, in the tuberculous lesion it is a hazardous procedure. Before leaving the subject it must be pointed out that nonspecific stimulation of the tuberculous patient (not involving local reactions) has been found very useful, both with certain drugs (and these include the commonly accepted ones such as creosote, succinides, arsenicals, etc.) as well as with serum injections such as Czerny and Eliasberger have recently reported (20). That the milder nonspecific injections seem to have a decidedly stimulating effect on the metabolism of infants has been previously reported.

The peculiar therapeutic importance that attaches to the diphasic character of the focal reaction has been previously discussed. Through the existence of inflammatory foci in various organs the omnicellular stimulation, by means of the various nonspecific agents (the ergotropie of v. Groer), becomes to a certain extent an organotropie. It is in this sense that we must consider the effect of the treatment of general paresis by means of tuberculin injections and similar therapeutic measures. A combined therapy of nonspecific and aetiotropic agents may be of value and experiments in this direction have been reported by Kyrle and Scherber (21) who have used milk injections in conjunction with mercury in the treatment of syphilis, or the use of milk injections and salicylates in the treatment of arthritis, the use of milk injections and luminal in the treatment of epilepsy, or the treatment of lupus with tuberculin and salvarsan (the latter in this case serving as the nonspecific agent). Our older method of treating syphilis with alternating courses of mercury

and iodides made use of this form of a combined specific and nonspecific method for many years. The nonspecific effect of the mercury is, however, not marked. The effect is to be sought rather in the effect of the iodides in facilitating the diffusion of the mercury. If a more active agent than iodide is used the mercurialization of the patient can be very rapidly brought about.

SUMMARY

In view of the demonstrated fact that tuberculous foci may react to nonspecific stimuli and conversely that nontuberculous foci may react to tuberculin, the fallacy of the purely specific concept of the focal reaction is discussed. Focal reactions can be classified according to the Schmidt scheme as those that occur (1) about inflammatory foci of infectious origin, (2) about localized foci endogenous or traumatic in origin and (3) on the basis of diathesis. Such reactions take place not only after specific stimulation, but after a wide variety of biological alterations in the organism. This widened concept of the focal reaction would seem to make clear a number of common clinical observations, such as the flaring up of gall bladder, appendiceal, arthritic or other localized inflammatory foci after remote trauma, vaccination, gastrointestinal disturbance, X-ray exposure, fatigue, chilling, etc.

The focal reaction, no matter how elicited, is essentially a diphasic phenomenon, consisting in an augmentation of inflammation, followed by a diminution, and a tendency to complete restoration to the normal. In a general way it may be stated that the second phase (positive or healing) is closely related to the intensity of the first (or negative) phase, that is, the more intense the inflammatory reaction, the greater the tendency to a complete restoration to the normal. In this phenomenon lies the therapeutic usefulness of the focal reaction.

In the mechanism of the reaction two general factors have heretofore been largely ignored. These include the effects of the (1) so called *Plasmaaktivation* (Weichardt)—an omnicellular stimulation by means of proteins, protein split products and a large number of other substances—and (2) the alteration in the permeability of cellular membranes (with vascular, nervous, glandular and other effects).

In the mechanism of the focal reaction in tuberculosis at least three factors are involved: (1) a true and strictly specific sensitization of the organism, (2) a general hypersensitiveness of the organism against proteins and (3) a nonspecific reaction about the tubercle. This latter

is of course greatly modified by the anatomical peculiarities of the tubercle (avascularity, encapsulation, accumulation of necrotic material, resistance of the tubercle bacillus, etc.). Schmidt's deduction that focal activation in tuberculosis may be beneficial, while theoretically defensible, is clinically unwarranted because the reactions are beyond our control and the digestive processes incident to the reaction may destroy protective connective tissue and result in the dissemination of the disease.

In the therapeutic application of the focal reaction in nontuberculous disease, attention is directed to the fact that favorable results have been reported from combined nonspecific stimulation and the use of aetiotropic agents. Thus milk injections and mercury (in syphilis), tuberculin and mercury (in general paresis), milk injections and salicylates (in arthritis), milk injections and luminal (in epilepsy), salvarsan and tuberculin (in lupus), etc., have been reported in this connection.

REFERENCES

(1) Much, H., Schmidt, H. and Peemöller, F.: München. med.Wchnschr., 1920, lxvii, 1057.
(2) Morgenroth, Biberstein and Schnitzer: Deutsch. med. Wchnschr., 1920, xlvi, 337.
(3) Berliner, M., and Citron, S.: Ibid., 1920, xlvi, 997.
(4) Menzer: Med. Klin., 1912, viii, 311.
(5) Schmidt, R.: Deutsch. Arch. f. kl. Med., 1919, cxxvi, 1.
(6) Schmidt, R., and Krauss, D.: Med. Klin., 1919, xv, 503.
(7) Petersen, W. F.: Arch. Int. Med., 1918, xxi, 14.
(8) Holler, G.: Beit. z. Klin. d. Infektionsk., 1917, vi, 92.
(9) Döllken: Berl. klin. Wchnschr., 1914, li, 1807 and 1841. München. med. Wchnschr., 1919, lxvi, 480.
(10) Josephson: Med. Rec., 1920, xcviii, 607.
(11) Wolff-Eisner, A.: München. med. Wchnschr., 1920, lxvii, 93.
(12) Königer, H.: Ibid., 1920, lxvii, 1136.
(13) Rosenow, E. C.: Jour. Inf. Dis., 1916, xviii, 383.
(14) Luithlen, F.: Wien. klin. Wchnschr., 1913, xxvi, 653.
(15) Starkenstein, E.: München. med. Wchnschr., 1919, lxvi, 205.
(16) Conradi and Bieling: Deutsch. med. Wchnschr., 1916, xlii, 1280.
(17) Bieling, R.: Zeit. f. Immunitätsforsch., 1919, xxviii, 246.
(18) Klemperer, F.: München. med. Wchnschr., 1920, lxvii, 1277.
(19) Feer, E.: Lehrbuch der Kinderheilkunde, Jena, 1911.
(20) Czerny and Eliasberger: Monatsh. f. Kinderheilk., 1920, xviii, 1.
(21) Kyrle and Scherber: Wien. klin. Wchnschr., 1917, no. 22.

SELECTIVE ORGAN STIMULATION BY ROENTGEN RAYS: ENZYME MOBILIZATION

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SELECTIVE ORGAN STIMULATION BY ROENTGEN RAYS: ENZYME MOBILIZATION

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AMONG the numerous biological phenomena which have been studied in connection with *x*-rays and other radiant agents, the effect on enzymes has received considerable attention. Usually such studies have been made by subjecting enzymes *in vitro* to rays of varying intensity. The opinion has been repeatedly expressed that intracellular enzyme activity must be altered following the raying of tissues, and a number of observations have been recorded which relate to this subject. Thus Heile[1] believed that the destruction of the leucocytes would liberate large amounts of proteolytic enzymes and that these liberated enzymes would then be able to attack other tissues. Neuberg,[2] who worked with the effect of radium on tumors, formulated his ideas in the following manner. The radiation causes a destruction of all the enzymes which have to do normally with the anabolic processes of the cell, while those that bring about autolysis are not altered. He showed that rayed carcinoma tissue autolyzed more rapidly *in vitro* than unrayed tissue. A number of other workers have reported experiments similar in character (Packard, Heile, Wohlgemuth, etc.).

So far no experiments have been reported which deal with the actual demonstration of alterations in the titer of the serum enzymes after *x*-ray or similar stimulation. Under the circumstances we thought it would be of interest to determine (a) whether such an alteration in titer does take place, (b) whether variations in the serum enzymes take place when different organs are stimulated, and (c) the influence of different degrees of stimulation on the alterations in titer.

In our experiments we have used dogs rather than the smaller laboratory animals. The larger serum amounts can be withdrawn from them for study without injury; the normal serum enzyme titers more closely resemble those obtained in the human, and in smaller animals the raying of organ groups is technically much more difficult and uncertain. Even in dogs it is of course impossible to confine the rays to any one organ, so that when the liver area is rayed, parts of the pancreas, the gastro-intestinal tract, etc., will necessarily be included to some extent and the results must be interpreted with this consideration in mind. In our preliminary experiments we used large doses (Coolidge tube, 10 inch distance from the skin, 8 ma., 5 to 8 inch back-spark without filter) for periods ranging from fifteen minutes to one hour. With the shorter periods of exposure we found that raying of the liver and intestinal areas resulted in some mobilization of enzymes, but that following the longer periods the titer of the enzymes diminished. We then proceeded with a filter (4 mm. aluminum) and reduced the time of exposure to the following periods—five minutes, ten minutes and twenty minutes, the latter with and without a filter. When so rayed, considerable alterations in the serum enzymes could be determined depending on the area rayed and on the duration of the exposure.

Our studies on the dogs included the nitrogen secretion, the non-coagulable nitrogen of the serum, the leucocyte and differential count, the coagulation time of the blood, the titer of the serum protease, peptidase, esterase (lipase), diastase, the anti-trypsin, and the complement titer. During the course of

the experiments a number of dogs were used for each regional exposure, but in the accompanying chart the average for two dogs has been used.

Nitrogen Excretion.—The animals were kept in metabolism cages and on a fixed diet. With the exception of the periods following the longest liver exposures there was no apparent increase of nitrogen excretion following the *x*-ray periods in the course of the experiments. Following the twenty minute exposure of the liver area the average nitrogen excretion was, however, increased approximately 60 per cent for a period of four days following the exposure.

Non-coagulable nitrogen of the serum.—This was altered to a considerable extent only following raying of the liver area where an increase of as much as 50 per cent was occasionally determined after raying for ten minutes or more. This increase persisted for several days in such animals. Hall and Whipple [3] in their experiments with lethal *x*-ray doses obtained such increases with considerable regularity.

The Leucocyte Count.—The leucocytic reaction showed considerable differences with the different regions stimulated. In the following tabulation the normal count taken before the *x*-ray exposure is contrasted with the average of the counts obtained for the one-half hour, one hour, five hour, twenty-four hour, forty-eight hour and seventy-two hour periods:

CHART I

	Liver	*Spleen*	*Intestine*
Normal	4,000	10,000	3,000
After 5 min. Exposure	7,600	16,700	3,400
Before	3,200	14,200	3,000
After 10 min. Exposure	5,600	13,400	8,200
Before	3,400	11,400	7,400
After 20 min. Exposure	10,000	11,300	12,900
Before	4,800	13,700	14,300
After 20 min. Exposure (without filter)	8,100	14,300	17,450

In Chart I the effect of the raying on the leucocytes is graphically apparent. It will be observed that following the raying of the liver there resulted a leucocytosis of transient nature; following raying of the intestinal area the effect of raying was a step-like increase until a relatively high leucocytosis (15,000) was maintained. The two dogs used for the spleen experiment commenced with a relatively high leucocyte count (as well as a high serum enzyme titer) and raying did not materially alter the count. The commonly observed leucopenia that follows raying in the human was not observed in this series of animals with the doses that we employed.

Differential Count.—Following the raying of the hepatic area three of four dogs observed showed a well marked eosinophilia. This ranged from 5 per cent to 20 per cent and persisted for a number of days after the exposure. Raying of the intestinal area and the splenic area resulted in general in a diminution of the mononuclear elements and a relative increase in the polymorphonuclear cell forms.

Blood Coagulation.—A number of European observers have recently discussed the increase in coagulability of the blood which they have observed after raying of the spleen. Our observations were made with the capillary tube method and gave us a normal clotting time that varied between three and four minutes. Promptly following the raying of the animals this was usually reduced from one to two minutes, the blood clotting so rapidly that the bleeding of the animals was at times very difficult. In our series there seemed very little difference whether the splenic or hepatic or intestinal area was rayed, the result being apparent no matter what region was stimulated. In studies reported in the following paper it was found that an increase in the thromboplastic substance as well as an increase in fibrinogen occurred after the raying.

The Serum Enzymes Protease.—The proteolytic titer of the serum was estimated by the chloroform method which has been described elsewhere.[4] While open to objections,

it nevertheless seems to give a fair index of the proteolytic capacity of the blood. As will be observed in the chart (the nitrogen digest of the serum is expressed in 1/10 milligrams), raying of the hepatic area increased the serum proteases after the ten minute exposure and the twenty minute exposure. The long exposure when unscreened was no longer effective. Protease appeared in the serum after raying the intestinal area, too, while raying the spleen seemed in general to be followed by a diminution of the originally high titer.

Peptidase.—Peptidase was titrated by allowing varying dilutions of serum to digest Witte peptone and determining the liberation of tryptophan by means of the simple bromine color reaction. Normal dog serum contains practically no peptidase; after raying the liver the enzyme makes its appearance but never to the extent that was observed after raying the intestinal area. Raying of the splenic area was never followed by such mobilization.

Lipase.—Serum esterase was determined by incubating ethyl butyrate with serum and titrating the resulting formation of acid by means of 1/50 NaOH. Moderate doses of *x*-rays seem to mobilize this enzyme after raying the hepatic as well as the intestinal area; raying of the splenic area, on the other hand, seemed to cause a gradual reduction in the amount of lipase in the serum. This was not, however, a constant finding in all of our animals, for in some raying of the spleen was at times followed by a well-marked mobilization of lipase, especially following a single dose of moderate intensity.

Serum Diastase.—The titer of the serum diastase was determined with the Wohlgemuth method of starch digestion by varying dilutions of serum, and the titer is expressed in units (24 hour digestion). Raying of the hepatic area was usually followed by a short sharp rise in the diastase curve. In the chart this does not become apparent because the average for the six bleedings after the *x*-ray exposure was not greatly altered. Raying of the intestinal area did not generally influence the titer, while raying of the splenic area was followed rather by a diminution.

Complement.—The complement titer (hemolytic titer) was followed in a number of animals, but seemed unaffected by the rays in the dosage that we employed. (Not charted.)

Anti-ferment.—Fluctuations in the titer of the serum anti-ferment were quite marked. As a rule the titer increased for a short time following the exposure, then diminished and gradually increased again for from forty-eight to seventy-two hours. The most marked effect followed the more intense periods exposure. (Not charted.)

DISCUSSION

While the clinical development of the *x*-ray and the related radiant agents has been confined largely to the field of diagnosis and local therapeusis, the possibility of remote therapeutic effect has not been uninteresting to medical observers. Among them Edsall and Pemberton [5] endeavored to utilize the effect of the *x*-ray in stimulating autolytic processes by their effort to hasten the autolysis of unresolved pneumonia by means of *x*-ray. Since their publications a number of observers have apparently sought similar applications. Perhaps the work of Manukhine [6] is of particular interest in this direction. Manukhine, aware of the influence that the spleen seems to have in favorably influencing the course of a tuberculous process, found that when he rayed the spleen of tuberculous animals (and patients) the tuberculous process improved. When, on the other hand, he rayed the liver the tuberculous process rapidly extended. He sought to explain the result because of the differences that follow in the leucocytic reaction with the different organs stimulated. While this is not to be excluded, we are nevertheless of the opinion that other factors must be taken into consideration, among them the effect of the serum enzymes which as we have shown in these studies takes place after *x*-ray stimulation.

Other observers besides Manukhine have

taken advantage of the remote effects of *x*-ray stimulation to bring about therapeutic effects. Drey and Losser [7] have but recently called attention to the effect of splenic *x*-ray stimulation on bronchial asthma, an effect first observed by Schilling;[8] Stettner [9] has used the stimulating property of *x*-rays in increasing healing and ossification and has also made the application in the stimulation of glands of internal secretion, as for instance in raying the head to stimulate the hypophysis to promote growth.[10] Stephan's [11] work in studying the effect of splenic raying on the blood coagulating mechanism will be discussed in the following paper.

The regional stimulation of the abdominal organs such as we have reported in this paper may perhaps be of some significance in the study of the intoxications brought about by *x*-rays and similar agents. Using small laboratory animals Denis, Martin and Aldrich [12] found that intoxication was dependent on exposure of some part of the gastro-intestinal tract and they are of the impression that the intoxication is closely concerned with a reduction of the alkali reserve found by them following raying of intestinal areas. Hall and Whipple [3] regard the intoxication as a protein intoxication following injury of the gastro-intestinal mucous membrane. If the effect is in the nature of a non-specific protein intoxication we must keep in mind that some of the remote therapeutic effects occasionally observed may be closely related to non-specific therapeutic results obtained by other means, such as vaccine, proteose, milk or tuberculine injections.

If pathological lesions are to some extent influenced by the serum enzymes it would seem to us possible that through *x*-ray organ stimulation or stimulation by other related agents a means of such therapeutic control is offered. We are of course at the present time unable to state definitely whether the metabolism of the normal cell is altered by the alteration in titer of serum enzymes; but where we deal with necrotic tissue it would seem plausible that an increase of the proteolytic serum enzymes would hasten the removal of such material provided that other factors that influence digestion (hydroxyl-hydrogen balance, anti-ferment concentration, etc.) are favorable. It might seem of interest to keep such enzyme mobilization in mind when studying the remote effects of radiant agents, not only where we have to deal with toxic manifestations (*x*-ray shock, etc.) but also where favorable therapeutic influences are made manifest.

BIBLIOGRAPHY

1. Heile. *Ztschr. f. klin. Med.*, 1904, iv, 508.
2. Neuberg. *Ztschr. f. Krebsforsch*, 1904, ii, 171.
3. Hall and Whipple. *Am. J. M. Sc.*, 1919, clvii, 453.
4. Jobling, J. W., Eggstein, A. A., and Petersen, W. F. *J. Exper. M.*, 1915, xxi, 239.
5. Edsall and Pemberton. *Am. J. M. Sc.*, 1907, cxxxiii, 286 and 426.
6. Manukhine, J. J. *Russky Vratch*, 1914, xv, 617.
7. Drey, L., and Losser, H. *Strählentherapie*, 1920, x, 1052.
8. Schilling, T. *Verhandl. d. 23ten Cong. f. innere Med.*, April 23, 1906, p. 436.
9. Stettner. *München. med. Wchnschr.*, 1919, lxvi, 1314.
10. Stettner. *München. med. Wchnschr.*, 1919, lxvi.
11. Stephan, R. *München. med. Wchnschr.*, 1920, lxvii, 309.
12. Denis, Martin and Aldrich. *Am. J. Med. Sc.*, 1920, clx, 555.

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ORGAN STIMULATION BY THE ROENTGEN RAY *

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Much,[1] in the preface to his "Pathologic Biology," has criticized, perhaps with justice, modern medical research. He says:

> There exists a pronounced tendency at the present time to overemphasize diagnostic methods. A diagnostic idea sweeps over the whole world with the greatest rapidity. If many therapeutic problems remain unanswered, it is possible that the reason lies less in the difficulty of the subject than in a lack of interest in biologic therapy. . . . Our only hope is that these diagnostic efforts may pave the way for later therapeutic discoveries.

A survey of modern roentgenology reveals this proclivity in clear outline. Day by day, diagnostic technic becomes more refined and useful; therapeutic use lags far behind. What, indeed, can we consider the yield in therapeusis? An occasional effect on malignant tissues or benign tumors; greater usefulness in glandular tuberculosis and in the leukemias; some application in certain goiters. The dermatologist and the gynecologist, too, find a limited field of indication.

Perhaps the early efforts to use the roentgen ray—and the radioactive agents—in malignant diseases is responsible to some degree for this relative paucity of therapeutic results. The roentgenologist has become obsessed with the idea that therapeutic results must be achieved through the destructive effects of the roentgen ray. We believe, on the contrary, that the therapeutic application of the roentgen ray will find far greater

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1. Much, H.: Pathologische Biologie, Ed. 3, Leipzig, 1920.

possibilities when once the simple biologic fact that cellular stimulation follows roentgen-ray exposure receives the consideration of the internist.

Heretofore we have emphasized almost exclusively the destructive effects to be achieved with the roentgen ray, either on malignant cells or on tuberculous granulation tissue, the effort being constantly made to intensify the effect on the pathologic tissue and to spare the normal tissues. But it is a biologic aphorism that agents which in large doses are toxic to cells act as stimulants in small doses. Pharmacologically, we constantly make use of just this principle.

If, then, the roentgen ray is active in a destructive sense, why should we not take advantage of its properties as a stimulant, the more so since we may thereby stimulate certain diseased organs whose metabolic functions are vital to the organism as a whole? From a nontechnical point of view it would seem that we could make use of no agent more suitable. We can with modern apparatus gage the dosage in a fairly satisfactory manner: it can be used selectively, that is, we can to some degree limit the effect to certain organs or tissues—and the effects should be promptly noticed—advantages that our pharmacologic methods do not all possess.

When we speak of organ or cellular stimulation it will be clear, of course, that we distinguish in this case between increased metabolic processes and reproductive stimulation. The latter is commonly implied when the roentgenologist speaks of stimulation.

At least two methods seem possible through which organ stimulation by means of roentgen rays might be of therapeutic use, the one direct, the other indirect. The former can be illustrated in a very simple manner by merely studying the rate of flow from some gland after roentgen-ray exposure.

EXPERIMENTAL WORK

EXPERIMENT 1.—*Stimulation of liver.*—The gallbladder of a 10-kg. dog was removed and a cannula inserted in the common bile duct; the operation was completed at 8:30. Collection of bile commenced at 9 and continued for three hours. Roentgen-ray exposure was made at noon for ten minutes (liver region, Coolidge tube, 10-inch focal distance, 8 ma., 5-inch back-up, unscreened). The bile secreted during the ten minutes' exposure was collected separately. The results

expressed in volume of bile secreted as well as the amount of bile pigment (colorimetrically determined) are given in Table 1.

TABLE 1.—SECRETION OF BILE IN EXPERIMENT 1

Time	Bile C.c.	Bile Pigment
9-10 a. m.	0.4	1.
10-11	1.5	0.99
11-12	2.4	0.96
12-12: 10 (roentgen-ray exposure)	1.3*	0.66†
12: 10-1: 10	3.5	1.6
1: 10-2: 10	3	1.05
2: 10-3: 10	2.75	0.95
3: 10-4: 10	4.7	1.35
4: 10-5: 10	2.6	1.4
5: 10-6: 10	2.8	1.45

* 7.8 c.c. per hour.
† 3.96 per hour.

EXPERIMENT 2.—*Kidney stimulation.*—In an 8-kg. dog, cannulas were inserted into both ureters in the evening, and hourly determinations of urine secretion were begun at 9 a. m. Roentgen-ray exposure of the right kidney was made at noon, and collection of urine was continued for five hours (Table 2).

TABLE 2.—URINE IN EXPERIMENT 2

Time	Volume of Urine—Left Kidney C.c.	Volume of Urine—Right Kidney (Irradiated) C.c.	Total Nitrogen Excretion Right Kidney Gm.
9-10	9	10.5	0.055
10-11	8	10	0.048
11-12	9	7	0.028
12-1 (Roentgen ray at noon)	6	7	0.03
1-2	5	13	0.058
2-3	7	10	0.032
3-4	4.2	4.7	0.012
4-5	3.5	4.2	0.01

The diuretic effect on the kidney that was exposed is apparent, and is associated with an increase in the amount of total nitrogen excreted.

EXPERIMENT 3.—*Pancreatic stimulation* (internal secretion).—A partial pancreatectomy was performed on a dog, Nov. 30, 1920, a portion of the tail of the pancreas being left in situ. After determination of sugar tolerance, the animal was placed on a constant diet containing 100 gm. of fat-free meat, 50 gm. of dried cracker meal, and 15 gm. of bone ash. The sugar determination of the urine is given in Table 3.

It will be observed that the only time that this animal was sugar free was on the 15th, two days after roentgen-ray stimulation.

CLINICAL APPLICATION

While it is possible that our indications for liver stimulation are at present indefinite, the clinical application of kidney stimulation and the stimulation of glands with an internal secretion is more apparent. Allen [2] has recently made the statement that "any positive means of augmenting the endocrine pancreatic function even by a little would give therapeutic results far surpassing those of the negative plan of sparing the function by diet." From preliminary clinical observations now in progress we have gained the impression that by means of such roentgen-ray stimulation a method of decided usefulness is offered for the treatment of diabetes. We may mention that we have irradiated various organs in wholly or partially depancreatized dogs without being able to influence the sugar tolerance in any degree.

TABLE 3.—SUGAR DETERMINATION IN THE URINE IN EXPERIMENT 3

Date	Gm.
January 9	5
January 10	5.5
January 11	2.8
January 12	3.8 *
January 13	6.8
January 14	3.12
January 15	0
January 16	2.5
January 17	5.32
January 18	8
January 19	2.76
January 20	6.8

* Roentgen-ray exposure over pancreatic area for ten minutes, afternoon of the 12th and morning of the 13th.

To illustrate the fact that the roentgenologist has constantly the factor of tissue depression or destruction in mind, rather than that of increasing metabolic function, we call attention to the paper of Dresel [3] in this particular connection. Dresel has endeavored to alter the sugar tolerance of diabetics by irradiating the suprarenals, expecting to lower their function and so indirectly affecting pancreatic secretion. He reports some transient lowering of the blood and urine sugar of diabetes, due, in our opinion, to an unintentional irradiation of portions of the pancreas.

2. Allen, F. M.: Am. J. M. Sc. **160**: 781, 1920.
3. Dresel, K.: Deutsch. med. Wchnschr., **46**, No. 45, 1920.

Fraenkel [4] seems to have been the first to make definite efforts to develop the use of stimulation by means of roentgen rays in the treatment of internal diseases. In a recent paper he calls attention to a number of clinical conditions so treated, including the irradiation of the ovaries in certain forms of dysmenorrhea, irradiation of the thymus and hypophysis in osteomalacia, irradiation of the periosteum to facilitate the healing of fractures, irradiation of the epiphysis of bones and the hypophysis of children to promote growth, and irradiation of the spleen and bone marrow in pernicious anemia, the spleen in tuberculosis, etc.

INDIRECT EFFECTS: ENZYMES; ANTIBODIES; THROMBOPLASTIC SUBSTANCES

The second effect of organ stimulation by means of the roentgen ray may be somewhat more complex in its mechanism, but therapeutically equally as useful. It involves the observation that following organ stimulation certain substances may be discharged from the cells which act on remote pathologic lesions.

Hektoen [5] has approached this problem from the antibody side and has endeavored to increase antibody production by irradiating various organs, particularly the hematopoietic system.

In a paper published elsewhere we have shown that it is possible to mobilize enzymes (protease, diastase, erepsin, etc.) after irradiating various organ complexes.

Stephan [6] observed that after irradiating the spleen a marked effect could be observed on the coagulation time, a finding that received immediate confirmation.[7]

The clinical application of such indirect effects has been found in the reported treatment of asthma by irradiating the spleen and the hypophysis,[8] and the favorable results which irradiation of the spleen is supposed to have on a tuberculous process.[9]

Indeed, when we come to the particular field of the roentgenotherapist—malignant diseases—in which the effect is supposedly a directly destructive one on the malignant tissue, it is possible that the effect is not

4. Fraenkel, M.: Deutsch. med. Wchnschr. **46**: 1309, 1920.
5. Hektoen, Ludwig: J. Infect. Dis. **27**: 23 (July) 1920.
6. Stephan, R.: München. med. Wchnschr. **67**: 309, 1920.
7. Neuffer, Hans: München. med. Wchnschr. **69**: 40, 1921.
8. Ascoli, M., and Fagiuoli, A.: Riforma Med. **36**: 622 (July 10) 1920; abstr. J. A. M. A. **75**: 903 (Sept. 25) 1920.
9. Manukhine, J. J.: Vrach. Gaz. **15**: 617, 1914.

selective per se, but depends first of all on the peculiar vascular instability of malignant tissues and secondly on indirect biologic alterations produced in the normal cells of the host. A discussion such as that of Keysser [10] in a recent paper is of interest in this connection.

SUMMARY

The roentgen ray, in proper dosage, has the property of stimulating cellular metabolism. When organs are selectively stimulated by roentgen rays, therapeutic results can be achieved either by direct stimulation of an external secretion (the kidney) or of an internal secretion (the pancreas in diabetes). A second method of possibly influencing remote pathologic lesions lies in the mobilization of antibodies, enzymes and thromboplastic substances following selective organ stimulation. The effects on tuberculosis (irradiation of the spleen), on hemophilia and purpura (irradiation of the spleen) and some of the effects on malignant tissues can possibly be examined from this point of view with profit. It is probable that the indications of roentgen-ray therapy in the treatment of internal diseases will find marked extension if proper recognition is given the possibility of organ stimulation by such physical means.

10. Keysser, F.: München med. Wchnschr. **69**: 4, 1921.

THE SACHS-GEORGI REACTION FOR SYPHILIS

A PRELIMINARY REPORT OF MORE THAN ONE THOUSAND COMPARATIVE WASSERMANN AND SACHS-GEORGI TESTS *

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The Wassermann reaction for syphilis, despite its wide clinical acceptance, cannot be regarded as free from certain inherent defects. The mere fact that the test requires a considerable number of biologic reagents for its performance, all of which are to some degree variable and uncertain, is perhaps one of the reasons that the results of the test are not always reliable even when in the hands of the conscientious worker. We must furthermore recognize that a definite number of cases clinically positive are serologically negative. The standardization of the biologic reagents might seem a logical improvement, and efforts are being made in this direction by the German authorities. Efforts to simplify the technic have repeatedly been made, particularly since the recognition that the reaction is essentially physicochemical, not depending on specific immunologic alterations in the serum of the syphilitic. Perhaps the flocculation reactions, such as those described by Porges and Meyer, Herman-Perutz, Bruck, Klausner and others, gave evidence of progress in this direction, although none of them offered a real substitute for the Wassermann test.

The increased use of tests for syphilis, as well as the general shortage of the necessary biologic reagents incident to the war, made a reinvestigation of the problem desirable. Two tests have been developed: that of Meinicke,[1] and that of Sachs-Georgi,[2] which are evidently of considerable usefulness. Of these, the Sachs-Georgi, because of its remarkable simplicity as well as its seeming specificity, has found most favor. The obvious importance of simplified serologic procedure would warrant a careful comparison of such tests with the Wassermann reaction, and we have therefore examined over one thousand serums and spinal fluids by the Sachs-Georgi technic.

TECHNIC

The technic of the Sachs-Georgi test which we have employed is the modification proposed by Mandelbaum,[3] which differs from the

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1. Meinicke, E.: Berl. klin. Wchnschr., **55**:83, 1918.
2. Sachs, H., and Georgi, W.: Med. Klin. **14**:610, 1918.
3. Mandelbaum, M.: München. med. Wchnschr. **65**:294, 1918.

original in the use of smaller amounts of serum and antigen. The serum or spinal fluid to be tested must be absolutely clear after being centrifuged, free from cotton fibers or other particles. It is inactivated in the water bath at 56 C. for one half hour. Three drops of serum are then mixed with 1 c.c. of sterile salt solution (0.85 per cent.). To this diluted serum, 0.5 c.c. of cholesterinized alcoholic beef heart antigen, which has been previously diluted 1 to 6 with 0.85 per cent. salt solution, is added. The tubes are then gently agitated and placed in the incubator at 37 C. for from eighteen to twenty-four hours. A reading is taken at this time; most of the positive serums will have flocculated. The tubes are kept at room temperature for the next twenty-four hours, and final readings are then made.

Apparatus.—Glassware must be perfectly clean. We have our tubes washed in tap water, then placed in an acid cleaning bath for several hours, washed in tap water and finally rinsed several times in distilled water. They are then dried in the hot air oven. Sterile pipets are used.

Serum.—The question that has been raised in the Wassermann technic concerning the use of active or inactive serum is also involved in the Sachs-Georgi test. Mandelbaum dilutes the fresh serum and then inactivates it before adding the antigen. We have used the inactivated serum, then diluted and added the antigen. Other observers prefer to keep the serum on ice several hours before inactivating assuming that because of certain alterations brought about by the chilling of the seroglobulins, the reaction is made more sensitive.

Diluting Agents.—In the Wassermann reaction, approximate isotonicity seems sufficient, minor variations in the concentration of the salt being without effect on the reaction. In the Sachs-Georgi reaction we have found that reliable results can be obtained only when the salt solution is made up to 0.85 per cent. The salt solution should, of course, be sterilized.

Antigens.—The preparation of the proper antigen is perhaps the fundamental factor in the success or failure of the Sachs-Georgi reaction. Extracts from human, beef, pig, guinea-pig and sheep heart have been used, as well as from beef and syphilitic fetal liver. Our most satisfactory results have been obtained with an antigen thus prepared: Beef heart cut into small pieces was ground in a meat grinder; 5 c.c. of alcohol (95 per cent.) per gram of moist heart substance was then poured over the mass and incubated at 37 C. for from eight to ten days. During this time the flask was shaken each day. The alcoholic extract was then filtered and cholesterinized. The amount of cholesterin needed varies with each antigen. We have found it good practice to titrate the antigen against positive and neg-

ative serums after adding various dilutions of cholesterin to small fractions of the antigen and selecting the proper amount of cholesterin to be added according to our actual serum titration. Sachs and Georgi thus prepared their antigen: To 100 c.c. of filtered beef heart extract and 200 c.c. of alcohol is added 13.5 c.c. of a 1 per cent. alcoholic solution of cholesterin.

Before use the antigen is diluted 1:6 with salt solution. The dilution must be made neither too slowly nor too rapidly. The extract when properly made is quite clear, the diluted antigen faintly opalescent and does not flocculate after standing several days. The antigen when diluted 1:10 serves very well as an antigen for the Wassermann reaction.

Incubation.—In the original Sachs-Georgi technic, the tubes were incubated for two hours and then placed at room temperature for twenty-four hours. Mandelbaum, Hauck,[4] Ammenshauser [5] and others have shown that better results are obtained if the incubation is prolonged for from eighteen to twenty-four hours. The tubes are then read and kept at room temperature for another twenty-four hours before the final reading is made. Sachs and Georgi have adopted this modification.

Reading of Results.—Most of the German workers have used the agglutinoscope devised by Kuhn and Voit to read the results. A good magnifying lens from a dissecting microscope or even the ocular from a microscope can, however, be used with very satisfactory results if the tubes are held against a dark background. The flocculation may be very fine—smoky, finely granular—driven snow, or may finally be precipitated at the bottom of the tube as a coarsely granular floccular mass. Gentle rotation or agitation of the tube aids observation. Negative cases remain perfectly clear for twenty-four hours, later a slight precipitate may collect at the bottom of the tube, but this can readily be differentiated from positive findings. In a general way, one can grade the degree of the reaction on a one to four plus basis; in a few cases we have recorded plus-minus readings. With the use of the agglutinoscope, the number of doubtful readings would probably be increased. We have found it useful to centrifuge doubtful tubes at low speed for half an hour and then pour off some of the supernatant fluid. By comparison with negative tubes similarly treated, minor differences become more apparent. We have also kept the tubes on ice after incubation, but have found no superiority over simple room temperature from this procedure.

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4. Hauck, L.: Influence of Temperature on Sachs-Georgi Reaction, München. med. Wchnschr. **67**:369 (March 26) 1920.

5. Ammenshauser: Centralbl. f. Bakteriol., **84**:521, 1920.

RESULTS OF EXAMINATION

In Table 1 are recorded the results of 1,042 examinations made with the Wassermann and the Sachs-Georgi tests. The serums and cerebrospinal fluids were obtained from the serologists of the Cook County Hospital and the city board of health; their Wassermann reports were based on tests with from two to four antigens.

TABLE 1.—Results with Wassermann and Sachs-Georgi Tests

Reaction	Result								
Wassermann	Positive			Negative			Anticomplementary		
Sachs-Georgi	+	±	−	+	±	−	+	±	−
	163	5	3	44	9	634	2	0	3
Total					866				

In Table 1 it will be observed that in 163 cases there was an agreement of positive cases (18.8 per cent.) and in 634 an agreement of negative cases (73.2 per cent.), i. e., an agreement of 92 per cent.

The results with cerebrospinal fluids are given in Table 2

TABLE 2.—Results with Cerebrospinal Fluid

Reaction	Result								
Wassermann	Positive			Negative			Anticomplementary		
Sachs-Georgi	+	±	−	+	±	−	+	±	−
	36	6	3	7	2	121	1	0	0
Total					176				

The total agreement in this series was 157, or 89.2 per cent.

Of the total number of serums and cerebrospinal fluids examined (1,042), sixty-two were found to give a positive or plus-minus Sachs-Georgi reaction while the Wassermann was negative. The details in our possession concerning these cases are recorded in Table 3

It will be observed that the clinical history or the physical findings in thirty-six of these patients made a diagnosis of syphilis probable (58 per cent.). In twenty, the reaction seems nonspecific (32 per cent.), while in six cases we have no information that would assist in the determination of the correctness of the serologic finding.

From a summary of 17,186 serum tests reported by a number of observers and collected by Baumgartel [6] (to which are added the 7,000 reported by him), Table 4 has been constructed.

It will be observed that in 5,808 positive cases and in 15,950 negative cases, there was complete agreement, as well as in 246 doubtful reactions, 91 per cent. of the total. Of the discrepancies (9 per cent.),

6. Baumgärtel, T.: Parallel Wassermann and Sachs-Georgi Tests, München. med. Wchnschr. **67**:421 (April 9) 1920.

TABLE 3.—Clinical Correlation of Cases with Positive Sachs-Georgi Reaction and Negative Wassermann Reaction

No. of Patient	Clinical Diagnosis on Admission	Clinical History	Treatment
xi-16	Suspected syphilis	Gonorrhea twice; chancre two months ago; now in second stage	None
ix-3	Chancroid	Primary syphilis; inguinal and epitrochlear glands enlarged, ulcer of scrotum	None
ix-32-34	Cerebral hemorrhage	History of chancre; rigid, irregular pupils; spinal fluid examination: pressure +++, globulins ++, 88 lymphocytes	None
ix-33	Cerebral hemorrhage	Paresis, left leg; patellar reflexes, +++; absent abdominal and cremasteric reflexes	Potassium iodid and mercury
viii-12	Acute arthritis	History of transient attacks of paraplegia; roentgen-ray, hypertrophic osteoarthritis; denies syphilitic infection	Improved under potassium iodid
viii-26	Chronic nephritis and endocarditis	Gonorrhea once, denies syphilis; pupils sluggish to light; absent patellar and cremasteric reflexes; shuffling gait; wife one miscarriage	None
vii-37	Bronchopneumonia and syphilis	Gonorrhea and buboes; punched-out scar on prepuce; slight adenopathy; epitrochlear glands	Intravenous medication 11/9/20 N. W. U. Post-Grad. M. Sch.
vii-64	General paresis	History of chancre and secondary syphilis; gastric crisis; tabetic gait and urinary incontinence; loss of memory	None
vi-8	General paresis	Chancre fourteen years ago; syphilitic erosion of nose; incontinence of urine; difficulty in walking at night; gastric crisis; patellar reflex, ++; Romberg sign, ++; Argyll Robertson pupils and scanning speech	Now on syphilitic treatment
vi-11-52	Syphilitic thrombosis	Chancre fifteen years ago; hemiplegia; amnesia; pupils sluggish; eyelids drooping; spinal fluid examination: pressure ++, globulin +, 16 cells	Has had six injections of arsphenamin
vi-24	Syphilitic epiphysitis	Congenital syphilis; palmar and plantar desquamation; rhagades about mouth; suggestive syphilitic nose	None
vi-51	Crossed paralysis of sixth and seventh cranial nerves	Chancre two years ago; paralysis, right side of face; secondaries, hands and face; spastic and ataxic gait; Romberg, +++; patellar, +++; spinal fluid examination: pressure ++, globulins ++; 12 cells	None
v-11	Syphilitic genital ulceration	Pregnant twenty times in twenty years; sixteen miscarriages, usually at two or three months	None
v-13	Chronic alcoholism, suspected tuberculosis	Chancre thirty-two years ago; rash twenty years ago; scars on penis, back and head	None
iii-1	Myocarditis; cardiac asthma	Chancre ten years ago; aneurism arch aorta	Arsphenamin 6 times, potassium iodid and mercury
iii-8	Syphilitic organic heart disease; general paresis	Perforated nasal septum; aortic regurgitation; tertiary syphilis; paresis, left side of face; cataract and posterior synechiae; aneurysmal dilatation of aorta	Syphilitic treatment, potassium iodid and mercury
iii-9	Cancer of stomach	Chancre four years ago; patellar reflex weak; gastric crisis	None
xii-58	Malaria	Chancre one year ago.....	None
xii-32	Hydrocele (syphilitic)	Chancre four years ago; secondary syphilis three years ago	None
xiii 23-50	Cerebrospinal syphilis	Chancre twenty-five years ago; loss of memory; scar on penis; drooping eyelids	None
xii-49	Chancre	Chancre three weeks ago.....	None
xiii-15	Acute epididymitis	Chancre three years ago; chronic gonorrheal urethritis; prostatic seminal vesiculitis and epididymitis	None
xiv-75	Cerebral syphilis	Chancre twenty-five years ago; Argyll Robertson pupils; spinal fluid examination: globulins ++, 80 cells	None
xiv-9	Traumatic epilepsy	Chancre twenty-four years ago; scar on penis and body	None

TABLE 3.—(*Continued*)

CASES IN WHICH THERE WAS NO COMPLETE RECORD AVAILABLE FROM HOSPITAL SOURCES, MOST OF THE SERUMS BEING OBTAINED FROM THE OUTPATIENT DEPARTMENT

	Clinical Diagnosis on Admission	Clinical History	Treatment
xii-49	.....	History of chancre.....	None
xiii-15	Syphilis	History of chancre.....	None
i-55	Tertiary syphilis	.....	None
ix-15	Fract. mandible	History of chancre.....	None
xii-4P	Syphilis	History of chancre.....	One injection
i-46	Tabes	.....	None
v-11	Syphilitic ulcer of leg	.....	None
xiv-2	Pulmonary tuberculosis and syphilis	History of chancre.....	None
i-6	.....	Chancre on lip.....	None
i-9	.....	History of chancre fifteen years ago.....	None
i-13	Syphilis	.....	None
xiii-62P	Syphilis	Syphilis.....	Treated case

TWENTY CASES IN WHICH THE WASSERMANN REACTION WAS NEGATIVE BUT THE SACHS-GEORGI POSITIVE WITHOUT ANY EVIDENCE OF SYPHILIS ON THE PART OF THE PATIENT, EITHER IN THE HISTORY OR CLINICAL FINDING

	Diagnosis or Symptoms		Diagnosis or Symptoms
iv-10	Pain in legs; optic neuritis	xiii-4	Bronchitis; asthma
vii-69	Dementia praecox	xiii-1	Typhoid
vii-68	Chronic alcoholism	xiv-30	Chronic nephritis
viii-22	Routine; no diagnosis	xiv-33	Bilateral salpingitis
viii-15	Ovarian cyst	xiv-54	Chronic bronchitis
ix-28	Routine; no diagnosis	xiv-55	Suspected carcinoma of colon
xii-31	Routine; no diagnosis	xiv-29	Routine; no diagnosis
xiii-24	Lobar pneumonia	xiv-13	Cerebral hemorrhage
xiii-14	Acute cystitis	xiv-8	Constipation
xiii-10	Acute gonorrhea; lobar pneumonia	xvii	Chronic arthritis and myocarditis

In six serums obtained from the serologist of the City Health Department, we obtained a positive Sachs-Georgi reaction with a negative Wassermann. These serums are sent in without diagnosis and we have no information concerning them.

TABLE 4.—RESULTS OF SERUM TESTS

Reaction	Result								
	Positive			Negative			Doubtful		
Wassermann.....	+	±	—	+	±	—	+	±	—
Sachs-Georgi.....	5,808	282	514	686	221	15,950	154	246	325
Total.....	24,186								

the cases in which the Wassermann reaction was positive and the Sachs-Georgi negative or doubtful, numbered 1,121, or 51 per cent.; those in which the Wassermann was negative while the Sachs-Georgi was positive or doubtful numbered 1,061, or 48 per cent. These collected results represent the observations made with various modifications of technic, particularly in regard to incubation time. The cases in which the Wassermann reaction was negative while the Sachs-Georgi was positive include a considerable number of treated cases of primary and parasyphilitic disease, of congenital syphilis, as well as a small group of diseases of nonsyphilitic basis. The latter may possibly be eliminated with further study of the preparation of the antigen. Just

as the Wassermann reaction becomes nonspecific to a considerable degree when the antigen is fortified with too much cholesterol, so perhaps the acuity of the Sachs-Georgi reaction can be modified when once we become familiar with its range.

DISCUSSION

An examination of the results tabulated herewith makes it evident that this flocculation test very closely approximates the results obtained with the classical Wassermann reaction and may in some ways prove superior to it. While for the time being we must accept the Wassermann test as our standard of comparison, we should by no means lose sight of the fact that this standard is not infallible. While the error does not lie in the direction of nonspecificity, a definite number of positive syphilitic cases do not react with the Wassermann technic and are either classed as negative or yield doubtful readings. It seems that this relatively large group of cases flocculate quite readily with the Sachs-Georgi method, as will be apparent from a perusal of some of the histories and clinical findings recorded in Table 2. Only in thirty-two cases of the series were the Sachs-Georgi reactions positive in the absence of positive clinical findings or history.

We can enter here but briefly into a discussion of the mechanism that seems to underlie the flocculation made grossly apparent in the Sachs-Georgi reaction, and which is closely related to the ultramicroscopic colloidal alterations that have been described as taking place in the Wassermann reaction when the antigen is added to the serum. Both the globulins and the lipoids of the syphilitic serum seem altered to some degree, and Schmidt,[7] Hirschfeld and Klinger,[8] Sachs,[9] and Meinicke[10] are of the opinion that the alteration involves a peculiar lipotropic property of the serum globulins, the lipoglobulin aggregate being easily precipitated in solutions that are iso-electric for the globulins. Neukirch[11] places the hydrogen-ion concentration represented in a 0.85 per cent. salt solution as the optimum for the precipitation of the globulins, i. e., the identical concentration found most satisfactory for use in the Sachs-Georgi technic. From the point of view of its theoretical interest, we wish to call attention to the two phase reaction of Meinicke[1] for syphilis. This reaction involves the precipitation of the globulins of the serum in both negative and positive serums by simple dilution after the addition of antigen; then the addition of varying dilutions of salt solution to the serums so altered. In positive cases, the lipoglobulin precipitate remains unchanged; in negative cases the precipitates redissolve.

7. Schmidt: Ztschr. f. Hyg. u. Infectionskrankh. **69**:513, 1911.
8. Hirschfeld and Klinger: Ztschr. f. Immunitätsforsch. **21**:40, 1914.
9. Sachs, H.: Kolloid-Ztschr. **24**:123, 1919.
10. Meinicke, E.: München. med. Wchnschr. **66**:932, 1919.

The Sachs-Georgi reaction is exceptionally simple, and once a suitable antigen has been prepared, it can be carried out with ease. The use of the complement-amboceptor-hemolytic system used in the Wassermann reaction is after all a cumbersome method of making apparent the colloidal alterations which are at the basis of these reactions for syphilis. Ultimately such a system must be replaced by a reaction simpler in character, requiring fewer reagents and less manipulation. Whether such a reaction will be of the flocculation type, such as the Sachs-Georgi, or will be purely chemical, is for the present not in the range of discussion. It is evident, however, that the Sachs-Georgi, reaction, even in its present form, offers a valuable contribution because of its simplicity and its apparent practicability. The fact, too, that it is frequently positive in those syphilitic cases which do not give a positive Wassermann reaction makes it of value as an additional test to be used in conjunction with the Wassermann technic. The fact that a relatively small percentage of nonspecific reactions is obtained should not detract from its ultimate usefulness; the earlier work with the Wassermann reaction was handicapped until the preparation of antigens was better understood.

CONCLUSIONS

An examination of 1,042 serums and cerebrospinal fluids by means of the Wassermann and the Sachs-Georgi reactions demonstrated a close parallelism of the two reactions (92 per cent.).

In sixty-two cases in which the Wassermann reaction was negative while the Sachs-Georgi reaction was positive or doubtful, the clinical history or examination revealed evidence of syphilis in 58 per cent.

The technic of the Sachs-Georgi reaction is simple, only one biologic reagent is required (antigen) instead of the four used in the Wassermann test (antigen, amboceptor, complement and red blood cells). This simplicity adds to the uniformity of the results. The ultimate specificity depends on the preparation of a proper antigen.

Because of its simplicity, and the fact that it is frequently positive in syphilitic cases when the Wassermann test is negative, we are of the opinion that the Sachs-Georgi reaction offers a valuable aid in the routine examination for syphilis when used in conjunction with the Wassermann reaction.

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11. Neukirch, P.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **29**:498, 1920.

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THE SACHS–GEORGI REACTION IN NEUROSYPHILIS

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THE SACHS–GEORGI REACTION IN NEUROSYPHILIS

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In a comparative study of the Wassermann reaction and the Sachs-Georgi reaction for syphilis recently published (1) we found that the Sachs-Georgi reaction was positive not only in practically every Wassermann positive serum, but was positive in a considerable number of cases (clinically syphilitic) in which the Wassermann reaction was negative. In our study it was observed that the majority of these sera (Wa—, SG+) were obtained from patients with syphilis of the nervous system. Under the circumstances we believed that a more detailed examination of the Sachs-Georgi reaction in syphilis of the nervous system might prove of value.

Methods useful in the early diagnosis of syphilis of the nervous system have received the attention and the careful study of neurologists and syphilographers because of the obvious clinical importance. Were we possessed of a certain means of diagnosing syphilis of the central nervous system before severe symptoms became manifest, untoward late results might at times be prevented or their frequency lessened. Under present conditions many cases of syphilis now dismissed as cured when the Wassermann reaction becomes negative after treatment, ultimately develop severe neurosyphilis before again coming under proper medical attention.

The Wassermann test has not been as successful in aiding our diagnosis of early syphilis of the nervous system as we might wish; this fact becomes evident when we observe the development of neurosyphilis in the so called cases of latent syphilis and of congenital syphilis with negative Wassermann findings. Of course when the clinical evidence has developed the serological reaction is usually merely of corroborative import. To illustrate the wide divergence between the clinical and the serological examination of these cases we may cite the statistics presented by Noguchi (2) concerning the percentages of positive Wassermann findings in the spinal fluid of neurosyphilitics.

Hereditary syphilis	80 per cent.
Cerebral and spinal syphilis	50 per cent.
Paresis	73 per cent.
Tabes	53 per cent.

None's (3) percentages of positive Wassermann reactions in neurosyphilis are somewhat higher, stating that even the blood examination gave him 60 per cent. positive in tabes and 95 per cent. in paresis.

In view of the advantage of the Sachs-Georgi reaction in the simplicity of its technic, in the fact that it appears earlier and remains positive longer after treatment in syphilitic infection, as well as in the fact that it is frequently positive in congenital, latent and parasyphilitic disease, its adoption in diseases of the central nervous system would seem desirable in permitting closer control of the therapy and more reliable correlation between clinical finding and serological examination.

Material and Technic

The technic used in these examinations and the reading of the results was similar to that previously described with the exception that we have used 6 drops of cerebrospinal fluid instead of 3 drops as in our former tests. Georgi (4) has modified the original technic of the Sachs-Georgi reaction for spinal fluid and recommends the use of 1.5 c.c. of spinal fluid to 0.75 c.c. of diluted extract. We have made use of inactivated sera and spinal fluids throughout, although we observed no difference in the reactions when fresh or inactivated sera or spinal fluids were used. Theoretically the inactivation of the fluids to be examined might have some advantage in that Noguchi (2) has shown that when serum is heated to 56° C. for 30″ the proteotropic group is destroyed thus eliminating the euglobulins and the paraglobulins from taking part in the reaction.

In carrying out the tests we used not only the antigens described in our previous paper, but in addition have used the original cholesterinized beef heart antigen No. 27 and cholesterinized human heart extract No. 1 which Professor Sachs kindly placed at our disposal. In the comparison we observed no marked differences in the results of the final 48 hour readings, although some variation was noted with different antigens in the 24 hour readings, *i.e.*, the relative speed of the reaction seemed to vary with some of the antigens used.

In the following tables the results of the examination of the serum and of the cerebrospinal fluid by the two methods of examination are recorded.

From the first table it will be observed that of the 39 serum

SERUM

Reaction									
Wassermann.....	Positive			Negative			Anticomplementary		
	+	±	−	+	±	−	+	±	−
Sachs-Georgi.....	16	0	0	7	1	15	0	0	0

CEREBROSPINAL FLUID

Reaction									
Wassermann.....	Positive			Negative			Anticomplementary		
	+	±	−	+	±	−	+	±	−
Sachs-Georgi.....	22	0	1	11	1	25	1	0	0

CLINICAL FINDINGS OF THE CASES IN WHICH THE BLOOD SERUM WASSERMANN REACTION WAS NEGATIVE AND THE SACHS-GEORGI REACTION POSITIVE

Patient	Clinical Diagnosis	Clinical History	
C–15.....	Cerebro-spinal Syphilis	Chancre 10 years ago Romberg +++ Argyll-Robertson pupils Mental disturbances	
D–53.....	Suspected Tabes	Shooting pains Tabetic crisis Chancre 10 years ago	
E–17.....	Cerebro-spinal Syphilis	Chancre 20 years ago Scanning speech	
O–2.....	Early Tabes	Gastric crisis Girdle pains Chancre 5 years ago	
O–4.....	Early Taboparesis	Gumma of septum Dementia Slurring speech	
O–23.....	Juvenile Paresis	Father paretic Slurring speech Underdeveloped	
O–29.....	Suspected General Paresis	Chancre 8 years ago Romberg +++ Argyll-Robertson pupils	

IN THE FOLLOWING CASES WE WERE ABLE TO SECURE SPINAL FLUIDS WHICH WERE WASSERMANN NEGATIVE, SACHS-GEORGI POSITIVE

Patient	Clinical Diagnosis	Clinical History	Spinal-fluid Findings
A–59.....	General Paresis	Argyll-Robertson pupil Dementia Romberg +++ Chancre 9 years ago	Clear Pressure increased Nonne ++ 25 cells per c.m.
D–56.....	General Paresis	A. R. pupils Reflexes exaggerated Dementia History of chancre	Clear Pressure increased Nonne ++ 30 cells per c.m.
D–58.....	General Paresis	A. R. pupils Reflexes exaggerated Romberg ++ Chancre 10 years ago	Clear Pressure increased Ross-Jones ++ 20 cells per c.m.
D–59.....	Suspected Tabes	Shooting pains Gastric crisis Blood Wassermann ++++	Clear Nonne ++ Pressure increased 11 cells per c.m.
D–61.....	General Paresis	Aortic aneurysm A. R. pupils Scanning speech Chancre 15 years ago	Clear Pressure increased Ross-Jones ++ 27 cells to c.m.
E–42.....	Tabo-paresis	Cerebral symptoms Blood Wassermann ++++	Clear Pressure increased Ross-Jones ++ 17 cells per c.m.
F–20.....	Cerebro-spinal Lues	Chancre 8 years ago A. R. Pupils Romberg +++ Reflexes exaggerated	Clear Pressure increased Nonne ++ 5 cells per c.m.
H–45.....	Suspected cerebro-spinal Syphilis	Epileptic attacks Hutchinson's teeth Saddle nose Slurring speech	Clear Pressure increased Ross-Jones ++ 50 cells per c.m.
H–47.....	Cerebro-spinal Syphilis	Syphilitic periostitis Tertiary syphilis 8 months ago	Clear Pressure increased Nonne ++ 12 cells per c.m.
O–39.....	Juvenile Paresis	Father paretic Hutchinson's teeth Slurring speech	Clear Pressure increased Nonne ++ 14 cells per c.m.
O–42.....	Suspected General Paresis	Chancre 18 years ago Tertiary lues 6 years ago Dementia A. R. pupils Under antispecific treatment	Clear Pressure increased Ross-Jones ++ 15 cells per c.m.

examinations there was an agreement of 80 per cent., with 7 cases in which Wassermann reaction was negative and the Sachs-Georgi positive, and one serum in which the Wassermann reaction was negative and the Sachs-Georgi doubtful.

Of the 61 cerebrospinal fluids examined there was an agreement of 77 per cent. with 11 negative to the Wassermann but positive with the Sachs-Georgi. One positive Wassermann fluid was negative with the Sachs-Georgi.

We have selected 100 clinically positive cases of neurosyphilis as diagnosed by members of the attending staff of the Cook County Hospital. We then made the serological comparison by means of the Wassermann and Sachs-Georgi reactions. In the following table we have briefly recorded the clinical findings of the cases in which the Wassermann reaction was negative and the Sachs-Georgi positive.

Discussion

Although the Wassermann reaction may be acknowledged the most valuable among the several methods for the serodiagnosis of syphilis, the complicated technic as well as the number of biologic reagents necessary to carry out the test, restricts its employment to some extent. Among clinicians too much importance is frequently attached to negative Wassermann findings; it cannot be too strongly emphasized that whereas a positive Wassermann test is evidence of syphilitic disease, a negative reaction by no means rules out the probability of the syphilitic etiology of symptoms under consideration. Particularly is this true of neurosyphilis.

Hoffmann, Wechselmann (5), Wile and Stokes (6), Wiley and Hosley (7) among others have called attention to the involvement of the nervous system during the primary stage of syphilis, while the Wassermann reaction of the spinal fluid does not at this time become positive. Kingery (8), studying the spinal fluid of congenital syphilitics, also demonstrated the unreliability of the Wassermann reaction in the fluids of these cases. Meirowsky and Leven (9) have shown that three patients who were given abortive intravenous treatment in primary syphilis, with constantly negative Wassermann findings, later developed neurosyphilis.

In the 100 cases of neurosyphilis here studied by both the Wassermann reaction and the Sachs-Georgi reaction the comparative results can be tabulated as follows:

Wassermann positive	39	Sachs-Georgi positive	57
Wassermann negative	60	Sachs-Georgi negative	41
Anticomplementary	1	Doubtful	2

From this tabulation the advantage of the Sachs-Georgi reaction

would seem manifest. This corresponds in general with the findings of Nathan and Weichbrodt (10), Schönfeld (11), Raabe (12), Eicke (13) and others. While the number of cases which we have been able to study is small, in view of the agreement of similar studies carried out by other observers, we have gained the impression that the Sachs-Georgi reaction may prove of considerable value in this particular field.[1]

SUMMARY

In an examination of the serum or the spinal fluid of 100 cases of neurosyphilis (tabes, paresis, cerebrospinal syphilis, etc.) an agreement of 78 per cent. was found between the Wassermann and the Sachs-Georgi reaction.

In 18 cases the Wassermann reaction was negative and the Sachs-Georgi reaction positive.

In view of the extreme simplicity of the Sachs-Georgi reaction as contrasted with the Wassermann reaction, we are of the opinion that it offers a valuable aid in the diagnosis of neurosyphilis, used alone or as a control of the Wassermann reaction and supplementing it.

BIBLIOGRAPHY

1. Levinson, S. A. and Petersen, W. F. The Sachs-Georgi Reaction for Syphilis. Arch Derm. and Syph., 1921, iii, no. 3.
2. Noguchi, H. Serum Diagnosis of Syphilis. 3d Ed. Lippincott, N. Y.
3. Nonne. Syphilis of the Nervous System. 1913, Lippincott, N. Y.
4. Georgi, F. K. Zur Frage der Empfindlichkeit der Sachs-Georgi Reaktion im Liquor. Munch. med. Wchnschr., 1920, 67, 1317.
5. Wechselmann, W. Deut. med. Wchnschr., 1912, 38, 1446.
6. Wile, U. J., and Stokes, J. H. Involvement of the Nervous System during the Primary Stage. J. A. M. A., 1915, 64, 979.
7. Wile, U. J., and Hosley, C. K. Involvement of the Nervous System, etc. J. A. M. A., 1921, 76, 8.
8. Kingery, L. B. A Study of the Spinal Fluid in 52 Cases of Congenital Syphilis. J. A. M. A., 1921, 76, 12.
9. Mierowsky and Leven. Primary Seronegative Syphilis. Munch. med. Wchnschr., 1920, 67, 1040.
10. Nathan, E., and Weichbrodt, R. Zur Sero- and Liquor Diagnostic, etc. Munch. med. Wchnschr., 1918, 65, 1280.
11. Schonfeld, W. Die Ergebnisse der Sachs-Georgischen Ausflockungsreaktion bei Blut und Liquor Untersuchungen. Munch. med. Wchnschr., 1920, 67, 399.
12. Raabe, A. Über Parallelversuche mit Serum und Liquor nach Wassermann und Sachs-Georgi. Berl. klin. Wchnschr., 1919, 46, 1012.
13. Eicke, H. Vergleichende Untersuchungen zwischen der Wassermann Reaktion, etc. Med. Klin., 1919, 1314.

[1] The following corroborative conclusions of W. W. Harryman have appeared in the Archives Derm. and Syph., Vol. 4, No. 3, Sept., 1921, page 299, since our manuscript was submitted for publication:

1. The results of the Sachs-Georgi reaction on the spinal fluid closely parallel those of the Wassermann test.

2. The Sachs-Georgi reaction is a substitute or may be a valuable addition to the Wassermann test on the spinal fluid.

3. The Sachs-Georgi reaction furnishes a means for an earlier serodiagnosis of central nervous system syphilis than the Wassermann test.

FLOCCULATION REACTIONS IN SYPHILIS. WITH ESPECIAL REFERENCE TO THE MEINICKE AND SACHS-GEORGI REACTIONS

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Ever since Landsteiner, Müller, and Poetzl[1] found that alcoholic extracts of nonsyphilitic organs could be used in the Wassermann reaction and thereby discredited the specific antibody conception of the mechanism, efforts have been made either to modify the original technic, or to supplant it with a simpler reaction. Sachs and Rondoni,[2] Noguchi[3], and others sought to prepare various artificial antigens; of these however, only the cholesterinized antigen has come into general use.

Other workers sought to standardize the technic. Kolmer[4] and his collaborators have demonstrated the large number of factors capable of producing divergent results. The German Health authorities are preparing an antigen issued by a central laboratory, hoping thus to secure greater uniformity in the test. However, when one takes into consideration the number of other biologic reagents necessary, such as red cells, amboceptor, and complement, one can readily see that uniformity of the antigen alone will not necessarily solve the problem.

Since the technic employed by different individuals often gives discordant results, because of the exact titrations necessary, a simplified technic for the serodiagnosis of syphilis would appear to be in order. The difficulty in the preparation of the antigen and the cumbersome technic in general warrants the search for a simpler reaction for syphilis.

The question of specificity and standardization of the Wassermann reaction is not within the range of our present discussion. To avoid the uncertainty of the complement-fixation reaction many investigators have tried other methods. Jakobaus[5], Streng and Karvonen[6]

have introduced the conglutination reaction, but their results were not as satisfactory as those obtained with the Wassermann reaction.

Efforts made to find other serum reactions to replace the Wassermann technic have resulted most often in methods of a precipitation either of the antigen by the serum, or of some serum component by a biologic or chemical reagent.

We will mention here very briefly some attempts to design simpler methods for the serodiagnosis of syphilis.

SERUM REACTIONS IN SYPHILIS OTHER THAN THE WASSERMANN REACTION

Hirschfeld-Klinger Coagulation Reaction[7].—When tissue extract, such as beef heart, is digested with syphilitic serum it looses its ability to coagulate blood. The effect depends on adsorption of lipoids of tissue extract by serum constituents and is fundamentally similar to the Wassermann reaction. The Hirschfeld-Klinger reaction because of its large number of nonspecific reactions, did not replace the Wassermann reaction as it was originally intended to do.

Fornet and Schereschewsky[8] have shown that serums from tabetics and paralytics give a precipitate with serums from positive luetics. Part of the serum used is undiluted, and is poured over the remainder which is diluted 5 to 10 times, and this mixture is placed in small test tubes. First the specific heavier mixture mixes with the lesser strongly diluted mixture, and then by slanting the tubes, the lighter specific fluid comes to the surface. For controls, definite syphilitic and nonsyphilitic serums are used. The tubes are placed at room temperature for two hours. The positive tubes show at the junction of the two fluids, a fine ring which is absent in the definite nonluetic sera.

Klausner's Serum Reaction.—When 0.6 c.c. distilled water is added to 0.2 c.c. of fresh, active, absolutely clear serum, a distinct flocculant precipitate separates out in from 7 to 15 hours and this property is more marked in syphilitic sera than in normal sera. While this reaction is not specific for syphilis, it is almost invariably present in certain stages of syphilis. This property is not due to excess of globulin present in syphilitic sera according to the later studies of Klausner, but probably to the high lipoid content of syphilitic serum.

Sachs[10] has shown that the serum globulins are less stable and more easily precipitated in syphilitic serums as well as in other infectious diseases due to changes in acidity. When serum is inactivated the

precipitate is increased to a certain extent because of an alteration in the OH and H ion balance. But Klausner's reaction can only be used in active serums. Sachs and Nathan have shown that on inactivating the serum with distilled water an ultramicroscopic precipitate is brought about when any pure chemical as normal HCl is added to it. This, however, did not bring practical results.

Porges-Meier Reaction[11] is based upon the fact that syphilitic serums produce floeculi in solutions of lecithin and similar salts. An equal amount of inactivated, clear patient's serum is mixed with 1 per cent sodium glycocholate in distilled water. This mixture (together with known normal and luetic controls) is kept at room temperature for from 18 to 24 hours. Positive reactions give distinct coarse floeculi; a turbidity or a faint precipitate is considered negative.

In this connection it may be mentioned that investigators such as Müller and Landsteiner[12], Elias, Neubauer, Porges and Salomon[13], Hermann and Perutz[14], Sachs and Altmann[15], Bruck and Hidaka[16], Teruuchi and Toyada[17], Levaditi and Yamanouchy[18], Fleischmann[19], Hecht[20], Hesberg[21], Munk[22], and others, have used lecithin, salts of bile acids, alkalies, sodium oleate, cholesterin, vaseline, courin, palmatin, stearin, sodium salts, potato extracts, shellac emulsions and numerous other combinations without definite results.

Jacobsthal[23] *has shown that by adding luetic serum to organ extract as in the Wassermann reaction, a precipitate is observed which can be demonstrated by means of a dark field. Bruck and Hidaka*[16] *could obtain the same results macroscopically.*

Hermann-Perutz Reaction[24] consists of a precipitation resulting from the interaction of clear inactive serums with a solution of sodium glycocholate, alcohol, and cholesterol in distilled water. The test is as follows: Solution 1 (stock solution diluted 1:20 with distilled water before use) consists of sodium glycocholate 2 gms., cholesterol 0.1 gms., 95 per cent alcohol 100 c.c.; solution 2 is a 2 per cent solution of sodium glycocholate in distilled water. Add 0.4 c.c. of clear inactive serum (heated at 56° C. for one half hour) in a small test tube to 0.2 c.c. of solution 1 and 0.2 c.c. of solution 2. The tubes are plugged with cotton and set aside at room temperature for 24 hours, after which the presence or absence of precipitation is noted. Known normal and luetic serums in distilled water are used as controls.

None of the above reactions has been found absolutely specific, and none has been generally adopted; the far greater accuracy of the

Wassermann reaction having made it the method of choice. Normal serum also precipitates lipoids, with this distinction, that in normal serum the zone of precipitation is smaller and longer delayed than in syphilitic serums.

The Bruck Test[25] is a serochemical reaction for syphilis. 0.5 c.c. patient's serum is mixed with 2 c.c. distilled water and allowed to remain at room temperature for 10 minutes and 0.3 c.c. of a 25 per cent solution of HNO_3 is added. The tube is shaken and again allowed to remain at room temperature for 10 minutes and 16 c.c. distilled water added to the contents. The tube is again allowed to remain at room temperature for 10 minutes, and is shaken several times for 30 minutes and allowed to remain at room temperature for 12 hours. When the solution is cloudy or presents opaqueness, the result is considered positive; but when the solution is transparent or presents but a little cloudiness the result is negative. This reaction does not compare favorably with the Wassermann reaction and is negative in a large number of clinically positive cases of syphilis.

Vernes Phenomenon[26]. In the Wassermann test a positive reaction is characterized by the absence of hemolysis, and a negative reaction by complete hemolysis. There are several degrees of color changes, the interpretation of which depends on the individual worker and is expressed by plus signs. Vernes devised a colorimetric scale consisting of 8 tints numbered from 0 to 8, zero representing the absence of hemolysis, and 8 the maximum hemolysis or deepest shade of red. By charting these results graphically Vernes at first believed that the curves obtained with syphilitic serums possessed certain fundamental characteristics, but other investigators showed they were not always able to differentiate normal from syphilitic serums by colorimetric readings, because under certain conditions the two gave the same results. These color changes were charted on a given scale and it was noted that syphilitic serums occupied a given part of the syphilitic chart or "graphique" as compared with normal serums.

Vernes began his experiment as follows: A series of approximately 40 tubes, each containing 2 c.c. of a colloidal suspension of ferric hydrate, 0.2 c.c. human serum and 0.9 per cent NaCl, were placed in an incubator at 37°C. for 40 minutes. Flocculation occurred in certain tubes, disappeared either abruptly or progressively in other tubes and reappeared in a later series of tubes, constituting periodic zones of flocculations. If other mineral substances in colloidal states were

substituted for ferric hydrate the periodic rhythmicity of flocculation was changed but variation between normal and syphilitic serums occurred constantly. In order then to produce flocculation with syphilitic serums and not with normal serums, an organic colloidal preparation was necessary, not a mineral suspension. An extract of horse heart diluted with distilled water was used to make the proper colloidal suspension. 0.4 per cent NaCl was used for the electrolyte. The complete reaction is a two-stage reaction as follows: Stage I: 0.2 c.c. of human serum which has been inactivated at 55°C. for 20 minutes, 0.8 c.c. horse heart extract diluted 1:40 in 0.9 per cent NaCl, and 0.8 c.c. pig serum. Tubes are placed in incubator at 37°C. for one hour and 25 minutes. Stage II: 0.8 c.c. sheep cells (titrated and made up in 50 per cent hypertonic saline solution) is added to each tube and again incubated for one half hour and centrifuged. In the first stage no flocculation results with normal or syphilitic serums because pig serum prevents flocculation by virtue of its antiflocculent power. After centrifugalization (as in the second stage) normal and hemolytic serums may be entirely free from a hemoglobin tinge but there may be intermediate degrees of color tints between deep red and colorless which constitute a syphilitic index which may be of some importance in the control of treatment.

Meinicke Reaction.[27] This test is based on the hypothesis that the reaction between serum and extract takes place when extract colloids disturb the isotonicity of salt solution permitting the union of seroglobulins and lipoid extract. This reaction is greatly intensified in the positive syphilitic serums as compared with negative. The various forms of Meinicke's reactions are (1) Water method; (2) Salt solution method; (3) Third Modification.

(1) *Water method.* Wassermann antigen diluted with distilled water flocculates all negative serums, while the positive serums show a characteristic opalescent turbidity. Positive serum does not flocculate as the globulin cannot be released in distilled water medium, but the lipoglobulin complex through the action of the lipoids compensates itself by keeping the globulins in distilled water solution. This method possessed many faults as weak flocculation would often result in strongly positive Wassermann serums, and heavy flocculations in negative Wassermann serums. Meinicke then devised the following method:

(2) *Salt Solution Method.* This method, intended to overcome the

difficulties of the previous method, consists of a two-phase reaction. Extract of syphilitic liver diluted with distilled water will flocculate both normal and syphilitic serums. The addition of salt solution in varying concentrations to both serums would dissolve the precipitate in the negative and not in the positive; in fact, the precipitate in the positive tubes becomes intensified. (See references 28 for those who investigated the Meinicke reaction).

For the reason that spinal fluid contains albumins, it will not flocculate with the first or second Meinicke methods, but flocculation will occur, however, in what is termed the Third Modification and also in the Sachs-Georgi reaction.

(3) *Third Modification.* Using an antigen prepared according to the method of Wassermann and with the addition of horse heart extract, he was able to get flocculation in the strongly positive serums. This is a one-phase reaction consisting of covering the contents of the tube with approximately a 2 per cent solution of NaCl. In many cases this modification gave a positive reaction earlier than the Wassermann reaction. In spinal fluids, however, the Third Modification will show flocculation and certain concentrations of NaCl will flocculate negative Wassermann serums. This phase of the question requires more study as Meinicke reports a difference of 5 to 10 per cent between Wassermann reaction, Meinicke (Phase I and II) reaction, and Third Modification. The reasons for these differences are not given by him.

The explanation of this reaction is as follows: The tissues of an organism immunized against a certain substance has the power to combine with an antigen faster and more intensely than through an organism which has not been so immunized. This specific reaction not only occurs with tissue cells but may also take place with blood serum. The various forms of immunity reactions are simply expressions of the numerous agents which react in various combinations in a progressively increasing manner so that it is possible that these various agents can combine with one another. This hypothesis then is based on the study of precipitation, agglutination, anaphylaxis, hemolysis, and bacteriolysis. By inactivating the serum the strength of the reaction is changed in two ways, namely, the seroglobulin molecule is heavier than the salt solution molecule, and conversely, the heavier the concentration of the solution the greater is the combining power with the albumin molecule in the inactive

serum over that in the active serum. The fundamental principle involving these two investigations is based upon the great binding power of the salt with the heated serums.

The water and salt solution methods are used as controls for the Third Modification of the Meinicke reaction. The results of this reaction compare favorably with the Wassermann reaction.

Sachs-Georgi Reaction.[29] This is a physico-chemical reaction between seroglobulins and lipoid extract. It has been shown that the globulins in syphilitic serums are increased in amount and that the flocculate which occurs in this reaction is a lipoglobulin aggregate. The modified technic of this reaction is as follows: Three drops of inactive serum or 6 drops of spinal fluid plus 1 c.c. of an 0.85 per cent NaCl plus 0.5 c.c. cholesterinized beef or human heart extract (cholesterinized beef heart extract will flocculate syphilitic serum only) which has been previously diluted 1:6 with 0.85 per cent NaCl is put in an incubator at 37° C. for from 18 to 24 hours. A reading is taken and positive tubes show a flocculation. Serum mixed with alcohol is used as a control for this reaction. The tubes are allowed to remain at room temperature overnight when a second reading is taken. Positive tubes show a flocculation, negative tubes remain clear. For reading the results of this reaction as well as for the Meinicke reaction, an agglutinoscope, a dissecting microscope with a number 8 or 9 lens, an ocular from a microscope, or an ordinary magnifying lens may be used.

The Sachs-Georgi reaction is the simplest of all the flocculation reactions. The preparation of the antigen and the technic of the test is so simple that the clinician can compare his data with the serologic results; and also the serologist may use this simple reaction as a check for the Wassermann reaction.

EXPERIMENTAL INVESTIGATION OF WASSERMANN, MEINICKE, AND SACHS-GEORGI REACTIONS WITH ANIMAL SERUMS

A comparison was made between the Wassermann, Meinicke, and Sachs-Georgi reactions using animal serums. It is known that the serums of certain animals will give a positive Wassermann reaction. Friedmann[30] has shown that by using inactivated beef, goat, dog, and rabbit serums one can easily obtain a positive Wassermann reaction. Inactivated cow, guinea pig, and goose serums did not give

a positive Wassermann reaction. This investigator has shown that in flocculation reactions, precipitation with lecithin, sodium glycocholate and distilled water may result with normal animal serums. Manwaring[31] examined active animal serums and was able to demonstrate a positive Wassermann reaction with beef, horse, and goat serums. The following tables show the relationship between the Wassermann, Meinicke, and Sachs-Georgi reactions using both active and inactive animal serums.

TABLE I

NORMAL ANIMAL SERUMS*

ANIMAL	WASSERMANN		SACHS-GEORGI		MEINICKE			
					PHASE I		PHASE II	
	ACT.†	INAC.†	ACT.	INAC.	ACT.	INAC.	ACT.	INAC.
Chicken	+	±	+	+	+	+	+	+
Duck	±	0	0	0	±	±	±	±
Cat	+	+	0	+	+	±	0	±
Horse	+	+	+	+	+	+	+	±
Beef	+	±	+	0	+	+	±	±
Steer	+	+	+	+	+	+	+	±
Goat	+	+	+	+	+	+	+	+
Pig	+	+	0	+	+	+	0	±
Rabbit	± — +	+	0	0	+	±	+	±

*Modified from Paul Konitzer, Ztschr. f. Immunitäts. u. Exper. Therap. Orig., 1920, # 30.
†Act. = Active; Inac. = Inactive Serum.

TABLE II

PRECIPITATING RABBIT SERUMS*

ANIMAL	WASSERMANN		SACHS-GEORGI		MEINICKE			
					PHASE I		PHASE II	
	ACT.†	INAC.†	ACT.	INAC.	ACT.	INAC.	ACT.	INAC.
Chicken	+	+	0	0	+	+	+	+
Duck	+	+	±	0	+	+	+	+
Cat	+	+	0	±	+	+	±	+
Horse	+	+	±	0	+	+	+	+
Beef	+	+	±	0	+	+	+	±
Steer	+	+	±	+	+	+	±	+
Goat	0	+	+	0	+	+	+	0
Pig	+	+	±	0	+	+	0	+
Deer	+	+	0	0	+	+	+	+
Goose	+	+	0	+	+	+	+	+

*Modified from Paul Konitzer, Ztschr. f. Immunitäts. u. Exper. Therap. Orig., 1920, # 30.
†Act. = Active; Inac. = Inactive Serum.

The above tables show that the Sachs-Georgi reaction and Meinicke reaction also give a positive flocculation reaction with certain animal serums; however, there exists no parallelism with the Was-

sermann reaction. There seems to be no definite advantage in using inactive serum. With certain serums flocculation seems to increase, with others it is less.

RELATION OF ABOVE REACTIONS TO THE CLINICAL DIAGNOSIS OF SYPHILIS

Most of the above tests were designed to supplant the Wassermann reaction, but it may readily be seen that not only are some of these reactions as complicated as the Wassermann reaction but they have also been proved inferior when the clinical diagnosis is considered, and certainly cannot be used to supplant the Wassermann reaction. Not only were some of the above mentioned flocculation reactions negative when the Wassermann reaction was positive, but they were negative also in definite clinical cases of syphilis with strongly positive Wassermann reactions.

Comparing the serologic data obtained of these reactions with the clinical studies of the cases, we find the Meinicke and Sachs-Georgi reactions most trustworthy. And because of the marked simplicity of the Sachs-Georgi reaction as compared with the Meinicke reaction we have made a more detailed study of this test, and have compared it with the Wassermann reaction and clinical symptoms both of syphilitics and nonsyphilitics.

The results of our previous investigations[32] show that in an examination of over 1000 cases with both the Wassermann and Sachs-Georgi tests, there was an agreement in 92 per cent. This percentage is in accord with the results of other investigators as Table III shows. Table IV gives a detailed comparison of the Sachs-Georgi and Wassermann reactions; also showing our results as compared with those of other investigators. In spinal fluid examination in cases of neurosyphilis,[33] there was an agreement between Wassermann and Sach-Georgi tests in 79 per cent. As our records show, the Sachs-Georgi reaction was positive in a larger number of clinically positive cases of syphilis than the Wassermann reaction, so that although the percentage of agreement between these two reactions may not be high, nevertheless, when the Sachs-Georgi reaction is compared with the clinical diagnosis of syphilis the percentage increase becomes higher. The response of the positive cases to antisyphilitic treatment as determined by the Sachs-Georgi reaction is also corroborative of its marked efficiency.

TABLE III

PERCENTAGE AGREEMENT BETWEEN WASSERMANN AND SACHS-GEORGI REACTIONS

INVESTIGATOR	PER CENT	INVESTIGATOR	PER CENT
Sachs-Georgi[29]	94.94	Konitzer[38]	86.
Lönns[34]	95.5	Schroder[39]	85.2
Sheer[35]	94.38	Weichard and Schroder[40]	82.
Gaethgens[36]	93.7	Hauck[41]	80.66
Meyer, Lipp, Nathan, Munster[37]	93.	Levinson and Petersen[33] (Neurosyphilis)	78.5
Levinson and Petersen[32]	92.		

The Sachs-Georgi reaction because it is simple and practical enables the clinician to better understand serologic tests and their relation to the clinical findings. It can be elicited earlier and remains positive later than the Wassermann reaction; it is not influenced by treatment to the extent of becoming negative after one or two injections of salvarsan; it is present in congenital cases of syphilis as well as in latent syphilis; and in cases of neurosyphilis.

Since there has not as yet been a sufficiently large number of cases reported by investigators to permit a trustworthy comparison, we are of the opinion that for the time being the Sachs-Georgi reaction should not supplant the Wassermann reaction, but may supplement it. For this reason, the various factors entering into this reaction will be analyzed, and an attempt made to explain the mechanism of the flocculation.

MATERIAL FOR SACHS-GEORGI REACTION

A detailed study of the material used and the technic employed in the Sachs-Georgi reaction will be made, especially in reference to the advisability of employing these various factors.

Inactivation of serum and its relation to the reaction. It is known that the complement varies in different specimens of serum, and for this reason Wassermann, Bauer,[52] and others heat the serum to 55° C. for one half hour to destroy the native complement. They substitute for this unknown factor in the Wassermann system a uniform amount of guinea pig complement of known activity. In the system of Hecht[53] and Tschernogubow[54] the serum is not heated, thus utilizing the native complement in the serum and making the reaction more sensitive. A study of the effect of active or inactive serums on the Wassermann reaction was undertaken by Noguchi,[55] who came to the following conclusions: "In the majority of active

human serums irrespective of sources there exists a constituent which fixes complement when mixed with certain proteins such as, nucleoproteins, peptone, albuminoses and many other autolytic decomposition products of proteins. This is known as the proteotropic fixation group in contradistinction to the lipotropic fixation due to the action of certain lipoids upon syphilitic serum. This proteotropic group is destroyed by heating to 55° C. for 20 minutes, while the lipotropic fixation is not abolished. It becomes evident, then, that when one employs as an antigen an alcoholic or aqueous extract of macerated organs inactivated serum must be used, because the antigen preparations contain various proteids and are liable to give a nonspecific proteotropic fixation with active serum."

TABLE IV

COMPARISON OF WASSERMANN REACTION AND SACHS-GEORGI REACTION SHOWING OUR RESULTS AS COMPARED WITH THOSE OF OTHER INVESTIGATORS

REACTION	RESULTS									TOTAL
Wassermann		+			±			0		
Sachs-Georgi	+	±	0	+	±	0	+	±	0	
Sachs-Georgi[29]	614	32	20	28	—	—	37	23	2016	2770
Georgi[42]	331	19	32	20	—	2	32	7	1228	1671
Nathan[43]	598	—	55	—	—	—	96	—	866	1615
Nathan-Weichbrodt[44]	39	—	—	—	—	5	7	—	141	192
Weichardt-Schroder[40]	31	—	2	—	—	—	2	—	2	37
Reich[28]	49	5	—	—	7	9	2	9	126	207
Lesser[28]	162	—	57	—	—	—	116	—	1165	1500
Meyer[37]	148	13	10	—	12	11	1	1	404	600
Konitzer[38]	175	—	31	—	—	—	44	—	438	688
Munster[37]	116	1	2	4	—	—	7	1	23	154
Löns[34]	183	—	8	4	—	—	3	5	337	540
Zurhelle[45]	663	—	133	—	—	—	71	—	1167	2034
Scheer[35]	129	1	4	—	28	—	17	—	199	378
Zimmern[46]	193	57	16	7	21	31	6	22	501	854
Gathgens[36]	110	1	11	13	2	9	23	1	530	700
Frankel[47]	83	—	6	—	3	—	6	—	179	277
Raabe[48]	569	—	5	—	—	47	8	37	1005	1671
Wolffenstein[49]	264	—	15	—	—	—	134	—	578	1000
Frommherz[50]	48	1	2	3	24	17	2	4	197	298
Baumgartel[51]	1303	152	105	75	129	194	72	111	4839	7000
Levinson-Petersen[32]	199	11	6	3	—	3	51	11	755	1042

In the Sachs-Georgi reaction the best results are obtained by inactivating the serum at 55° C. for 20 minutes. Mandelbaum[56] has carried out experiments along this line in which positive serums were

inactivated at various degrees ranging between 45° C. to 65° C. Above 55° C. the number of positive cases became less in number. It is best to inactivate the serum undiluted. Hauck[57] states that cold temperatures influence the flocculation in some way and that more nonspecific reactions occur at lower temperatures than higher temperatures. The best results obtained which are characteristic for flocculation as well as for the Wassermann reaction is when the serum is heated at 55° C. for one-half hour.

Diluting solution. Neukirch[58] has shown that a larger number of nonspecific reactions occur in undiluted serums than in diluted serums. For the Wassermann reaction physiologic salt solution can be used as a diluting agent, but in the Sachs-Georgi reaction, an 0.85 per cent NaCl must be used. This salt is equimolecular and has an isoelectrical point for the seroglobulins and is the optimum concentration needed to produce flocculation. Neukirch[58] has also tried other salts as sodium nitrate, sodium sulphate, sodium acetate, sodium bromide, potassium chlorate, and magnesium chloride, but these salts which were used in varying concentrations did not prove as satisfactory as an 0.85 per cent NaCl solution. The diluting agent must not be added to the antigen too rapidly or too slowly. In the diluting of the serum this factor plays no appreciable rôle.

Antigen. The most important factor in the standardization of the Wassermann reaction as well as for the specificity of the Sachs-Georgi reaction is the antigen. Although artificial antigens such as, lecithin, salts of bile acids and soaps have been used with some degree of specificity in syphilitic serums, it is entirely a matter of choice, as the ultimate usefulness of an antigen is dependent on the care with which the extract was standardized.

The preparation of the proper antigen is perhaps the fundamental factor in the success or nonsuccess of the Sachs-Georgi reaction. Extracts of various organs can be used but the most satisfactory results have been obtained with extracts prepared from human and beef heart. An alcoholic solution of cholesterin is added to the extract, the exact amount, however, being determined by titrating the antigen against negative and positive serums using varying amounts of cholesterin. This method of titration has been described in a previous article.[32]

When the extract is properly made it is quite clear, but before use

when the antigen is diluted 1:6 with salt solution it is faintly opalescent and does not flocculate after standing several days. For the Wassermann reaction this antigen can be used in dilution of 1:10.

Several antigens which we prepared 8 months ago, and which have been standing at room temperature, were tested recently in the Sachs-Georgi reaction and the Wassermann reaction and found as active as when first prepared.

Incubation. The specificity of the flocculation reaction also is dependent upon the period of incubation. In the original technic of Sachs-Georgi the tubes were incubated for 2 hours and read, then allowed to remain at room temperature for 24 hours before the final reading. A large number of nonspecific reactions resulted especially in cases of tuberculosis, tumors, and typhus. Steiling,[59] Gaethgens,[36] Schoenfeld[60] and others have shown that by incubating the tubes at 37° C. for from 18 to 24 hours and a first reading taken, and then allowing the tubes to remain at room temperature overnight, these nonspecific reactions could be obviated. By this procedure the agreement between the Wassermann reaction and the Sachs-Georgi reaction reached a higher percentage. Sachs and Georgi have also adopted the latter incubation time.

Reading of Results. The positive cases present a flocculation which either may be finely granular, having a snow storm appearance, or which may be finally precipitated as a coarsely granular mass at the bottom of the tube. The very definite cases may be precipitated at the end of 2 hours' incubation; however, by allowing the tubes to remain at room temperature overnight all positive cases may show the same results. The negative cases may remain perfectly clear, or a very slight precipitate may collect at the bottom of the tube which can readily be differentiated from positive findings. By experience one may grade the degree of the reaction on a 1 to 4-plus basis.

As a rule most of the readings can be made with the naked eye. The earlier investigators of the Sachs-Georgi reaction, as well as of the Meinicke reaction, used the agglutinoscope devised by Kuhn and Voit. A good magnifying lens from a dissecting microscope, or an ordinary ocular may be used with satisfactory results if the tubes are held against a black background.

Meyer[61] and Gaethgens[36] have found it advisable to centrifuge the tubes for 20 minutes at low speed after incubation thus saving time

Also by concentrating the precipitate one is able to eliminate nonspecific reactions. In doubtful cases we have centrifuged the tubes at low speed for 20 minutes and by pouring off one-half the supernatant liquid we were able to concentrate the flocculation into one-half the amount of fluid, thus making the reaction doubly certain.

MECHANISM OF FLOCCULATION REACTIONS

The mechanism of the Wassermann reaction is based upon the disappearance of complement in the mixture of antigen and antibody and is called "complement fixation;" if the complement has been deviated by the combination of antigen and antibody thus preventing participation in the hemolytic process, it is called "complement deviation." However, the question of hemolysis does not enter into the mechanism of the Sachs-Georgi reaction as only one biologic reagent (antigen) is used, instead of 4 (antigen, red blood cells, amboceptor, complement) in the Wassermann system.

The numerous investigations into the phenomena of complement-fixation reaction resulted in the presentation of 2 theories: One being the specific antibody-antigen reaction in connection with Ehrlich's side chain theory; the other the Bordet adsorption theory based upon a physical or a physicochemical concept. With further advances into the study of the chemistry of colloids a large number of investigators came to the conclusion that the Bordet-Gengou phenomenon is simply a precipitation of colloids.

At first Zangger as well as Landsteiner,[62] studying the Bordet adsorption theory, came to the conclusion that the reaction between the immune bodies and antigens is in its entirety a growth of colloidal particles, and as to specific complement fixation, Moreschi[63] concludes that it is a precipitation of antigen by antibody. Gay[64] and others have shown that the adsorption of the complement is a phase of the reaction similar to the aggregation of ultramicroscopic colloidal particles.

The mechanism underlying the reaction discovered by Bordet and Gengou remained in doubt for a long time until Moreschi[63] demonstrated the complement-binding reaction between the antialbumin group and its accompanying antigens.

We cannot enter here into a detailed discussion of the explanation of the mechanism of the Wassermann reaction, but it is the con-

sensus of opinion among investigators that this reaction is a physical or a physicochemical reaction. Wassermann[70] has recently revised his old conception of the mechanism of his reaction as being an antigen-antibody reaction, for he has shown that when this reaction was first formulated, antigens as normal lipoids, such as lecithin, or alcoholic extracts of normal organs were not used. It was not known, in the early stages of this reaction, that the amboceptor may be lipotropic. Wassermann thoroughly investigated this subject and was able to demonstrate that syphilitic serum contains a substance which he calls "Wassermann substance." This substance is capable of combining with lipoids forming a new aggregate which he calls "Wassermann aggregate." These Wassermann aggregates are evenly composed and can easily go back into solution, as the two specific components (amboceptor and lipoid) are held together in a loose combination. Under favorable conditions these two elements may enter into a combination again. There is a reversible binding power between the Wassermann substance and the added antigen in which the complement is bound together very strongly. This newly isolated substance has all the characteristics of an amboceptor. According to the definition, the Wassermann substance undoubtedly belongs to the class of antibodies, but primarily it is certainly an antibody for human lipoids, and probably later this substance may be isolated from animal cells.

It was very interesting from this standpoint to note the relationship the various serum reactions for syphilis, especially the flocculation reactions, have with the Wassermann reaction. Wassermann studied the Sachs-Georgi reaction to determine if this reaction showed a qualitative relationship to the Wassermann reaction. The precipitate from the Sachs-Georgi reaction was washed, dissolved in salt solution and tested to show the relationship the isolated partial antigen has to the Wassermann substance. When the latter is added to the partial antigen solution a positive reaction is the result, and conversely, when the partial antigen from the Sachs-Georgi reaction is added to the Wassermann system, a positive reaction also takes place. *This is conclusive evidence that the Sachs-Georgi reaction is qualitatively similar to the Wassermann reaction, and that the foundation for all serodiagnostic methods for syphilis is the formation of the Wassermann aggregates.*

It becomes evident then, that the newer flocculation reactions, such as the Meinicke and Sachs-Georgi reactions might throw some light on the colloidal changes of the seroglobulins characteristic for syphilitic serum, without attempting to explain complement binding action. Certain antibodies are called upon to unite with the complement (Wassermann reaction), but the conditions of the serum such as disturb the electrochemical affinity and dispersion, may be so altered, as to bring about a rearrangement of the serum globulins which enables them to unite with the lipoidal extract to form a colloidal precipitate. In the Wassermann and flocculation reactions definite colloidal aggregates result, and for syphilis there appears to be a special affinity between the serum globulins and lipoidal extract resulting in the formation of colloidal aggregates. According to Sachs the difference between the Wassermann reaction and the flocculation reactions is that in the former there is no definite macroscopic precipitate at the beginning, but instead there is a growth of the globulin particles which continues until gross precipitation takes place.

Acids increase and alkalies decrease the speed of these reactions. (Nathan[65] added acids, inulin, and bacterial suspensions to negative Wassermann serums and was able to obtain a positive Wassermann reaction; the Sachs-Georgi reaction became positive later on. The flocculations which occurred with the Sachs-Georgi reaction were not characteristic for syphilis, since the seroglobulins were affected in some way.) In the Meinicke reaction, when normal HCl of 1:500 dilution was used, some tubes showed flocculation, others not; but in the Sachs-Georgi reaction there is a scant flocculation when this chemical is added which can easily be differentiated from positive flocculations.

Herzfeld and Klinger,[28] Saligmann,[66] Much,[67] Elias, Porges, Neubauer and Solomon[13] investigated the Wassermann and flocculation reactions with various reagents and came to the conclusion that no actual difference exists between the two. Hecht,[53] Meinicke,[27] and others have shown that the union of extracts and antigen resulted in an anticomplementary precipitate. Michaelis and Davidsohn[68] are of the opinion that with immunity reactions, especially the Wassermann reaction, the affinity between antigen and antibody cannot be explained on the basis of adsorption.

Meinicke explains the flocculation phenomenon resulting from the union of luetic globulins with extract by saying, "that the combination of these 2 elements takes place in a salt solution medium. In positive serum the reaction is intensified and there is adsorption of salt from solution, while with the negative serum there is a weak adsorption of NaCl. The positive serums contain, therefore, flocculi which have a strong affinity for NaCl, the negative serums having a weak affinity for NaCl."

According to Sachs and Georgi flocculations result from the union of extract and seroglobulins which are characteristic for lues, and these two elements have a definite affinity for each other.

There are two possible factors which enter into the mechanism of flocculation reactions: (1) The OH ion is increased when luetic serum is heated to 55° C.; (2). Michaelis[69] has also shown that heat changes the isoelectrical point of the serum and that when the globulins are changed through inactivating the serum, the isoelectrical point is disturbed and the H ion concentration is lessened; thus normal inactive serum can be made to flocculate upon the addition of HCl. Sachs, Schmidt, and Hecht have demonstrated that a rearrangement of the isoelectrical point of the seroglobulins in luetic serum is the result of the formation of acid albuminate bodies. Flocculation in normal and luetic serums do not take place suddenly but gradually.

Alcohol and NaCl used in diluting the extract have no effect upon the globulins, or upon the inactivated serum mixed with diluted extract. Solutions of alcohol and NaCl in higher concentration may produce precipitation but alone and in such high concentration they are not used in flocculation reactions.

Meinicke is of the opinion that in the first phase of his reaction the precipitating power of extract and distilled water is brought about through a diminution of its binding power with the globulins. (This is not characteristic for precipitation in luetic serum and certainly is not true in a medium of high salt concentration, but that NaCl, under ordinary circumstances adsorbs water from the precipitated globulins. Bechhold investigating this subject says, "that globulin flocculi under certain conditions is bound with water as a result of its weight.") Also Meinicke's water method in which

flocculation takes place in certain zones in negative serum which has been diluted with water, is in accord with the above explanation.

Spinal fluid does not flocculate in the first phase of the Meinicke reaction, and it is explained that because of the relatively high water content of the spinal fluid, the water diluted extract will not flocculate the spinal fluid upon the removal of NaCl from solution, while the Sachs-Georgi reaction and Third Meinicke reaction will flocculate spinal fluid.

The above explanations would seem to indicate that flocculation comes to a stand still when the individual colloidal globulin particles adsorb water, and that these particles through adsorptive powers become larger complex units. In order to determine whether the extract lipoids play a rôle in flocculation, Meinicke used Sudan IV to stain the extract particles. The stain is not thrown down when organ extract and luetic serum is mixed. Sudan IV stains the lipoglobulin aggregate, but this condition does not explain the reason that lipoids do not combine with NaCl. Meinicke came to the conclusion then, that since Sudan IV stains extract lipoids only, the latter do not enter into the process of flocculation. Hecht[53] having the opposite opinion, that from his studies of the complement with flocculations resulting in positive serums, IX gave nothing else than an antigen complex characteristic for precipitation in luetic serum.

Joel[28] used Osmic acid to stain the extract lipoids and reached the same conclusion Meinicke did. The black staining particles were mixed with luetic serum, and the flocculations centrifuged; by this means the black staining flocculations can be recognized. Joel, drawing his conclusion from Meinicke, says, "that the precipitation of lipoidal extracts is not characteristic for the Meinicke reaction, although one cannot say very definitely that there are no extract lipoids in the precipitation. But that in the course of the reaction there is set up a new individual chemical process of an entirely different nature."

In a dark-field examination of diluted extract, one can observe small, round, strongly opaque bodies, which cannot be seen in undiluted extract. In precipitated tubes—the dark-field examination shows no distinction between primary precipitation in the Meinicke reaction, and between positive and negative series—there are very few colloidal particles in the extract, while in the nonprecipitation series, or upon

the addition of NaCl to the serum extract mixtures, one can easily see the result of the molecular rearrangement in the growth of the colloidal particles in the positive serums of Phase I Meinicke reaction, and in the Sachs-Georgi reaction. Here and there one may observe a netlike structure in the flocculi, each being strongly opaque, which makes these particles easily seen.

If Joel's and Meinicke's original explanation is correct, namely, that the efficacious colloidal particles take part in the flocculation, then in a positive Sachs-Georgi reaction, serum plus extract after precipitation of formed flocculi should not precipitate upon the addition of more serum; since the lipoids have been thrown down in large numbers during centrifuging. But this is not true, there being as marked a precipitation in the centrifuged tubes after the addition of serum, as in the control tubes. This question, however, needs more study.

The 0.85 per cent salt solution used in the Sachs-Georgi technic is the optimum concentration needed for the precipitation of the globulins for which it is isoelectric. The horse heart extract used in the Meinicke reaction has two optima: one for distilled water, the other for a 2 per cent NaCl solution. The salt solution in no way affects the antigen or seroglobulins. Although it makes no apparent difference whether or not the blood serum is inactivated at 55° C. for one-half hour, all questionable errors such as the complement are done away with. The latter when present, plays no appreciable rôle in the reaction.

The flocculation, as is seen in the Sachs-Georgi reaction, is the result of the union of the serum globulins with the lipoidal antigen and is very similar to the ultramicroscopic colloidal alteration which takes place in the Wassermann system. The degree of flocculation may depend on the amount of serum globulins present in the syphilitic serum which in turn may be due to the degree of irritation resulting from the action of the Treponema pallidum on the tissue cells. It becomes evident then, that in the very active cases of syphilis, although the clinical symptoms may not be markedly apparent, there may be an increased amount of globulins present in the patient's serum (Noguchi[55]). These various amounts of increased serum globulins uniting with lipoidal antigen result in flocculations varying from 1 to 4-plus.

In the negative cases, however, the serum globulins found in syphilitic serum are absent, although nonsyphilitic serum may contain an increased amount of globulins. In these negative cases the salt solution may be isoelectric and equimolecular with the serum globulins and is kept in suspension along with the lipoidal antigen resulting in a clear nonflocculating solution.

Heretofore, we have discussed the various contributions presented by different investigators to explain the mechanism of flocculation. We offer the following suggestions in the hope that they may be of some assistance in explaining and clarifying the subject of mechanism of flocculation reaction in syphilis. In this connection it would be of interest to note the reason for the syphilitic serum showing an affinity for nonspecific lipoidal antigens. As has been mentioned above, there are certain conditions in which the serum globulins may be increased over that of normal, if not in a greater percentage than that found in syphilitic serum. A detailed discussion of this question must be left for a later paper, but we can only mention the various factors which probably play a role in this mechanism.

The Treponema pallidum as it irritates the tissue cells causes the globulins to become increased, and a toxin is liberated by the Spirocheta pallidum which is specific for syphilis. When this occurs the globulins combine with this toxin, thus causing this sensitized globulin to have a greater affinity for the lipoidal extract particle, resulting in colloidal aggregates which appear in positive flocculation reactions. In negative cases, although the globulins may be increased in number, there is no specific syphilitic toxin liberated by the spirochete which may combine with the globulin, and no flocculation can occur. This method of reasoning does not explain the flocculations which occur in nonspecific reactions.

In tuberculosis, for example, we know that the serum globulins are increased in number, and that there is also a specific toxin liberated by the tubercle bacillus. These specific toxins may combine with the globulins in the same manner as the syphilitic toxin may combine with its globulins. Inasmuch as the flocculations occurring in nonspecific reactions are not numerous, it is possible that the sensitized syphilitic globulin has a greater affinity for the nonspecific lipoidal extract than any of the other sensitized globulins

have, but perhaps there may be some other reasonable explanation for this mechanism.

What rôle do electrolytes play in the growth of colloidal particles? When NaCl of a certain concentration is added to the antigen and serum, the globulins and lipoidal extract have a tendency to retain certain quantities of the electrolyte. In the preparation of most inorganic colloids small quantities of the electrolytes are retained by the colloids, and these tend to bring about coagulation (Woudstra[71]). With organic colloids a similar phenomenon may occur which becomes manifest, in this connection, to a greater extent with syphilitic globulins than with nonsyphilitic globulins. The influence of age may also be of some significance in bringing about a greater affinity for these colloids.

In flocculation reactions, certain characteristic phenomena may occur, such as a strong flocculation taking place when the tubes are kept in the incubator at 37° C. at any time between 2 and 24 hours, or after standing at room temperature for 24 hours. In some cases after the flocculation has appeared in the first 24 hours, the flocculi may disappear within the following 24 hours. A similar condition also takes place with the Wassermann reaction (as weak positive reactions). In the latter case, it is the opinion of some serologists that weak positive Wassermann reactions may have been strongly positive during the period of incubation, but that some of the clumped red cells may have undergone hemolysis and gone into solution. The explanation for these occurrences is possibly as follows: A colloidal particle is changed in some way by the addition of the electrolyte resulting in coagulation taking place very early, and in the course of time this union becomes stronger. A change in the state of the colloid may also be brought about by electrolytes where the union of colloidal elements decreases after a certain time giving a weak or a negative reaction.

Colloidal solutions have a characteristic electric behavior which may explain many of their peculiar properties; and the magnitude of the electric charge, which most substances in colloidal solution carry, varies greatly. The syphilitic globulin may be so charged that it has a greater electrochemical affinity for the lipoidal extract, producing flocculations, and in the negative cases, where no flocculation takes place the lipoidal extract and serum globulin may

both contain negative charges and they repel each other, since it is a known fact that oppositely charged colloids precipitate each other. In those cases, where there is a weak or a doubtful reaction, the positive and negative charged colloidal particles are held in a loose combination.

CONCLUSIONS

1. A brief résumé of the numerous studies of the Wassermann reaction discloses two schools of thought; (1) That which believes that the Wassermann reaction is an antigen-antibody reaction, and has attempted to modify and simplify this reaction, (2) That school which follows the study of the chemistry of the colloids, and has attempted to show a parallelism between the Wassermann reaction and certain colloidal reactions. The latter has led to the Meinicke and Sachs-Georgi reactions.

2. Many investigators have studied the practical value of the Meinicke reaction (Phase I and II) and have reached the following conclusions: There was 89.2 per cent agreement with the Wassermann reaction; this reaction is in all respects characteristic for syphilis and it is more simple than the so-called Third Modification or the Sachs-Georgi reaction; it cannot be used in spinal fluid; the overlapping of the Meinicke Phase I reaction by the Phase II reaction did not prove of any practical value; the theory upon which the Meinicke reaction is based does not explain the kind of flocculation produced.

3. The Third Modification of the Meinicke reaction is more simple and is therefore recommended. The agreement with the Wassermann reaction is 88.8 per cent. In many cases it is positive earlier, and often remains longer than the Wassermann reaction. When Meinicke's horse heart extract was used all Wassermann positive serums did not flocculate, and it is hoped that the acuity of the reaction, and a better antigen will be reached so that the precipitate can be detected with the naked eye.

4. The Sachs-Georgi reaction has met with the approval of many investigators. The nonspecific reactions are less frequent with this reaction. Our agreement with the Wassermann reaction was 92 per cent, as compared with the general averaged agreement of 91 per cent reported by other investigators.

5. A comparative study of the Sachs-Georgi, Meinicke, and Wassermann reactions gives the Sachs-Georgi reaction an advantage over the Meinicke reaction.

6. None of these reactions can at present supplant the Wassermann reaction but may be used in conjunction with it. Our investigations, as well as those of others, have shown that the Sachs-Georgi reaction becomes positive earlier and remains positive (and also in some treated cases of syphilis) longer than does the Wassermann reaction.

7. In an investigation of animal serums with the Wassermann, Meinicke, and Sachs-Georgi reactions, no parallelism was noted.

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REFERENCES

[1]Landsteiner, Müller, Poetzl: Wien. klin. Wchnschr., 1907, xx.
[2]Sachs and Rondoni: Berl. klin. Wchnschr., xlv, 1968. Ztschr. f. Immunitatsforch, Orig., i, 132.
[3]Noguchi, H.: Jour. Exp. Med., 1909, xi, 84.
[4]Kolmer, J.A. and Collaborators: Am. Jour. Syph., January, April, July, October, 1919, January, July, 1920, January, 1921.
[5]Jakobaus: Ztschr. f. Immunitatsforch., iv, p. 1, No. 2.
[6]Streng and Karvonen: Arch. f. Dermat. u. Syph., cviii.
[7]Hirschfeld and Klinger: Deutsch. med. Wchnschr., 1914, xl.
[8]Fornet and Schereschewsky: Deutsch. med. Wchnschr., 1907, xli. München. med. Wchnschr., 1907, xxx. Berl. klin. Wchnschr., 1908, xviii.
[9]Klausner: Biochem. Ztschr., 1912, xlvii.
[10]Sachs, H.: in Kolle-Wassermann Handbuch. d. Pathog. Mikroorgan. Ed. 2.
[11]Porges-Meier: Wien. klin. Wchnschr., 1908, xxxi. Berl. klin. Wchnschr., 1908, xlv.
[12]Müller, and Landsteiner: Wien. klin. Wchnschr., 1907, xvii.
[13]Elias, Neubauer, Porges, and Salomon: Wien. klin. Wchnschr., xxi; ibid, 1918, xxxix.
[14]Hermann and Perutz: Med. Klin., 1911, ii.
[15]Sachs and Altmann: Berl. klin. Wchnschr., 1908, x.
[16]Bruck and Hidaka: Ztschr. f. Immunitatsforch., viii, p. 4.
[17]Teruchi and Toyada: Wien. klin. Wchnschr., 1910, xxv.
[18]Levaditi and Yamanouchy: La Serodiagnostic de la Syphilis.
[19]Fleischmann: Berl. klin. Wchnschr., 1908, x.
[20]Hecht: Berl. klin. Wchnschr., 1909, x.
[21]Hesberg: Cited by Bechhold in Die Kolloide in Biologie u. Medizin, Dresden and Leipzig, 1919.
[22]Munk: Deutsch. med. Wchnschr., 1912, xxix. Ztschr. f. Chemotherap., Ref., 793 to 808; 973 to 985.
[23]Jakobsthal: Ref. Deutsch. med. Wchnschr., 1909, xxx. München. med. Wchnschr., 1910, xiii.
[24]Hermann-Perutz: Wien. klin. Wchnschr., 1918, xxxix.
[25]Bruck: Munchen. med. Wchnschr., 1917, lxiv.

[26]Vernes, A.: Les signes humoraux de la Syphilis, Paris, 1913. Compt. rend. Acad. d. sc., 1917, clxv; 1918, clxvi, ibid clxvii.

[27]Meinicke, E.: Munchen. med. Wchnschr., 1918, xlv, xlix, li; ibid, 1919, xxxiii, li. Deutsch. med. Wchnschr., 1919, vii, xii, xxx, ibid, 1920, ii. Berl. klin. Wchnschr., 1917, xxv, 1; ibid, 1918, iv. Med. Klin., 1918, xxxvi. Ztschr. f. Immunitatsforch. Orig., 1918, xxvii; ibid, 1919, xxviii; ibid, 1920, xxviii.

[28]Lesser: Munchen. med. Wchnschr., 1918, xxxii. Deutsch. med. Wchnschr., 1918, xlii. Berl. klin. Wchnschr., 1919, x.

Luza: Ned. Tijdsch. v. Geneesk., 1920, xii.

Herzfeld and Klinger: Berl. klin. Wchnschr., 1918, xxix.

v. Vagedes and Korpsch: Deutsch. med. Wchnschr., 1918, li.

Reich: Deutsch. med. Wchnschr., 1919, vii.

v. Kaufmann: Med. Klin., 1918, xxxiii.

Kafka: Munchen. med. Wchnschr., 1918, l.

Konitzer: Med. Klin., 1919, xiv.

Blumenthal: Med. Klin., 1919, xxxi.

Joel: Inaug. Dissert., Jena, 1919.

Felke and Wetzell: Munchen. med. Wchnschr., 1919, xlvii.

[29]Sachs and Georgi: Med. Klin. 1918, xiv, ibid, xxxiii. Munchen. med. Wchnschr., 1919, lxvi, iii, xxxiii, ibid, 1920, lxvii. Arb. a. d. Inst. f. exper. Therap., Frankfurt a. M. x, 1920.

[30]Friedmann: Ztschr. f. Hyg. u. Infectionskrankh., 1910, lxvii.

[31]Manwaring: Ztschr. f. Immunitatsforch. u. exper. Therap., 1910, iii.

[32]Levinson and Petersen: Arch. Derm. Syph., 1921, iii.

[33]Levinson and Petersen: in print.

[34]Löns: Deutsch. med. Wchnschr., 1919, xxi.

[35]Sheer: Munchen. med. Wchnschr., 1919, xxxii.

[36]Gaethgens: Munchen. med. Wchnschr., 1919, xxxiii.

[37]Meyer, Lipp, Nathan, Munster: Med. Klin., 1918, 1; 1919, xi. Munchen. med. Wchnschr., 1919, xix.

[38]Konitzer: Med. Klin., 1919, xiv.

[39]Schroeder: Med. Klin., 1919, xxi.

[40]Weichardt and Schroder: Med. Klin., 1919, vi.

[41]Hauck: Munchen. med. Wchnschr., 1919, xlix.

[42]Georgi: Munchen. med. Wchnschr., 1920, lxvii.

[43]Nathan: Med. Klin., 1918, xli.

[44]Nathan and Weichbrodt: Munchen. med. Wchnschr., 1918, xlvi.

[45]Zurhelle: Derm. Ztschr., 1919, xxviii.

[46]Zimmern: Derm. Ztschr., 1919, xxxii.

[47]Frankel: Deut. med. Wchnschr., 1919, xxxi.

[48]Raabe: Berl. klin. Wchnschr., 1919, xliii.

[49]Wolffenstein: Berl. klin. Wchnschr., 1919, xlvii.

[50]Frommherz: Wurttemb. Kon. 1919, lii.

[51]Baumgartel: Munchen. med. Wchnschr., 1920, lxvii.

[52]Bauer: Deut. med. Wchnschr., 1908, xxxiv.

[53]Hecht: Ztschr. f. Immunitatsforch., 1915, xxiv, H. iii. Wien. klin. Wchnschr., 1908, xxi; 1909, xxii.

[54]Tschernogubow: Berl. klin. Wchnschr., 1908, vii. Deut. med. Wchnschr., 1909, xv.

[55]Noguchi: Serum Diagnosis of Syphilis, Lippincott, ed. 2.

[56]Mandelbaum: Munchen. med. Wchnschr., 1918, lxv.

[57]Hauck: Munchen. med. Wchnschr., 1920, lxvii.

[58]Neukirch: Arb. a. d. Inst. f. exper. Therap. zu Frankfurt a. M., 1920, x, p. 45. Ztschr. f. Immunitatsforch., xxix.

[59]Stilling: Med. Klin., 1920, ii.

[60]Schoenfeld: Munchen. med. Wchnschr., 1920, lxvii.

[61]Meyer: Med. Klin., 1919, ii.

[62]Landsteiner: Kolle-Wassermann Handbuch d. path. Mikroorgan., 1913, ii, Jena.
[63]Moreschi: Berl. klin. Wehnschr., 1905, xxxvii; 1906, iv, xxxviii; 1907, xxxviii. Centrl. f. Bak., 1906, xxxviii, Ref.
[64]Gay: Centralbl. f. Bakt., 1905, xxxix; 1906, xl.
[65]Nathan: Ztschr. f. Immunitatsforch., 1920, xxix, H. 6.
[66]Seligmann: Berl. klin. Wehnschr., 1907, xxxii. Biochem. Ztschr., 1908, x. Ztschr. f. Immunitatsforch., 1909, i.
[67]Much: Med. Klin., xxviii, xxix.
[68]Michaelis and Davidsohn: Biochem. Ztschr., 1912, xlvii.
[69]Michaelis and Rona: Biochem. Ztschr., 1910, xxvii, xxviii.
[70]Wassermann von, A.: Berl. klin. Wehnschr., 1921, No. 9, p. 193.
[71]Woudstra, H. W.: Ztschr. f. physik. Chem., 1908, lxiii, 619.

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ANAPHYLAXIS AND ALLIED PHENOMENA IN RELATION TO DISEASE*

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THE term anaphylaxis was introduced by Richet[1] in 1898 to describe a condition of increased susceptibility to a toxic protein (eel serum) but has later been extended, as a result of the work especially of Arthus[2] and Theobald Smith, to include the toxic action of nontoxic proteins. Probably the first recorded observation on anaphylaxis is one by Jenner in his *Inquiry into the Causes and Effects of Variolæ Vaccinæ,* in 1798. He found that quite regularly in persons who had had either cowpox or smallpox previously the inoculation of variolous matter produced only "a tingling sensation" and "an erysipelatous look appearing on the skin near the punctured parts" but it "died away in a few days without producing any variolous symptoms." In a footnote to Case 4 he says, "It is remarkable that variolous matter, when the system is disposed to reject it, should excite inflammation on the part to which it is applied more speedily than when it produces the smallpox. Indeed it becomes almost a criterion by which we can determine whether the infection will be received or not. It seems as though a change which endures through life had been produced in the action or disposition to action in the vessels of the skin; and it is remarkable, too, that whether this change has been effected by the smallpox or the cowpox, the disposition to sudden cuticular change is the same on the application of variolous matter."

Magendie[3] in 1839 (injecting egg albumen into dogs) and Flexner[4] in 1894 (injecting dog serum into rabbits) recorded typical instances of experimental anaphylaxis, but these early observations were apparently lost sight of, and the phenomenon was rediscovered, and was experimentally analyzed by Arthus,[5] Pirquet and Schick,[6] Wolff-Eisner,[7] Otto,[8] Rosenau and Anderson,[9] Vaughan,[10] Gay and Southard,[11] Besredka,[12] and since 1908 by a large number of observers.

The typical anaphylactic experiment is too well known to warrant repetition. Suffice it to say, however, that while the typical experiment results in definite shock or death following the second injection of a protein, yet we recognize many irregularities in the reaction. For instance there are great individual variations in the response of different animals to the experiment, so that it is extremely difficult to put this work on a definite quantitative basis further than to establish maximums, minimums, and averages. Thus I have had a group of four guinea pigs of the same size and all treated in the same way, and following the second injection, one died promptly; one had a severe reaction,

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but recovered; one had a mild reaction; and one had no reaction at all. I have had one lot of pigs in which it was difficult to produce a distinct shock without killing the animal, and a year later, another lot of pigs in which it was difficult to produce a fatal shock by the same methods. One pig, after surviving a shock, will be completely refractory to later injections of whatever size. Another pig, under the same conditions, after surviving a shock, will succumb the next day to a dose of the same size. One animal, if injected at weekly intervals, will become completely refractory and never develop anaphylaxis, while another, under similar conditions, will develop fatal anaphylaxis after the third or fourth injection. We should expect, and, indeed, we do find as great if not greater variations among human beings in the clinical conditions that we ascribe to anaphylaxis. And these great individual variations that exist in the type of reaction must be borne in mind if the full clinical significance of anaphylactic states is to be appreciated.

Anaphylaxis manifests itself in one or both of two ways—local and general. In the general reaction we may have either increase or decrease in temperature, a fall in blood pressure, cyanosis, delayed coagulability of the blood, leucopenia, dyspnea, pain in chest or head, dizziness, convulsions, and unconsciousness. In the local reaction, we see evidences of vasomotor disturbance—hyperemia and edema, and sometimes vesicle or pustule formation, as in urticaria, angioneurotic edema, and the local manifestations of serum disease, and in such clinical tests as the Schick, Pirquet, Calmette, luetin, and the various protein skin tests used in asthma, eczema, and related conditions.

The phenomenon of anaphylaxis is very highly specific, but not absolutely so. Thus an animal sensitized with the serum of one species may react partially to the serum of a closely related species. This has also been shown by Wells and Osborne[53] to be true of the isolated proteins of wheat and barley. This corresponds to group agglutination in bacteriology, and to that elusive form of partial immunity to one infectious disease that sometimes seems to follow recovery from another infectious disease (as seen particularly by the statistical method in public health studies).

The term "anaphylaxis" has been objected to since it implies the direct opposite to immunity, whereas it might better be said to include immunity. The term "allergy," which was introduced to indicate a state of altered immunity, is undoubtedly better, but has not been generally adopted in this sense, and has been used by some writers to indicate the altered immunologic state existing in infections as distinguished from that produced by nonorganized proteins, and by others to indicate congenital hypersusceptibility. The term "protein intoxication," while expressive and definite, is cumbersome. The original expression of Richet is no more misleading than any of the substitutes suggested, and has the sanction of age and custom. It is simpler to make a slight change in the interpretation of a word than to change the word completely.

THEORIES

The various theories evolved to explain anaphylaxis may be grouped into two classes: the humoral and the cellular. One group of the supporters of the humoral theory have held that the reaction, which took place in the blood stream,

was essentially of the nature of an antigen-antibody reaction. The work especially of Doerr and Russ[13] lent great weight to this conception. The work of Vaughan and Wheeler[14] was of especial importance as providing a definite and demonstrable chemical explanation of the phenomena. They believe that the first injection of protein stimulates the production of a specific lysin capable of splitting the protein molecule into a toxic and a nontoxic portion, and that on the second injection this splitting takes place very rapidly, and the liberated toxic fraction produces the symptoms of shock. Every true protein investigated by Vaughan was found to be capable of being split by chemical means into a toxic and a nontoxic fraction. The toxic fraction when injected into animals produces symptoms similar to anaphylaxis. The writer[116] has found that repeated injections of this toxic fraction into animals produce histologic lesions similar to those of chronic anaphylaxis. It has been shown, however, by Manwaring, Kusama, and Crowe[15] and others that the toxic fraction is derived not from the splitting of the injected antigen, but from the splitting of the serum proteins of the individual.

Another group believe that while the reaction takes place in the blood stream, yet it is not so specific in nature as the antigen-antibody assumption would necessitate, since the reaction (or at least a very similar reaction) can be elicited by nonspecific substances. Jobling and Peterson[16] showed that during anaphylactic shock there was a marked increase in serum ferment, and in the end-products of protein disintegration, and a decrease in the serum antiferment. They believe that the injection of protein stimulates the production of a nonspecific proteolytic ferment which, following the second injection, splits the serum proteins, and liberates their toxic fraction. Friedberger[17] noted that the injection of inert substances, such as kaolin, was followed by anaphylactic symptoms, and Jobling and Peterson[18] found almost the same serum changes following kaolin injections as in typical anaphylaxis, and concluded that the kaolin by adsorption removed the antiferment and allowed the native serum ferment to split off the toxic fraction from the serum proteins.

Novy[19] has taken the middle ground between these positions. He believes that the toxic substance, anaphylatoxin, is produced by an "inducing body" which is itself the result of antigen-antibody union. The specificity of the reaction, then, concerns the production of this inducing substance, and not the production of the anaphylatoxin. Very diverse substances such as bacteria, starch, kaolin, agar, peptone, organ cells or extracts, and even distilled water are also able to act as "inducers" or accelerators of this reaction by which the matrix of the poison is broken down, and the poison liberated. Novy was able to produce a typical shock by the injection of agar, even so small an amount as 9 milligrams of dry agar per kilo of guinea pig proving fatal. He calls attention to the fact that this dose is less than that of most pathogenic bacteria, and suggests that the results of bacterial injections may depend on the physical effect of bacteria on the serum colloids.

Schultz[20] showed that the excised smooth muscle of a sensitized animal is hypersensitive, and the work of Pearce and Eisenbrey,[21] Dale,[22] Weil,[23] and Coca[24] has supported and extended his original findings, and lent great weight to the contention that the seat of the reaction in anaphylaxis is in the body cells and

not in the blood. Undoubtedly the last word has not been said, but the acceptable theory that explains anaphylaxis must take into account these various apparently contradictory findings.

It is probable that in immunity, as we ordinarily understand the term, at least in acquired immunity, we are dealing with a process of the same nature as anaphylaxis. It is probable that an antibody, perhaps a ferment, increased in amount by the process of immunization, acts upon the toxic substance to break it down into simpler nontoxic fractions. Such a ferment may be specific, or more probably be nonspecific (complement?) but activated or liberated by a specific factor. This process may be conceived of as one stage in anaphylaxis.

PASSIVE ANAPHYLAXIS

The phenomenon of passive anaphylaxis was first described by Gay and Southard,[25] and later in the same year by Otto.[26] If a guinea pig is injected with horse serum, and after he is sensitized his serum is withdrawn and injected into a normal pig, the second pig now becomes sensitive to horse serum, and develops an anaphylactic shock on the first injection. In the earlier experiments it was thought necessary to wait fifteen to twenty-four hours before the second pig became passively sensitized, but Doerr and Russ, and Friedmann later showed that if the amounts of guinea pig serum and horse serum were rightly proportioned, an immediate reaction could be obtained. A female pig thus passively sensitized may transmit this sensitization to her offspring.

ANTIANAPHYLAXIS

If an animal responds to a second injection of a protein with a typical nonfatal anaphylactic shock, he is usually refractory to subsequent injections of the protein. This condition is called antianaphylaxis, or desensitization, and may persist for a few months in guinea pigs. If an animal is injected with a protein, and then before sensitization is complete (say about the sixth or seventh day) he is injected with a fairly large dose of the same antigen, he then becomes refractory without ever having been sensitive. If an animal has been sensitized to a certain protein, he can also be desensitized by injecting a small dose of the same protein—a dose too small to produce shock. He then becomes entirely refractory to subsequent doses that would otherwise have proved fatal. It is also possible to desensitize animals nonspecifically. If an animal be injected with horse serum, and after sensitization is complete he be given an injection of egg albumen, he will now be found partially desensitized, and a subsequent injection of horse serum may produce a mild shock, but will not kill him. Injections of many other proteins and of peptone are able to desensitize more or less completely. Many drugs have been used, such as ether, chloroform, chloral, atropine, urethane, paraldehyde, but while they may mask the symptoms, or delay death, they do not prevent death. When an animal is rendered antianaphylactic by any of the methods mentioned, this refractory condition may persist for some time, but will eventually disappear, and the animal become sensitive again. If blood is withdrawn from a refractory animal, and the serum injected into a normal animal, that animal may become more highly sensitized than when injected with serum from a sensitized animal. No theory yet advanced accounts satisfactorily for all of the known facts of antianaphylaxis.

Since anaphylaxis has been shown to depend upon sensitization to proteins and to nothing else, and since bacteria contain 50 to 80 per cent of protein and protein derivatives, this phenomenon has been offered as an explanation of bacterial disease. Vaughan has shown that the bacterial proteins may be split chemically to yield his protein poison, and that properly spaced injections of killed pathogenic or nonpathogenic bacteria may produce typical anaphylaxis in guinea pigs. The "endotoxin" of Pfeiffer then would correspond to the toxic fraction split off from the protein molecule. The characteristics of bacterial disease—the period of incubation, the sudden onset of the symptoms, the fever, the skin eruption, the crisis, the subsequent immunity—all have their counterpart in experimental anaphylaxis. The immunity against certain infections that may be conferred by injecting bacterial vaccines has its counterpart in desensitization.

RELATED PHENOMENA

There is a group of phenomena that are closely related to anaphylaxis. Biedl and Kraus,[27] Arthus,[28] DeWaele,[108] and others have shown that injections of peptone produce in animals symptoms very similar to anaphylaxis. It produces in dogs the same fall in blood pressure, delayed coagulability of the blood, and leucopenia, and in guinea pigs the same bronchial spasms. Injections of peptone into sensitized animals produce some degree of desensitization toward the specific protein used. I[29] have shown that the histologic lesions produced in guinea pigs by repeated injections of peptone are closely similar to those in chronic anaphylaxis. Phillippson[30] noted that a solution of peptone applied to the scarified skin produces local urticarial wheals similar to those found in anaphylactic states. Vaughan[107] noted a similar action of his protein poison.

Witte's peptone contains variable amounts of toxic secondary proteoses and of peptone. Pullitzer[31] in 1885 first separated the proteoses from the peptone, and studied their physiologic action. Grosjean[32] in 1892 isolated the individual proteoses and studied their effects when injected into animals. The effects of proteose intoxication have since been investigated carefully by Thompson,[33] Chittenden, Mendel, and Henderson,[34] Underhill[35] and others. Injections of proteoses into the blood stream of dogs produce rapid and marked fall of blood pressure, delayed coagulation, increased lymph flow, narcosis, and other toxic symptoms and some immunity (tolerance) for later injections. DeKruif and Eggerth[36] have shown that the toxic principle of Witte's peptone easily passes membranes that hold back all of the anaphylatoxin, hence "peptone" or proteose intoxication is probably not identical with anaphylaxis. Injections of toxic proteoses, however, have been used in the treatment of infectious diseases in the hope of producing a condition similar to anaphylaxis, and thus benefiting the clinical condition.

Barger and Dale[37] and Kutscher[38] simultaneously but independently discovered the physiologic activity of histamine. This substance is β-imidazolylethylamine, and is produced by the decarboxylation of histidine. Its action has been further studied by Dale and Laidlaw,[39] Ackermann and Kutscher,[40] Barbour,[41] Oehme,[42] and Jackson and Mills.[43] Its effects on blood pressure and on smooth muscle are very similar to those found in anaphylaxis, and Barger and Dale have

suggested that histamine may be a factor in producing the symptoms of anaphylaxis. Eppinger,[44] and Sollmann and Pilcher[45] found that the local application of histamine in high dilution to the human skin produces redness, swelling, and an urticarial wheal. I[46] have found that repeated injections of histamine in animals produce histologic lesions somewhat similar to those found in chronic anaphylaxis. Barger and Dale[47] have recovered histamine from intestinal mucosa. Berthelot and Bertrand,[48] Mellanby and Twort,[49] and Jones[50] have described methods of isolating an organism (B. aminophilis intestinalis) from feces which is able to produce histamine from histidine, and Koessler and Hanke[51] have found that the B.coli can do the same thing under proper conditions. Dale and Laidlaw[52] have pointed out certain similarities in the action of histamine to the manifestations of surgical shock, and have suggested that the ease with which surgical shock is brought on by traumatizing the intestine is related to the histamine content of that organ. Histamine acts partly as an endothelial poison relaxing capillaries and increasing their permeability. I have found definite histologic evidence of endothelial damage. Locally applied, it produces an urticarial wheal similar to that seen in local anaphylaxis. But a sharp blow on the skin may produce the same wheal. A definite relationship may be perceived between anaphylactic shock and surgical shock, though this relationship may extend no farther than a similarity of mechanism.

CLINICAL CONSIDERATIONS

One of the best known and most important manifestations of anaphylaxis, serum disease, has been well described by Pirquet and Schick,[54] Axenow,[55] Weaver,[56] Bokay,[57] Schultz,[58] Goodall,[59] Davidson,[60] and others, and is too well known to require further description. Most of the reported cases have been due to horse serum injected for curative purposes. Netter,[61] however, has reported the case of a child with poliomyelitis who developed serum disease after the second injection of human serum. There were joint pains, elevated temperature, and rash; the child recovered.

Within recent years several diseases, previously little understood, have been explained as due to anaphylaxis. As a result of the work especially of I. Chandler Walker,[62] and others, about 50 per cent of cases of bronchial asthma have been shown to be due to anaphylaxis, and the specific protein to which the patient is sensitized may be determined by skin tests. These proteins are classified as those from (1) animal hair (horse, cat, rabbit); (2) foods (eggs, meat, milk, etc.); (3) bacteria; and (4) pollens. Sometimes an individual may be sensitized to several different proteins, and the degree of specificity involved is often remarkable. Thus an individual may be sensitized to horse hair protein, but not to horse serum protein, or to only a single protein of horse hair, and not to the others. Hay fever is anaphylaxis induced by pollens.

Most cases of urticaria are known to have a very definite relation to anaphylaxis as shown by Strickler,[63] McBride and Schorer,[64] Schwann,[65] Widal,[66] and others. Blackfan[68] found positive cutaneous reactions with egg white, cow's milk, and human milk in 22 of 27 cases of eczema. Strickler[69] obtained endermic reactions with foods in 20 per cent of cases of eczema, and White[70] found that nearly 50 per cent of his eczema cases reacted to foods. The same phenom-

enon of protein sensitization has been found to be at the bottom of certain cases of angioneurotic edema. This condition has also been shown to have a definite hereditary tendency, though the exciting cause may be different in related individuals.

Idiosyncrasies against certain foods such as shell-fish, eggs, strawberries, etc., are also evidences of anaphylaxis. This has been discussed by Talbot.[106] It is probable that in those individuals who show such sensitization, the course of digestion is somewhat abnormal or that the intestinal mucosa allows the absorption of certain products of protein digestion that are ordinarily excluded. In some cases, drug idiosyncrasies are also due to anaphylaxis.

Sensitization to foreign proteins occurs in some cases through inhalation. This is especially true in hay fever, and in asthma due to animal hair. In some cases, as in foods, sensitization occurs by way of the intestinal tract, though the exact mechanism is not known. In cases of serum disease the sensitization may probably occur by either of these routes (inhalation of horse hair and skin secretions, or ingestion of horse meat) or by direct inoculation (anti-toxin). The duration of sensitization is variable: in many cases it appears to persist through life, but occasionally it disappears spontaneously.

If human beings could be desensitized as readily as laboratory animals it would be of enormous clinical importance. Deaths from anaphylaxis are occasionally reported (I[71] have reviewed this literature elsewhere) and many of these could be prevented if we could safely and surely desensitize patients who are likely to develop anaphylaxis following serum injections. Besredka[72] devised a method which he called the method of "doses subintrantes" by which he hoped to render serum injections completely safe. He injected sensitized guinea pigs with several doses of horse serum beginning with a dose too small to produce symptoms, and rapidly increasing the size of the injections until within a few hours the animal could safely withstand a dose of 40 to 200 times the fatal dose for sensitized control pigs. All of Besredka's animals, however, were sensitized with a small dose of antigen. If an animal has been sensitized with a large dose of protein, a large dose is required to desensitize him—a dose, indeed, larger than the fatal dose for an animal sensitized with a minimum dose of antigen. This method is therefore impractical unless we know the size of the sensitizing dose, and in human beings this is almost never possible. Moreover, as Weil[73] has pointed out, this repetition of preliminary doses does not always produce desensitization, but some animals respond to successively increasing doses with symptoms of successively increasing severity, and eventually death. Netter,[74] Grysez and Dupuich,[75] and Doerr[76] have reported similar observations in patients. Because of the uncertainty of the method itself, and because of the marked variations in their manner of response which many individuals show, this method of desensitization, as a clinical procedure is practically useless.

Another method of desensitization has been employed with greater success. If an individual sensitized against a certain protein receive injections of a very dilute solution of the protein at intervals of a few days, and the amount injected be cautiously increased, the individual may become entirely refractory, and this refractory condition may last for a long time. This method has been worked out

in great detail by Walker[77] in the treatment of asthma, and is applicable also to such chronic conditions as eczema and food sensitization.

This is essentially the method that is used in the administration of vaccines for chronic infections, with the exception that the bacterial protein injected is primarily toxic. Injections of killed bacteria in the treatment of infectious disease were used empirically before our ideas on immunity were at all definite, and long before the nature of anaphylaxis was appreciated. It has never been possible to explain either the good results or the frequent disappointments met with in the use of bacterial vaccines in the treatment of disease, on the basis exclusively of immune antibodies produced, since the patient may be better when those antibodies detectable in the test tube are low, or he may be worse when the antibodies are high. All that we know of bacterial vaccines in disease, and of anaphylaxis tends to support the contention that injections of dead bacteria when they produce any effects at all, produce anaphylaxis, or desensitization, which, as we have seen, should be considered as one phase of anaphylaxis. (The injection of dead bacteria for prophylactic purposes, as in the antityphoid inoculation, should be considered an exception to this statement, for here we are dealing with specific easily demonstrable antibodies.) The mobilization of the serum ferments, and the elevation of temperature (frequently seen in bacterial injections, and obtainable by very small doses of simple proteins[67] are evidently a means of defense. There is no reason to think that there is any difference whatever in the anaphylaxis produced by different proteins. If these assumptions are true (that vaccines act by inducing anaphylaxis, and that the nature of anaphylaxis does not vary with the inducing protein), then it is not necessary to use cultures of the specific infecting organism, and it is possible that any foreign protein or protein derivative that will produce a mild anaphylactic shock will accomplish as much good as can be accomplished by bacterial injections. It may easily be that bacteria represent a type of protein that is more readily acted upon, and hence more "stimulating" than are unorganized proteins or protein derivatives. Not all strains of bacteria produce equally satisfactory vaccines for clinical use. There is some evidence that a particular strain of bacteria that produces good results when injected as a vaccine in one case may produce just as good results in other cases regardless of the type of infecting organism. Of course the problem of dosage, and of the degree of the anaphylactic shock desired, as well as variations in the susceptibility of different patients make the whole question very uncertain, and emphasizes the fact that this procedure (the injection of bacteria or of proteins) is still almost wholly empirical.

In support of the view that anaphylaxis therapeutically induced need not be specific, it may be mentioned that many observers have reported good results by the use of nonspecific vaccines, especially in typhoid and arthritis, but also in a variety of infections including puerperal sepsis, gonorrheal complications, asthma, influenza, pneumonia, and some skin diseases. Rumpf[78] in 1893 treated typhoid fever with a vaccine of B. pyocyaneus, and in the last four years Luedke,[79] Kraus,[80] Miller and Lusk,[81] Culver,[82] Engman and McGarry,[83] Cowie and Calhoun,[84] Roberts and Cary,[85] Cowie and Beaver,[86] Snyder,[93] Reibmayr,[104] Dansyz,[105] Cadbury[117] and many others have reported good results from the use of nonspecific vaccines.

Less favorable testimony, however, is at hand. Sholly, Blum, and Smith[87] in pertussis, Whittington[88] in typhoid fever, and Bumpus[89] in prostatic cases found that though their cases seemed at first glance to do well, a careful analysis showed that they did not do quite so well as the cases that received no vaccine. A partial explanation of these diverse results is found in the work of Herrmann[90] who showed that in rabbits "sensitized" (immunized) to streptococcus or meningococcus, a definite liberation of specific opsonins and agglutinins followed the injection of foreign proteins (human serum and ascitic fluid), but similar treatment of typhoid sensitized rabbits gave no results. But on the other hand, Dunklin[91] found that injections of proteose in typhoid immune rabbits produced an increase in the antibodies of the serum. Jobling and Peterson[92] showed that in animals, injections of bacteria, of kaolin, and of protein split products are followed by mobilization of serum protease, and this presumably might aid in the destruction of bacteria, and would explain some of the good results obtained by nonspecific means.

Since nonspecific vaccines have given good results in many cases, it is only a step to the use of simple toxic proteins. Matthes[93] in 1894 found that injections of secondary proteose produced about the same results as tuberculin. Luedke[79] in 1915 treated typhoid fever successfully by means of injections of secondary proteose. With the same substance good results were obtained in typhoid fever and arthritis by Miller and Lusk,[81] in arthritis and other complications of gonorrhea by Culver,[94] in arthritis, gonorrheal epididymitis, and in erysipelas by Jobling, Peterson, and Manier.[95] Beebe[96] used a protein prepared from the seeds of millet and alfalfa in the treatment of arthritis, and Brooks and Stanton[97] used "hemoprotein" from ox-blood in the same condition, both with success. Nolf[98] found good results in the treatment of a variety of infections with intravenous injections of Witte's peptone. Baldwin and L'Esperance[99] found that injections of typhoid bacteria produced improvement in tuberculous guinea pigs, but Krause and Willis[100] found that animals receiving repeated injections of egg white were less resistant to subsequent injections of tuberculous material, and repeated anaphylactic shocks had no effect on the course of an established tuberculosis. Peterson[118] has pointed out that resistance to tuberculosis depends more on the ferment-antiferment balance than on specific immune factors, and that therefore appropriate nonspecific therapy might be expected to produce better results in tuberculosis than that calculated to increase specific immune antibodies. Wells[101] obtained good results in the treatment of influenzal pneumonia by injection of the residue left after the alcoholic extraction of typhoid bacilli, and Lamb and Brannin[102] in the same condition used a variety of antitoxic serums, and the bacterial filtrates of organisms commonly found in influenza and pneumonia, but they "make no great claims for this treatment." This diversity of methods and of results shows the condition of uncertainty that exists with respect to nonspecific protein therapy. All writers agree, however, on one point, and that is that the administration of foreign proteins by the intravenous route is potentially highly dangerous, and must be performed with great care. Thomas[103] says he knows of several deaths due to the unskillful use of this method.

Passive anaphylaxis is of some practical importance clinically. Ramirez[109]

has reported the case of a man who acquired horse asthma (was passively sensitized to horse hair protein) by receiving a blood transfusion from an asthmatic. This constitutes a new hazard in transfusion cases. DeBesche[110] records two cases of horse asthma and one of cat asthma in which guinea pigs were passively sensitized by injections of patient's serum, and later killed by injecting the specific antigen. Bruck[111] had a similar experience with the serum of patients sensitized to pork and to iodoform. Schloss,[112] working with the serum of a patient sensitized to egg, and Koessler[113] with the serum of hay fever patients, obtained the same results. It is possible that this reaction might be of diagnostic value in some cases. Of course the skin test is much simpler, but it is not completely reliable, for cases have been found in which anaphylaxis developed after the administration of therapeutic serums, even when the skin test was negative for horse serum.

The frequency with which vaccines and other foreign proteins are being injected today for the treatment of all manner of diseases makes it appropriate to inquire whether there may be any harmful results aside from the immediate effects of the injection. Longcope[114] has shown that repeated anaphylactic shocks in animals produce degenerative and inflammatory changes in kidney, liver, and heart. I[115] have obtained similar results, and have further shown that there is a distinct degenerative change in the arteries in many organs. Just how far these results are applicable to human beings has not yet been determined, though I have studied one case of anaphylaxis[116] in which the microscopic findings were very similar to those of experimental anaphylaxis in animals. It is well to remember that protein therapy is still in the experimental stage, that it is a potent instrument for harm, and that at least a few deaths are traceable to its injudicious use.

REFERENCES

[1]Richet and Hericourt: Compt. rend. Soc. de biol., 1898, l, 137.
[2]Arthus and Breton: Compt. rend. Soc. biol., 1903, lv, 1478.
[3]Magendie: Vorlesung ueber das Blut, 1839.
[4]Fiexner: Med. News, 1894, lxv, 116.
[5]Arthus: Compt. rend. Soc. biol., 1903, lv, 817.
[6]Pirquet and Schick: Wien. klin. Wchnschr., 1903, xvi, 758. Die Serumkrankheit, Franz Deuticke, Leipzig, 1905.
[7]Wolff-Eisner: Ztschr. f. Bakteriol., 1904; Berl. klin. Wchnschr., 1904, xli, 1105, 1131, 1156, 1273.
[8]Otto: Das Theobald Smithsche Phaenomen, v. Leuthold Gedenkschrift, i, 1905.
[9]Rosenau, M. J., and Anderson, J. F.: Hygienic Lab. Bull., 1906, No. 29, ibid., 1907, No. 36; ibid., 1908, No. 45.
[10]Vaughan, V. C.: Jour. Infect. Dis., 1907, iv, 476.
[11]Gay, F. P., and Southard, E. E.: Jour. Med. Research, 1907, xvi, 143; ibid., 1908, xviii, 407; ibid., 1908, xix, 1, 5, 17.
[12]Besredka: Ann. de l'Inst. Pasteur, 1907, xxi, 384, 777, 950. Compt. rend. Soc. biol., 1907, lxii, 1053; ibid., 1907, lxiii, 294; ibid., 1908, lxv, 478.
[13]Doerr and Russ: Ztschr., f. Immunitat., 1909, ii, 208.
[14]Vaughan and Wheeler: Jour. Infect. Dis., 1907, iv, 476.
Protein Split Products, Philadelphia, 1913, Lea and Febiger.
[15]Manwaring, W. H., Kusama, Y., and Crowe, H. E.: Jour. Immunol., 1917, ii, 511.
[16]Jobling and Peterson: Jour. Exper. Med., 1915, xxii, 401; Bull. Johns Hopkins Hosp., 1915, xxvi, 296.
[17]Friedberger, E., and Kumagai, T.: Ztschr. f. Immun., Orig., 1912, xiii, 127.
Friedberger, E., and Tsunooka, R.: Ibid., 1913, xx, 405.
[18]Jobling and Peterson: Jour. Exper. Med., 1915, xxii, 590.
[19]Novy, F. G.: Jour. Infect. Dis., 1917, xx, 490.
[20]Schultz: Jour. Pharm. and Exper. Therap., 1910, i, 549, ibid., 1910, ii, 221.

[21]Pearce and Eisenbrey: Trans. Congr. Am. Phys. Surg., 1910, viii.
[22]Dale, H. H.: Jour. Pharm. and Exper. Therap., 1913, iv, 167.
[23]Weil, R.: Jour. Med. Research, 1913, xxvii, 497; ibid., 1914, xxx, 299.
[24]Coca, A.: Ztschr. f. Immun., 1914, xx, 622.
[25]Gay, F. P., and Southard, E. E.: Jour. Med. Research, 1907, xvi, 143.
[26]Otto: München. med. Wchnschr., 1907, liv, 1665.
[27]Biedl and Kraus: Wien. klin. Wchnschr., 1909, xxii, 363.
[28]Arthus: Compt. rend. l'Acad. de Sc., 1909, cxlviii, 999, 1002.
[29]Boughton, T. H.: Jour. Immunol., September, 1919, iv, No. 5.
[30]Phillippson: Gior. ital. d. mal. ven., 1899.
[31]Pullitzer: Jour. Physiol., 1885, vii, 283.
[32]Grosjean: Arch. de biol., 1892, xi, 381.
[33]Thompson: Jour. Physiol., 1896, xx, 455.
[34]Chittenden, R. H., Mendel, L. B., and Henderson, Y.: Am. Jour. Physiol., 1898, ii, 142.
[35]Underhill, F. P.: Am. Jour. Physiol., 1903, ix, 345; Jour. Biol. Chem., 1915, xxii, 465.
[36]DeKruif, P. H., and Eggerth, A. H.: Jour. Infec. Dis., 1919, xxiv, 505.
[37]Barger, G., and Dale, H. H.: Proc. Chem. Soc., 1910, xxvi, 128.
[38]Kutscher: Zentralbl. f. Physiol., 1910, xxiv, 163.
[39]Dale, H. H., and Laidlaw, P. P.: Jour. Physiol., 1910, xli, 318.
[40]Ackermann and Kutscher: Ztschr. f. Biol., 1910, liv, 387.
[41]Barbour, J.: Jour. Pharm. and Exper. Therap., 1912, iv, 245.
[42]Oehme, C.: Arch. f. exper. Path. u. Pharmacol., 1913, lxii, 76.
[43]Jackson, D. E., and Mills, C. A.: Jour. Lab. and Clin. Med., 1919, v, 2.
[44]Eppinger, H.: Wien. klin. Wchnschr., 1913, xxiii, 1414.
[45]Sollmann and Pilcher: Jour. Pharm. and Exper. Therap., 1917, ix, 309.
[46]Boughton, T. H.: In press.
[47]Barger, G., and Dale, H. H.: Jour. Physiol., 1910, xli, 499.
[48]Berthelot and Bertrand: Compt. rend. de l'Acad de Sc., 1912, cliv, 1643, 1826.
[49]Mellanby, E., and Twort, F. W.: Jour. Physiol., 1912, xlv, 53.
[50]Jones, H. M.: Jour. Infect. Dis., 1918, xxii, 125.
[51]Koessler, K. K., and Hanke, M. T.: Jour. Biol. Chem., 1919, xxxix, 539.
[52]Dale, H. H., and Laidlaw, P. P.: Jour. Physiol., 1919, lii, 355.
[53]Wells and Osborne: Jour. Infect. Dis., 1913, xii, 341.
[54]Pirquet and Schick: Die Serumkrankheit, Franz Deuticke, Leipzig, 1905.
[55]Axenow: Jahrb. f. Kinderh., 1913, lxxviii, 565.
[56]Weaver, G. H.: Arch. Int. Med., 1909, iii, 485.
[57]Bokay: Deutsch. med. Wchnschr., 1911, xxxvii, 9.
[58]Schultz: Berl. klin. Wchnschr., 1914, li, 349, 401.
[59]Goodall: Jour. Hyg., 1907, vii, 607; Brit. Med. Jour., ii, 1359.
[60]Davidson: Glasgow Med. Jour., 1919, xci, 321.
[61]Netter: Compt, rend. Soc. de Biol., 1915, lxxviii, 505.
[62]Walker, I. C.: Jour. Med. Research, 1917-8, xxxv, xxxvi, xxxvii.
Jour. Am. Med. Assn., 1918, lxx, 897.
[63]Strickler, A.: New York Med. Jour., 1916, civ, 198.
[64]McBride and Schorer: Jour. Cutan. Dis., 1916, xxxiv, 70.
[65]Schwann, A. W.: Jour. Am. Med. Assn., 1915, lxiv, 737.
[66]Widal, F.: Bull. et mém. Soc. méd d. hôp. de Paris, 1914, xxxvii, 256.
[67]Friedberger: Deutsch. med. Wchnschr., 1911, i, 61; Ztschr. f. Immun., 1911, Orig. x, 216.
[68]Blackfan, K. D.: Am. Jour. Dis. Child., 1916, xi, 441.
[69]Strickler, A.: Jour. Am. Med. Assn., 1916, lxvii, 70.
[70]White, J. C.: Jour. Am. Med. Assn., 1917, lxviii, 81.
[71]Boughton, T. H.: Jour. Am. Med. Assn., Dec. 27, 1919, lxxiii.
[72]Besredka: Ann. de l'Inst. Pasteur, 1910, xxiv, 879.
[73]Weil, R.: Jour. Med. Research, 1913, xxix, 233.
[74]Netter: Bull. et mém Soc. méd. d. hôp. de Paris, 1912, xxxiii, 401.
[75]Grysez and Dupuich: Ibid., 1912, xxxiii, 374.
[76]Doerr in Kolle and Wassermann: Handbuch der Pathogenen Mikroorganismen, 1913, p. 1082.
[77]Walker, I. C.: Arch. Int. Med., 1918, xxii, 466.
[78]Rumpf, T. R.: Deutsch med. Wchnschr., 1893, xix, 987.
[79]Luedke, H.: München, med. Wchnschr., 1915, lxii, 327.
[80]Kraus, R.: Wien. klin. Wchnschr., 1915, xxxviii, 29.
[81]Miller, J., and Lusk, F.: Jour. Am. Med. Assn., 1916, lxvi, 1756; ibid., 1916, lxvii, 2010; ibid., 1917, lxix, 765.
[82]Culver, H.: Jour. Am. Med. Assn., 1917, lxviii, 362.
[83]Engman, M., and McGarry, R.: Jour. Am. Med. Assn., 1916, lxvii, 1741.
[84]Cowie, D. M., and Calhoun, H.: Arch. Int. Med., 1919, xxiii, 69.
[85]Roberts, D., and Cary, E. G.: Jour. Am. Med. Assn., 1919, lxxii, 922.

[86]Cowie, D. M., and Beaver, P. W.: Jour. Am. Med. Assn., Apr. 19, 1919, lxxii, 1117.
[87]Schelly, A. I., Blum, J., and Smith, L.: Jour. Am. Med. Assn., 1917, lxviii, 1451.
[88]Whittington, T. H.: Lancet, London, 1916, i, 759.
[89]Bumpus, H. C.: Jour. Am. Med. Assn., 1918, lxxvi, 213.
[90]Herrmann, S. J.: Jour. Infect. Dis., 1918, xxiii, 45.
[91]Dunklin: quoted by Jobling and Peterson: Jour. Am. Med. Assn., 1916, lxvi, 1753.
[92]Jobling and Peterson: Jour. Am. Med. Assn., 1915, lxv, 515.
[93]Matthes, M.: Deutsch. Arch. f. klin. Med. 1894, liv, 39.
[94]Culver, H.: Jour. Lab. and Clin. Med., 1917, iii, 11.
[95]Jobling, Peterson, and Manier: Arch. Int. Med., 1917, xix, 1042.
[96]Beebe, S. P.: Med. Rec., New York, July 21, 1917, xcii.
[97]Brooks, C., and Stanton, F. M.: New York Med. Jour., 1919, cix, 452.
[98]Nolf, P.: Presse Méd., Feb. 24, 1919, xxvii, 93; Jour. Am. Med. Assn., Nov. 22, 1919, lxxiii, 1579.
[99]Baldwin, H., and L'Esperance, E.: Jour. Immun., 1917, ii, 283.
[100]Krause, A. K., and Willis, H. S.: Am. Rev. of Tuberculosis, 1919, iii, 153.
[101]Wells, C. N.: Jour. Am. Med. Assn., 1919, lxxii, 1813.
[102]Lamb, F. H., and Brannin, E. B.: Jour. Am. Med. Assn., Apr. 12, 1919, lxxii, 1056.
[103]Thomas, H. B.: Jour. Am. Med. Assn., 1917, lxix, 770.
[104]Reibmayr, H.: München. med. Wchnschr., 1915, lxii, 610.
[105]Dansyz, J.: Presse méd., 1918, xxvi, 367.
[106]Talbot, F. B.: Med. Rec., New York, 1917, xci, 875.
[107]Vaughan, V. C.: Jour. Am. Med. Assn., 1916, lxvii, 1559.
[108]De Waele: Bull. Acad. roy. de méd. de Belg., 1907, xxi, 715.
[109]Ramirez, M. A.: Jour. Am. Med. Assn., Sept. 27, 1919, lxxiii, 984.
[110]DeBesche, A.: Jour. Infec. Dis., 1918, xxii, 594.
[111]Bruck: Arch. f. Dermatol., 1909, xcvi, 241.
[112]Schloss: Am. Jour. Child. Dis., 1912, iii, 341.
[113]Koessler, K.: in Forschheimer's Therapeusis, v, p. 671.
[114]Longcope, W. T.: Jour. Exper. Med., 1913, xviii, 678.
[115]Boughton, T. H.: Jour. Immunol., 1916, i, 105; ibid, 1917, ii, 501; ibid., 1919, iv, 213.
[116]Boughton, T. H.: In press.
[117]Cadbury, W. W.: China Med. Jour., May, 1919, xxxiii, 213.
[118]Peterson, W. F.: Arch. Int. Med., 1918, xxi, 14.

The Limiting Hydrogen-ion Concentration of Various Types of Pneumococci

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THE LIMITING HYDROGEN-ION CONCENTRATION OF VARIOUS TYPES OF PNEUMOCOCCI

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The final reaction produced by an organism when grown in a medium containing a utilizable carbohydrate is known as the limiting hydrogen-ion concentration of that organism. In a previous paper [1] it was shown that this reaction varies for a given organism, depending on factors which favor or hinder abundant growth. As the growth of the pneumococcus is markedly influenced by slight changes in environment, it was necessary to establish rather definite conditions under which the final H-ion concentrations were developed.

Glucose broth, with the initial reaction of P_H 7.0, is commonly used in making final H-ion concentration determinations, but in dealing with such delicately growing organisms as the pneumococcus, this medium was found unsuited for the purpose, because of the irregularity with which growth was obtained with some of the strains.

Table 1 shows the wide variations in the final H-ion concentrations of a number of strains of each of the four types of pneumococcus, when such a medium was used.

TABLE 1

Inoculations from Rapidly Growing 24-Hour Blood-Agar Cultures Made Into Broth Containing 1% Peptone, 0.3% Beef Extract, 0.7% NaCl, 1% Glucose, and Having an Initial Reaction of Ph 7.0

Organisms	Viability	Final Ph
1	+	6.6
2	+	5.8
3	+	6.4
4	+	5.8
1 R	+	5.7
2 R	—	7.0
3 R	+	5.6
4 R	+	5.7
1 Vir.	+	6.2
2 Vir.	—	7.0
3 Vir.	+	6.4
4 Vir.	+	6.6
1 Vac.	+	6.0
2 Vac.	+	6.5
3 Vac.	+	5.7
4 Vac.	+	5.6

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"Viability" of the organism in table 1 does not imply growth. It was proved by simply plating on blood-agar at the end of the four-day incubation period. The H-ion concentration determinations were made by the hydrogen electrode described elsewhere.[2]

None of the strains developed a greater final concentration than P_H 5.6. Lack of growth macroscopically was no indication of the lack of change in reaction; and visible growth was, likewise, no indication that the P_H had reached its highest possible concentration. To show the effect on the limiting H-ion concentrations, and also in diminishing these wide variations in P_H shown in table 1, contrast the results shown in table 2, in which the same series of organisms were inoculated into broth differing from that used in the previous experiment only in having an initial reaction of P_H 7.6, instead of P_H 7.0.

TABLE 2

Inoculations from 24-Hour Blood-Agar Slants Made Into 1% Glucose Broth Having an Initial Reaction of Ph 7.6

Organisms	Viability	Final Ph
1	+	5.2
2	+	5.3
3	+	5.6
4	+	5.4
1 R	+	5.0
2 R	—	7.6
3 R	+	5.1
4 R	+	5.3
1 Vir.	+	5.2
2 Vir.	+	5.2
3 Vir.	+	5.2
4 Vir.	+	5.1
1 Vac.	+	5.4
2 Vac.	+	5.0
3 Vac.	+	5.4
4 Vac.	+	5.2

A surprising difference in the abundance of growth was also noted. The range of the final H-ion concentration of this series was from P_H 5.0 to P_H 5.6, which is about the same range of final concentrations for hemolytic streptococci of virulent type. The hope of utilizing this method for the differentiation of the types of pneumococci from each other, or from the streptococci, was therefore abandoned.

A comparison of tables 1 and 2, however, very clearly shows how the final concentration of the H-ion is affected by the initial concentration. The initial alkaline reaction of P_H 7.6 was very obviously favorable to abundant growth, and abundant growth, in turn, was favorable to final concentrations which were more uniform throughout the series. Lord [3] noted that the final H-ion concentrations for his

[2] Ibid., 1919, 25, p. 262.
[3] Jour. Am. Med. Assn., 1919, 72, p. 1364.

series of pneumococcus strains were approximately within these same limits.

Cullen and Chesney,[4] however, found that "acidification during growth in beef infusion media proceeds until a P_H of about 7.0 is reached. At this point growth stops." The error of their findings is not difficult to explain when it is recalled that they did not add glucose to their medium. That growth does not stop at P_H 7.0 was easily shown by inoculating some of our strains into glucose broth with an initial reaction of P_H 6.8 and making plates of the cultures after one

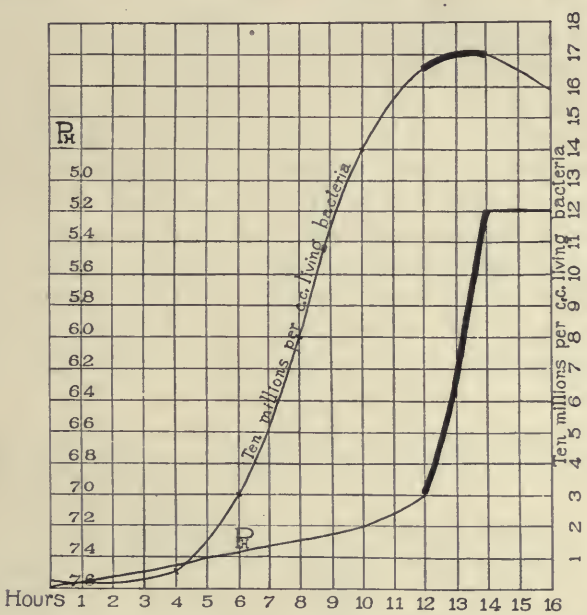

Fig. 1.—Curves of rate of change of PH, coincident with rate of growth. Note that the greatest period of growth occurs while the reaction is still near neutrality, the interval indicated by light lines; and that the greatest period of sugar utilization occurs after the growth rate slows down — the interval indicated by heavy lines.

hour and again after 24 hours. Strain 3 R, for example, in 24 hours had increased from 24,000 to 52,000,000 viable bacteria per c c of culture. The P_H in the meantime had risen to P_H 5.9.

That their cultures developed an acid reaction in spite of the fact that glucose was not added to the medium, is explained by the fact that beef infusion medium contains considerable quantities of "muscle sugar." The final P_H reported by them would have been higher had

[4] Jour. Exper. Med., 1918, 28, p. 289.

they added more sugar, or lower if they had added a buffer, as disodiumphosphate. To obtain the final H-ion concentration of a given strain of bacteria, which will be reproducible in subsequent determinations, one should use a medium containing carbohydrate in excess of the amount which the given strain can remove from that medium.

A similar oversight is seen in the work of Dernby and Avery [5] who also used a beef infusion medium without addition of glucose. Their final concentrations would have been higher had glucose been added, as doubtless also would their bacterial counts. Their contention, that an initial reaction of about P_H 7.8 is the optimum for getting growth started is valid, but that growth does not continue at a P_H higher than 7.0 is not true, for then we should have here an obvious and simple basis for differentiation of this organism from Streptococcus hemolyticus. However, when abundant growth had occurred in a given culture in which an excess of glucose is present, the change in concentration of H-ion proceeds with such rapidity toward the higher ranges that the increase in growth is not proportionate. For example, in a culture of type 1, the P_H had risen from 7.6 to 7.0, the count had increased from 11,000 to 165,000,000 per c c in 12 hours, but in two hours more the P_H had suddenly risen to its maximum of P_H 5.2, while the count had had time to change only appreciably, as shown in figure 1.

TABLE 3

The Effect of an Initial Reaction of Ph 7.0 in Hindering the Development of Pneumococci Introduced, with 2 c c of Pure Blood, into 100 c c of Glucose Broth

Strain	Glucose Broth Initial Ph 7.0 Ph After 24 Hours	Growth per c c After 24 Hours	2% Blood in Glucose Broth Initial Ph 7.0 Ph After 24 Hours	Growth per c c After 24 Hours
1	6.6	15,000	4.8	800,000,000
2 Vac.	6.5	23,000	4.8	1,200,000,000
4 Vir.	6.6	2 500	5.0	2,000,000,000

This inability of the pneumococcus to grow in a medium of the usual reaction employed in ordinary bacteriologic mediums, namely P_H 7.0, is pronounced in some strains. For example, strains 1 of type 1, 2 Vac. of type 2, and 4 Vir. of type 4, produced no visible growth in such a medium, and, as seen in table 1, only very slight change in the P_H of the medium. The fact that blood cultures in pneumococcic septicemia often fail, seemed probably due to the fact that a broth of this unfavorable reaction may prevent the development of organisms, even though they may have been present in the blood under

[5] Ibid., p. 345.

examination. Accordingly, the effect of two parts of pure blood when introduced into 100 parts of glucose broth of this reaction, was tested, using those strains which grew poorly in glucose broth of P_H 7.0. Incidentally, the addition of the blood was shown to have no measurable effect in changing the reaction of the medium.

The results show that the introduction of 2% of blood is sufficient to cause profuse growth, even at the same reaction, P_H 7.0, at which growth in ordinary glucose broth fails. The experiment was repeated, using ascitic fluid in place of blood. In general, the effect was the same, both as to the abundance of growth, and the higher concentrations of H-ion developed by these strains. Long boiling renders these fluids useless in stimulating the profuse growths which resulted in the experiment described above.

In a previous paper [1] attention was called to the effect which these body fluids have in increasing the tolerance of Streptococcus hemolyticus of virulent type to the toxic ion of hydrogen. In other words, when these fluids are added to glucose broth, which is frequently done to insure growth, such a P_H is then developed that the virulent is made to imitate in P_H the avirulent types of streptococci. Table 3 show that the pneumococcus enjoys, with the streptococcus, this same increase of tolerance to the toxic ion of hydrogen, when these body fluids are added to the medium, which is of importance in connection with the fact that the virulent strains of streptococci are sometimes differentiated with great difficulty from the avirulent strains on the one hand, and the pneumococcus on the other.

No explanation of the action of the body fluids in stimulation of growth and increasing the organism's tolerance to hydrogen-ion, is attempted here, but since strong acidification, long boiling, etc., destroy this unknown "active principle," further search may reveal still other phenomena having "vitamins" as a basis of their explanation.

SUMMARY

The final hydrogen-ion concentration produced by pneumococci of various types when grown in glucose broth varies, with different strains, between P_H 5.0 and 5.6, being indistinguishable in this respect from various strains of Streptococcus hemolyticus of virulent type.

The regularity with which these final hydrogen-ion concentration values can be reproduced depends largely on the initial reaction, ordi-

nary glucose broth of P_H 7.0 being useless for this purpose. None of the strains failed to grow, however, when the initial reaction was set at 7.6.

This failure to grow in broth of P_H 7.0 does not account for the often observed failure to secure growth of the pneumococcus as when blood cultures are being made, since the addition of 2% of whole blood renders the medium of P_H 7.0 even superior to glucose broth of P_H 7.6 in stimulating growth.

A marked increase in tolerance toward the hydrogen-ion is also observed, as is also the case with Streptococcus hemolyticus.

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EFFECT OF CARBOHYDRATE ON AMINO ACID UTILIZATION OF CERTAIN BACTERIA

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That a utilizable carbohydrate influences the changes in protein and amino-acids in bacterial cultures, is so well known as to require no comment. The phenomenon has assumed undue prominence in the literature because various investigators do not agree in their interpretations. The two outstanding features of bacterial metabolism, which have been emphasized by Kendall, Walker and Day[1] in a series of experiments extending over several years, are: (1) Protein in the presence of a fermentable sugar is spared. (2) When sugar is present, protein metabolism is reduced to the minimum required for structural purposes. Herman and Rettger,[2] however, using a simple buffered medium in their series of experiments, came to the conclusion that "Kendall and Walker's conception that the presence of glucose delays the production of the proteolytic enzyme cannot be accepted. In the tests in which the buffer reagent was employed the proteolytic enzyme appeared as soon in the sugar media as in the plain bouillon." They ran parallel tests on mediums containing 0.2% and 0.4% dextrose, with 0.25% beef extract, 0.5% peptone, and 0.5% NaCl, one series without, and the other series with 0.5% K_2HPO_4. By following the changes by tests on successive days, an interesting sequence, when properly interpreted, is recorded in their table. Why they would limit the concentration of sugar to any percentage is not clear. The action of the buffer in their experiments is clearly to hold the concentration of hydrogen-ion below the toxic limit, and thus enable the organisms to exhaust the last trace of such small quantities of sugar. The sugar having been exhausted, the organisms of course then turn to the amino acids for their energy needs, which accounts for the sequence of changes shown in their table.

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[1] Jour. Am. Chem. Soc., 1913, 135, p. 1201.
[2] J. Bacteriol., 3, 389, 1918.

They conclude that "the presence of sufficient buffer in a medium encourages continued normal nitrogen metabolism." In other words, they argue for keeping the hydrogen-ion concentration nearer neutrality. In compliance with this requirement, I have used 4% dextrose, with normal sodium hydroxid for titrating the acid as rapidly as formed with brom-cresol-purple in the culture as an indicator. After 12 hours the culture had to be watched continuously because of the rapid formation of acid, but after a period of 36 hours of this marked activity, the reaction became stationary. Although still maintained at the neutral point (P_H 7.5, by the hydrogen electrode) and kept for a period of 32 days, no evidence of "continued normal nitrogen metabolism" could be detected. The proteolytic enzyme had not appeared, indol was negative, ammonia formation was not greater than in the untitrated control, and sugar was still present.

Aside from the interesting fact that the activity of a culture can be arrested by the accumulation of products of its metabolism other than the toxic ion of hydrogen, perhaps in this instance the lactate ion principally, it is seen that even under the most favorable conditions of hydrogen-ion concentration, i. e., P_H 7.5, amino acid utilization for energy needs is definitely inhibited as long as sugar is available.

The paradoxic result of finding sugar in the phosphate medium but not in the 0.2% dextrose phosphate-free medium, requires explanation. Why was it not present in the unbuffered medium? That such a small trace of sugar was detected in the presence of 0.5% phosphate is most unusual. In regard to this, Berman and Rettger insist that "these results were indeed unexpected, and the tests were repeated, with identical results."

Whether sugar was present or absent, therefore, decides the interpretation of what follows. Here is the crux of the whole matter. The following points are offered as evidence against the claim that sugar was present: (1) It should be remembered that there is a common practice among bacteriologists of rendering beef infusion sugar free by incubating for 24 hours with dextrose fermenting organisms. How is such a practice to be justified if there is a "residuary carbohydrate" left in the culture even after 27 days' incubation? (2) To test the correctness of these findings one has only to kill such a culture, divide it into two portions, add at least 0.1% of sugar to one portion, and test both portions with Benedict's solution to discover that even with this extra 0.1% of sugar added to the "residuary carbohydrate" spoken of by these authors, the precipitate of cupric phos-

phate is so heavy in both tubes as to obscure entirely any positive sugar reaction. Even after repeated boiling and standing for 24 hours the tubes differ in no respect in appearance from an absolutely sugar free control. (3) When phosphate in such a concentration is known to be present, failure to remove it from the solution before testing for small traces of sugar, would be considered a serious oversight by any one acquainted with the limitations of Benedict's solution as a reagent for sugar in the presence of certain other substances.

Berman and Rettger claim that "fermentable sugars in moderate amounts do not affect the nitrogen metabolism of bacteria—under conditions of favorable environment," and that "the common belief in a so-called 'sparing action' of sugar in a protein medium is untenable in the light of these experiments. According to this idea protein is spared from all participation in the metabolism." I am unable to find anywhere in the reports of the work which they refute, any statement that "protein is spared from all participation in the metabolism," under any conditions. Growth requirements imply participation of protein in the metabolism.

The disagreement in our interpretations throughout probably originates from the experiment in which they found "a residuary carbohydrate" by Benedict's method, even in the presence of 0.5% phosphate, after 27 days' incubation, and though only 0.2% carbohydrate was present in the beginning. According to my experiments, the limiting hydrogen-ion concentration of B. proteus i. e., P_H 4.8, is sufficiently high to allow complete removal of 0.2% dextrose in 36 hours even in the usual (unbuffered) medium. Their table shows practically the same result in the 0.2% dextrose unbuffered medium, namely, positive sugar test up to 24 hours, but negative after 3 days. But why should it persist in the buffered medium? Phosphate should facilitate its removal rather than give rise to a "residuary carbohydrate."

Further disagreement arises from the fact that they do not make clear the distinction between protein hydrolysis and amino-acid utilization. These must be specified by more definite terms than the inclusive term "protein metabolism." Both should not be included when only one is meant. When the one occurs we see liquefaction without putrefaction; when the other occurs we find the formation of indol, H_2S and the genuine putrefactive changes characteristic of amino acid disintegration. They usually occur together but not neces-

sarily. For example, many organisms which do not liquefy plain gelatin will produce a decided softening (acid hydrolysis) if a utilizable sugar is present. In other words, in the sugar-free gelatin cultures of, say, B. coli, putrefaction but not liquefaction occurs; in the sugar gelatin, liquefaction but not putrefaction, showing that either phase of "protein metabolism" may occur alone. To demonstrate this softening of gelatin by acid hydrolysis, one has only to acidify a tube of sterile gelatin medium to P_H of about 4.5, with some acid, e.g., lactic, and compare its consistency after a few days at 37 C. with a similar control tube to which has been added a proportionate quantity of sterile water. Since the medium is kept sterile, this effect on the gelatin certainly is not one of "protein metabolism" and yet this softening of gelatin has often been erroneously offered as evidence that protein metabolism occurs in the presence of sugar.

It is not impossible that certain organisms have been or will be found which could utilize both amino acids and sugars at the same time, but such strains will be the rarest exception to the rule, and their occurrence would not in any sense invalidate the general proposition set forth in the work of Kendall, Walker and Day as a valuable working hypothesis and basis of interpretation in metabolic studies.

SUMMARY

A culture of B. proteus containing sufficient carbohydrate shows no evidence of amino acid utilization, even though the reaction of the culture was maintained at neutrality during its entire period of active growth, and for an additional period of one month following cessation of activity.

The softening of gelatin occurring in sugar-gelatin medium is an acid rather than an enzymic-hydrolysis, and should not be interpreted as a part of protein metabolism.

A Simple Device for Measuring Rate of Metabolism

HORRY M. JONES, PH.D.
CHICAGO

A SIMPLE DEVICE FOR MEASURING RATE OF METABOLISM *

HORRY M. JONES, PH.D.
CHICAGO

Of those laboratory tests useful in the diagnosis of certain forms of disease; perhaps none is more valuable to the clinician than the recent test devised to measure basal metabolism.

The sudden interest which the test has stimulated among clinicians has spread with surprising rapidity, perhaps for the reason that, from the standpoint of efficiency in the differentiation of certain disease, its results are so spectacular in uniformity and conclusiveness. Moreover, it was just this rapid spread of interest in the test which has so effectually confirmed its usefulness as a diagnostic aid. The question as to its value, so far, has been singularly free from controversy.

Lusk [1] and his associates, on the basis of very accurate and elaborate measurements, have emphasized chiefly the scientific aspects of the test.

The problem of making the test available to those clinicians not having access to the elaborate equipment of the nutritions laboratory was solved by Benedict,[2] who designed a portable apparatus for measuring the basal metabolism of human subjects, and by DuBois,[3] who has devised a "linear formula" for use in indirect respiration calorimetry.

In a recent article, McCaskey [4] says of the method originated by Benedict: "Unless its clinical value can be shown to be commensurate with the time, labor and equipment required, and in this instance these items are rather large, it cannot and should not endure." His paper is offered as an additional contribution to this end. In other words, he believes that the urgent need for such a test justifies the fairly considerable expenditure of time, labor and equipment necessary to its performance.

To extend still further its usefulness by reducing to a minimum this expenditure of time, labor and equipment, I undertook to devise an apparatus which is simple and accurate in operation and yet sufficiently compact for the surgeon, clinician or general practitioner to carry it to the patient's home or bedside, an apparatus which is portable in a practical sense. In designing it, I have kept this feature constantly in mind.

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1. Lusk: Arch. Int. Med. **15**:793 (May) 1915.
2. Benedict: Boston M. & S. J. **178**:667, 1918.
3. DuBois: Arch. Int. Med. **15**:793 (May) 1915; **17**:855 (June) 1916.
4. McCaskey: J. A. M. A. **74**:927 (April) 1920.

A second feature also deserves special emphasis in this connection. All mathematical computations have been eliminated. The units expressing the results of the test are arrived at immediately and without calculation. A method which involves the use of logarithms, slide rule and considerable time for calculations, as in the case of the Benedict method, has not properly considered the inability of the average busy clinician to deal with this kind of mathematical procedure.

PRINCIPLE OF THE METHOD

The ratio of the quantity of carbon dioxid eliminated to the quantity of oxygen consumed is called the respiratory quotient. In the oxidation of fat this R Q (respiratory quotient) is about 70: 100, or 0.70; of carbohydrate it is 100: 100, or 1. Obviously, when a combination of fat and carbohydrate is oxidized, the R Q lies somewhere between 0.70 and 1. It is necessary to know the R Q because the

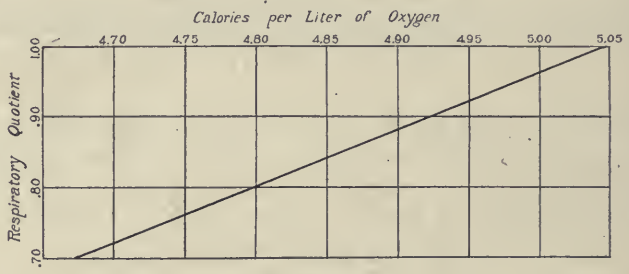

Figure 1.

caloric value of a liter of oxygen, a factor which must be known, varies with the R Q, as shown in Figure 1.

With appropriate apparatus, the determination of the R Q, i. e., the amount of carbon dioxid eliminated and oxygen consumed, is an ordinary laboratory procedure. Having determined the R Q, reference to Figure 1 gives the caloric value of each liter of oxygen so consumed. The number of liters of oxygen consumed,[5] multiplied by the caloric value of one liter at this R Q then gives the total caloric radiation or rate of metabolism.

In figure 1 it is seen that the caloric value of oxygen is not markedly affected by varying the value R Q. In fact, in clinical work, it is

5. A measure of the carbon dioxid eliminated could also be made the basis for calculating the heat output according to this same reasoning. The objections to this method, however, are that the caloric value represented by the elimination of a liter of carbon dioxid varies too widely, depending on whether it was derived from the burning of fat or of carbohydrate, and also to the fact that irregularities in the respiration causing overventilation of the lungs increases this error in determinations extending over such short periods.

customary to assume the R Q as 0.82, which has been found to be the average of a large number of determinations. By so doing, the task of measuring the carbon dioxid to ascertain the ratio is obviated, and it is necessary then only to measure the oxygen intake.

To calculate the caloric output by measuring the rate of oxygen intake and multiplying this by the caloric value of one liter at the assumed R Q of 0.82 is, therefore, the principle on which the apparatus here described is designed to operate.

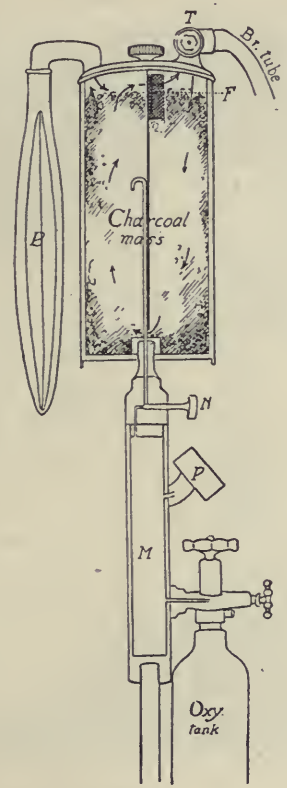

Fig. 2.—Cross-section of apparatus: Arrows indicate direction of movement of inspired and expired air; Br. tube, breathing tube; T, three-way cock; F, flutter valve for directing circulation of gases; b, rubber bag to contain measured amount of gas and to allow for expansions and contractions in respiration; N, needle valve for releasing the measured amount of oxygen from M, the measuring cylinder, into the charcoal-alkali tower above; P, pressure gauge with room temperature scale on dial to indicate when one liter of dry oxygen at 0 C. and 760 mm. has been released at any given room temperature from the oxygen tank.

CLINICAL APPLICATION OF THE METHOD

There are three principal ways in which the living body expends its store of heat. 1. Following the ingestion of food there is a marked

rise in the rate of heat loss due to the specific dynamic action of foods. 2. Muscular exertion requires an expenditure of its caloric equivalent. Its effect on the heat loss ceases with complete muscular relaxation usually within thirty minutes following ordinary muscular effort. 3. Certain vital processes, such as respiration, circulation, secretion, the maintenance of body temperature, etc., must be supported irrespective of the first two and at the expense of the food stores or tissues of the body.

The first two represent variable quantities and may be eliminated by fasting and muscular relaxation, as indicated above. However, the third is a remarkably constant quantity for a given individual. Ordinarily this regular and ever present demand for the maintenance of the vital functions amounts to more than one-half of the body's daily expenditure of heat; and because of the constancy with which it takes this toll, even in conditions of extreme starvation it has been given the name basal metabolism.

Under conditions of basal metabolism, the rate of heat loss of different individuals is surprisingly similar. It varies with sex and age, being higher in youth and in males, but these factors need only to be taken into account to determine what is normal in any given case. So true is this, that when the basal metabolism of a given individual varies more than 10 per cent. from the normal for that patient's sex and age, certain diseases may be diagnosed on the basis of this change from the normal rate.

DESCRIPTION OF THE APPARATUS

The apparatus (Fig. 2) consists merely of a mouthpiece with wide flexible tube leading the expired air into the apparatus; a tower of small pieces of charcoal, soaked in alkali, for the purpose of removing the carbon dioxid; a gas anesthetic rubber bag to allow for expiration and inspiration and to contain the oxygen supply; a piece of aluminum pipe serving as a support to the alkali tower, and also as a measuring apparatus for delivering into the rubber bag a known quantity of oxygen. The measuring cylinder is also provided with an attachment for the small forty gallon oxygen cylinder, and with a pressure gage with special dial to indicate when the desired quantity of oxygen has been released.

The instructions which have been found to cover the points in technic and principle of the method sufficiently to enable one of ordinary skill to carry out the test are as follows:

PREPARATION OF THE PATIENT

(1) Have subject take no food (nothing but water), for from fourteen to eighteen hours previous to test, preferably from 6 o'clock evening meal, to 10 o'clock next morning, when test is made.

(2) Subject should be lying comfortably and quietly from fifteen to thirty minutes before test begins.

TECHNIC OF THE TEST

Attach nose clip and test for air leak at nose by having subject close mouth and exert moderate pressure. Turn three-way cock open to air. Insert rubber shield of mouth piece inside of lips but outside of teeth, drawing lips up about neck of mouth piece. Open needle valve of measuring cylinder. Admit gas slowly from oxygen tank until bag distends sufficiently to just touch the side of the alkali tower. This quantity is used merely to establish what is called the *beginning point.* Now *close* needle valve. Admit gas *slowly* again

Fig. 3.—Apparatus in use.

from oxygen tank while indicator is driven around, and, after tapping gage with finger, stands exactly over room temperature point of scale on dial. When released, later, this quantity will be 1,000 c.c. (± 2 c.c.) of gas at 0 C. and 760 mm. Hg. It is held in the measuring cylinder ready for release at the *beginning point.* Approximately at *beginning of expiration* quickly turn three-way cock closed. Subject is now breathing gases confined in the tower and bag. Observe the quantity of gas in bag as it gradually diminishes in volume. Watch point where bag makes contact with the side of alkali tower. That expiration, when the bag, at its fullest distention, just fails to touch the side of alkali tower, is counted *one.* If the expiration following this one fails to cause the bag to touch the alkali tower it is counted *two;* and the one following this *three.* This establishes the *beginning point.* At this instant release

the stop watch and then discharge the 1,000 c.c. of gas now in measuring cylinder by opening needle valve. *Close* needle valve again and admit a second liter of gas into measuring cylinder. After a few minutes bag will have again diminished in volume to same condition described for the beginning point (namely, three successive normal expirations, when the bag, at its fullest distention, just fails to touch side of alkali tower). Watch for this as before, and when it occurs note time by stop watch. Release second liter to bag, as before. Close needle valve again and admit a third liter to measuring cylinder. Proceed in this way, observing exact time required by subject to consume each successive liter. Two liters is usually sufficient but average of three is better. Finally at the end point of the last liter used, stop the watch, turn three-way cock to open, and remove the mouth piece. Before removing nose clip, test again for air leaks as at beginning of test. (If air leak has developed during test discard the readings.) Total time on watch divided by number of liters consumed equals *average time required to consume one liter,* and from this the subject's rate of metabolism is made known as follows:

TO CALCULATE METABOLIC RATE

To illustrate: Male, age, 30; height, 66 inches; nude weight, 153 pounds; averaged 3.1 minutes to consume 1 liter of oxygen. Referring to Table 1, his height and weight lines intersect in a point between oblique lines 1.7 and 1.8, say at 1.79; 1.79 square meters is, therefore, his body-area. Referring to Table 2, this body-area line, 1.79, and his 3.1 minute line, intersect in a point between 50 and 55, say 53. Rate is, therefore, 53 calories per square meter per hour or + 34 per cent., i. e., 34 per cent. above the normal for his age and sex, as seen from Table 3.

CARE OF ALKALI TOWER

Pour about 400 c.c. of a *saturated* solution of commercial sodium hydrate over coal mass. About 100 c.c. of this will settle to bottom. A few minutes before beginning on a test, hold back coal particles with hand and drain this bottom fluid into a glass or beaker, then pour it over coal mass again to redistribute the alkali. Run the test and duplicate test, then drain this bottom liquid off again and discard it. For another test and duplicate test use another fresh 100 c.c. portion of the alkali solution. After test, drain and discard as before. Before putting apparatus away always drain off this bottom fluid, and then pour in about 200 c.c. water to wash off the exhausted alkali on the coal particles. Drain this off, and then pour in another 200 c.c. water to stand in bottom of tower until used again. This prevents crystallization of sodium bicarbonate in bottom and clogging of coal mass. Before using apparatus again, drain this water off and pour on about 100 c.c. of saturated solution of sodium hydrate, which will be sufficient for a test, and duplicate test, as before.

The height-weight table (Fig. 4) is modified after the table of DuBois [3] according to his "linear formula": Area $= W\,0.425 \times H\,0.725 \times 71.84$.

The body area-minute table (Fig. 2) is constructed according to the formula $\frac{4.823 \times \text{liters of oxygen consumed per hour}}{\text{Surface area of patient}} =$ calories per hour per square meter, in which 4.823 is the caloric value of one liter oxygen at the assumed R Q 0.82, and in which the liters of oxygen consumed per hour was calculated on the basis of the average number of minutes required by the patient to consume one liter of oxygen.

SOURCES OF VARIATIONS

1. *Age and Sex.*—The effect of age and sex on the rate of metabolism is shown in Table 1 by Aub and DuBois.[6]

TABLE 1.—Variations Due to Age and Sex, Expressed as Calories Per Square Meter Per Hour

Age	Males	Females
14–16	46.0	43.0
16–18	43.0	40.0
18–20	41.0	38.0
20–30	39.5	37.5
30–40	39.5	36.5
40–50	38.5	36.0
50–60	37.5	35.0
60–70	36.5	34.0
70–80	35.5	33.0

In expressing the rate of metabolism as $\pm\%$, the above factors must therefore be taken into account.

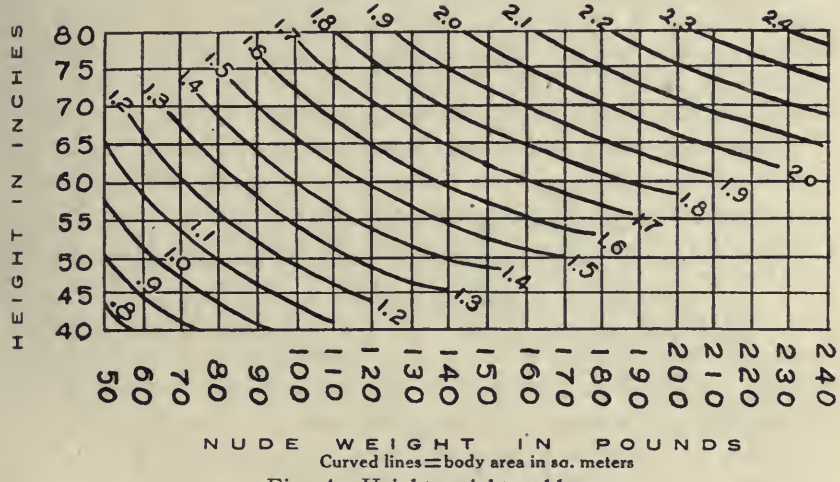

Fig. 4.—Height-weight table.

2. *The Effect of Foods.*—The specific dynamic action of food may be defined as that effect which the ingestion of food has in stimulating the metabolic rate to an increase above the basal rate. For proteins this stimulating action amounts to an increase of 33 per cent. of the caloric value of the amount of protein ingested; for fats it is 11 per cent, for carbohydrates, 5 per cent. The patient is instructed to take no food after the six o'clock evening meal, the test being made at about 10 o'clock the next morning, i. e., sixteen hours after the last meal. If these instructions are not made clear, or the subject forgets, or uses deception in regard to them, this specific dynamic action of food enters as a source of considerable variation, and may take the observer unawares. Another source of error which may also

6. Aub and DuBois: Arch. Int. Med. **19**:831 (July) 1917.

take the observer by surprise is that due to the effect of caffein [7] in increasing the rate of metabolism. If the patient is allowed his morning cup of coffee or tea on the assumption that it contains nothing of any food value, the observer is then at a loss to explain the 10 or 20 per cent. rise above the normal basal rate.

3. *Muscular Tension and Psychic States.*—The effect of muscular tension in increasing the rate of metabolism is obvious. If the subject is restless, or lies in an uncomfortable or tense position, or is subjected to various psychic disturbances as fear of the test, or embarrassment, the effect in increasing the metabolism above the basal rate may be even greater than in the last mentioned source of variation. For example, in a demonstration of this apparatus given before the Chicago Society of Internal Medicine, the subject's rate rose to 28 per cent. above her basal rate; which later, under less exciting conditions, was normal. In spite of her attempts to assume complete muscular relaxation, the rate of metabolism, more than anything else, revealed the attack of stage fright, which the subject later admitted. Some day the criminologist, by the aid of appropriate controls and setting, may find in this stimulating action of fear a dependable ally in the detection of crime. The point of therapeutic value is that a patient, put to bed to reduce his rate of metabolism, defeats the purpose in a large measure, sometimes by worry, by homesickness, by entertaining visitors, or by a number of other ways in which the psychic element has not been considered.

4. *Variations Due to Certain Diseases.*—When the basal rate of metabolism of a subject is found to be constantly above or below the average basal rate for persons of his age and sex, certain diseases are diagnosed, chief among which are disturbed functions of the thyroid and pituitary. Severity of the disturbance is proportional to the variation from the normal rate, and, therefore, by following the variation of the rate from the normal by successive tests, the effect of therapy can be demonstrated. Following the effect of treatment is, in fact, one of the firmly established uses of the test.

TECHNICAL SOURCES OF VARIATIONS

The Gas Measuring Apparatus: The purpose of the pressure gage is to indicate that pressure at which the measuring cylinder will deliver 1,000 c.c. of dry oxygen at 0 C. and 760 mm. Hg pressure. This volume at 23 C. and 750 mm. Hg becomes 1,098.8 c.c.; if measured over water it is further increased to about 1,128.8 c.c. since the vapor tension at 23 C. is 21 mm. Hg. In operation, however, this

7. Means, Aub and DuBois: Arch. Int. Med. **19**:832 (July) 1917.

measuring apparatus is independent of temperature and pressure changes. The influence of temperature on the gas volume is eliminated by having the pointer of the gage come to rest over various points on the dial representing various room temperatures. The construction of the measuring apparatus is essential in principle to that of the anaeroid barometer, and is, therefore, independent of changes in atmospheric pressure, i. e., the indicator merely represents the tension of gas inside the cylinder as against the tension outside of it, regardless of what this outside tension may be. In spite of these features, the apparatus is not a delicate mechanism requiring frequent readjustment

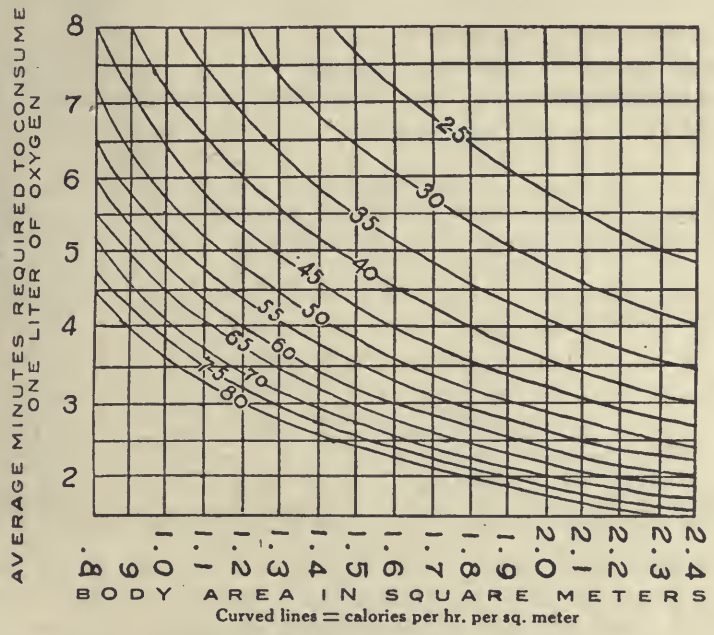

Fig. 5.—Body area-minute table.

or repair. By actual trial the accuracy of the measurement is about ± 2 c.c. out of 1,000 c.c., or 0.2 per cent. error. This degree of accuracy is possible, however, only if the operator is careful each time when reading the pressure to tap on the side of the gage with the tip of the finger, to make sure that the indicator has not encountered some slight resistance in its cog mechanism.

(b) Effect of Oxygen Rich Air on Rate of Oxygen Absorption: Benedict and Higgins[8] have shown that the breathing of oxygen rich air, even up to 90 per cent. oxygen, has no effect on the rate of oxygen absorption. The concentration of oxygen in the

8. Benedict and Higgins: Am. J. Physiol. **28**:1, 1911.

test as described here is never above about 80 per cent. at the beginning, and never below 20 per cent. at the end of the test.

(c) Effect of Temperature: The effect of temperature on the volume of gas contained in the alkali tower is very slight. The rise in temperature averages only 2 C. and the volume of the contained air is only about from 500 to 700 c.c. Error from this source is negligible.

(d) The Nose Clamp: Special caution is necessary in adjusting the nose clamp. If too tight, the patient is in considerable distress in a few moments; if too loose enormous errors will result, since the air of expiration is forced out through small leaks very rapidly. The patient should use only moderate pressure, however, in testing for such leaks. To guard against this source of error, consistent care in adjusting the nose clamp is absolutely essential, regardless of the type of nose clamp employed.

TABLE 2.—Variations in End-Points in Successive Tests on the Same Individual, Without Disconnecting the Subject from the Apparatus *

Test	Stop-watch	Minutes	Percentage Variation from Average
1	4.71	4.71	+0.4
2	9.34	4.63	—1.3
3	14.01	4.67	—0.4
4	18.76	4.75	+1.3
5	23.42	4.66	—0.4
6	28.04	4.62	—1.5
7	32.72	4.68	—0.2
8	37.46	4.74	+1.1
9	42.15	4.69	0.0
10	46.90	4.75	+1.3

* The patient from whom these data were obtained was in coma, showing that knowledge of the test or cooperation on the part of the patient is not essential to accuracy.

(e) The End Point of the Test: The volume of gas in the bag is reduced at the rate of about 15 c.c. per respiration. The bag, therefore, approaches the empty condition gradually. Moreover, the point in the cycle of respiration which gives us the beginning and end-point, namely, the end of expiration, is also constant regardless of the rate and amplitude of respiration, since this point in the cycle represents the position of passivity of the respiratory organs.

The percentage variation in the time required by the patient to consume each successive liter is usually within $\pm$ 2 per cent. of the average of the total time, as seen in Table 2. In patients suffering from severe degrees of thyreotoxicosis who are invariably more or less intractable and restless, it is, therefore, of greater importance, clinically, to make two separate duplicate one liter tests, than a single test of two or more liters in succession. In this way the method is rendered practically free from chance errors due to the operator's technical routine.

COMPUTING THE TOTAL CALORIC REQUIREMENTS OF THE PATIENT

When the total caloric loss of an individual under basal metabolism conditions is determined (Fig. 6) for the twenty-four hour period, this does not mean that this caloric equivalent in food will keep him in caloric equilibrium, even with complete rest in bed. A subject with a caloric output of 1,600 calories for twenty-four hours will require about 400 calories in addition to offset the caloric waste caused by the specific dynamic action of the food. This additional amount of food will depend on the proportion of the various food elements, since these vary, as stated before, in their effect in stimulating the

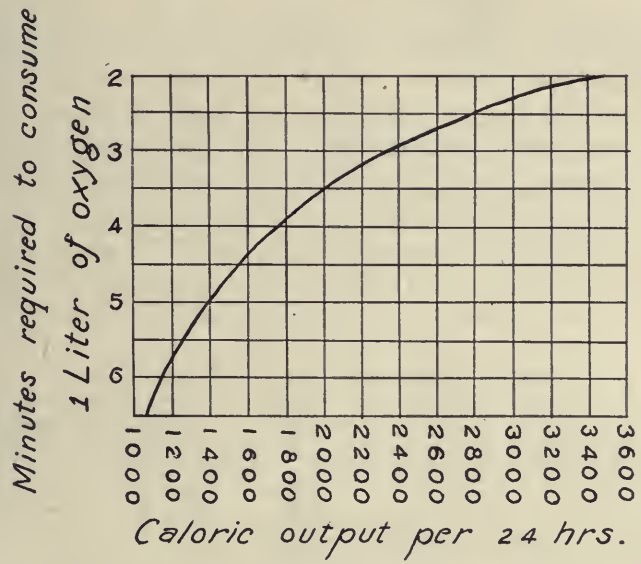

Figure 6.

metabolism above its basal rate. Ordinarily, their combined effect amounts to a waste of between from 20 to 25 per cent. of the total caloric intake. If the subject is not at rest in bed, a still further addition in the caloric intake must, of course, be allowed, the amount depending on the amount of work done by the subject. This is of importance in connection with the regulation of the diet of patients who are abnormally low or high in body weight.

COMPARATIVE TESTS

In comparing the results of tests made by this apparatus with those obtained by the Benedict apparatus, the two instruments agree within very narrow limits. Strict agreement in the reading of the gas volumes could not be expected since the oxygen volume as measured by

the Benedict method is made over water and no allowance made for aqueous tension. This, at 23 C., introduces an error of 3 per cent. in the reading by the Benedict apparatus, and at higher temperatures the error would be disproportionately greater. When correction is

TABLE 3.—Basal Rate in Calories Per Square Meter Per Hour*

Subject	Age	Sex	Rate
S. T.	25	F	36.5
Wy.	26	F	37.0
J. J. T.	32	M	39.6
L. P. G.	24	M	39.2
F. G.	22	F	37.5
M. W.	16	F	41.0
S. P.	13	M	44.0

* These results should be compared with the values given in the table by Aub and DuBois (Charts 1 and 2).

made for this factor, the liter of gas, as measured in the apparatus described here, when discharged into the spirometer in the Benedict apparatus showed a rise of the spirometer corresponding very closely to one liter as measured in the latter. With successive tests the agreement was extremely close, $\pm$ 3 or 4 c.c. when the same end of the spirometer was used. Three different models of the Benedict apparatus were used in this comparative test with equally close results.

TABLE 4.—Percentage Rise Above Basal Rate*

Patient	Age	Sex	Rise, per Cent.
C. C. H.	35	M	+112
H. P.	47	F	+ 87
P. P.	28	F	+ 51
F. C.	28	M	+ 35
W. S.	52	F	+ 63
A. H.	44	F	+ 26
M. P.	38	F	+ 22

* The rates given in this table are those of persons suffering from hyperthyroidism of varying degrees of severity. All of them had been diagnosed as such by Dr. Charles S. Williamson before being tested. The results show the same ranges of variation reported by others on many cases of this disease.

In comparing the percentage rates of metabolism of normal persons, and also hyperthyroid subjects showing varying grades of severity, the values shown in Table 5 represent the usual degree of agreement of the two instruments, when the particular instrument in use is protected against leaks, and when the carbon dioxid absorbing reagent is working properly.

The conclusion is, therefore, justified that either apparatus is quite satisfactory for clinical studies in metabolism, the question of differences in the two instruments centering mainly around the proposition of arriving more easily and quickly at the end result with less chance for error when operated by persons of only average skill.

This instrument has also been used for many months in hundreds of tests on normal persons of all ages and both sexes. The results agree (within the limits of physiological variation) with the average values of normal subjects of given age and sex (see table by Aub and DuBois given on a preceding page). As reported in a previous paper,[9] the apparatus is also being used regularly by the author in

TABLE 5.—Percentage Rates of Metabolism with Benedict and Jones Apparatus

	Benedict	Jones
Miss G. H.	104–107	103–101
Miss S. F.	157–160	158–150
Mr. N. A.	99–102	98–99
Miss E. P.	123–120	120–121
Mr. H. L.	103–105	104–107

clinical research and also in the diagnosis of suspected or borderline cases of myxedema and hyperthyroidism. Duplicates of the instrument have been distributed among clinicians and general practitioners in various parts of the country, who are using it for a like purpose. So far no inherent inaccuracies of technical difficulties have been reported from these sources.

SUMMARY

An apparatus for measuring the rate of oxygen consumption, designed to be portable in a practical sense, is described and illustrated.

Sources of error are discussed and their percentages reduced to a minimum consistent with simplicity.

Mathematical procedures necessary for calculation of the rate of metabolism (from the respiratory quotient, the body area, and the rate of oxygen consumption) are eliminated from the test. The reading is made directly, in terms of calories per hour per square meter of body area.

Independent and comparative tests show its technical variations to be within physiologic and individual variations, and, therefore, adequate to the needs of the clinician as an instrument for measuring basal metabolism.

9. Jones, H. M.: J. A. M. A. **75**:538 (Aug. 21) 1920.

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PAPILLOMA OF THE URETER

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Primary neoplasms of the ureters are very rare, as evidenced by the few instances in medical literature and by almost complete absence of clinical or pathological records of such cases in large general hospitals. The difficulty in making an accurate clinical diagnosis together with the failure of making complete necropsies in all cases of death regardless of the apparent cause, no doubt, has tended to minimize the number of cases on record. Some of the known cases of ureteral neoplasm were incidental to acute infections which caused death and were discovered by complete organ examinations at necropsy.

The ureter, like other structures of the urinary tract, develops practically only neoplasms of epithelial origin. Speiss in 1915 was able to collect but twenty-four instances of primary epithelial ureteral tumors from the literature, including nine benign and fifteen malignant growths. Indeed, the ureter is more frequently involved in new growths secondarily, either by extension from the renal pelvis or the urinary bladder, in both of which structures these growths are quite frequently primary. While it would be hardly possible to state in which structure the tumor is primary in all such instances, only those are considered as true primary ureteral tumors which are confined to the ureter at the time of the examination, or present adequate clinical or pathological evidence supporting this assumption to justify such classification.

The rarity of benign epithelial ureteral tumor, together with a fairly typical clinical course and symptomatology of an available instance of such disease justifies this report.

A married woman, age thirty-five years, entered the hospital complaining of blood in the urine and severe colicky pain in the left side of the abdomen and back. Symptoms had been present intermittently for two years. Her initial symptom was pain in the region of the left kidney, associated with tenderness of this organ. This pain was deep and boring, sudden in onset and did not radiate; it disappeared as suddenly as it appeared and was followed by the appearance of blood in the urine. The hematuria, while really constantly present since the onset became intermittently profuse and was then associated with pain and tenderness of the left kidney.

Cystoscopy about one year after the beginning of symptoms established the facts that the bladder was normal and the blood was coming from the left ureteral orifice. Separated urines were negative for tuberculous or pyogenic infections and rentgenograms were negative for calculi.

Owing to a gradual increase in the frequency and severity of the attacks of pain and profuse bleeding, a left nephrectomy was done. The pathological report, from a reliable source stated that the kidney specimen showed a mild chronic diffuse nephritis. There was no evidence of neoplasm, stone or infection.

Following nephrectomy there were ten days of relief from symptoms, then bleeding returned associated with the most severe attacks of pain yet experienced. The character of the pain, however, had changed to a typical left ureteral colic and occurred with such frequency and severity that frequent hypodermics of morphine were necessary.

The patient came under my observation three weeks following the nephrectomy. There was then profuse hematuria and pain and tenderness along the course of the left ureter.

General examination was negative. The urine showed macroscopic blood but was otherwise normal. The hemoglobin estimation was 75 per cent (Dare).

Cystoscopy. The urethra easily admitted a no. 24 instrument without pain or obstruction. The bladder tolerance was good, its capacity being 15 ounces. One bladder washing was necessary to get a clear medium. A blood clot was seen protruding from the left ureteral orifice, the right ureteral efflux was normal. Excepting for blood stained vesical mucosa about the left ureteral orifice, the bladder was normal. The blood clot of the left orifice was washed away and close inspection of this region revealed no new growth. A no. 6 skiagraphic ureteral catheter was passed up the left ureter for 23 cm. before obstruc-

tion was met. Bright red blood dropped steadily from the ureteral catheter. An ureterogram demonstrated that the ureter extended about 4 cm. above the obstruction. There were no tortousity, dilation or filling defect of the ureter; neither were there any shadows suggesting calculus.

These findings strongly indicating ureteral neoplasm, complete ureterectomy was advised and accepted.

The ureter was readily exposed extraperitoneally by extending the previous nephrectomy incision. The ureter was ligated and removed near its entrance into the bladder wall. It did not appear indicated to remove the intramural and intravesical portions of the ureter as there was no evidence in this instance of tumor involvement here although they are the most frequent sites of tumor growth.

Surgical specimen. (4 cm. of the ureter were removed with the kidney at the first operation).

The section of the ureter removed measured 24 cm. Upon opening the ureter longitudinally for its entire length it is seen that the mucosa is blood stained throughout its course. Four centimeters from its upper end there is a distinct papillary growth (fig. 1) which consists of one long villus 3 cm. in length and many rather indistinct shorter ones springing from the base of the long growth. The adjacent mucosa, to which the pedicle is attached, is normal in appearance and consistence. A second small sessile growth is present about 1 cm. below it.

The ureteral musculature is very much thickened throughout; otherwise there are no further appreciable changes.

Microscopic examination of tissue. The mucosa of the ureteral wall is directly continuous with that of the neoplasm (fig. 2) and at no point is there any malignant tendency. The mucosa of the tumors consists of several layers, the most superficial of which is made up of flattened cells; as the basement membrane is approached the cells become columnar just as is seen in the normal ureter. The submucous tissue of the ureteral wall is connected directly with the framework of the tumor. This tissue carries large blood sinuses both in the ureteral wall and tumor. There is a considerable inflammatory reaction of all of the tissues examined microscopically, as evidenced by polymorphonuclear and round cell infiltration which extends superficially into the ureteral musculature.

After history. The convalescence was short and the clinical result striking. The bleeding stopped at once and eight months after the second operation there had been no symptoms referable to the urinary tract.

A complete search of the literature has been made and but 16 instances of benign epithelial new growths of the ureter were found. A table presenting the essential features of all the reported cases is shown (table 1).

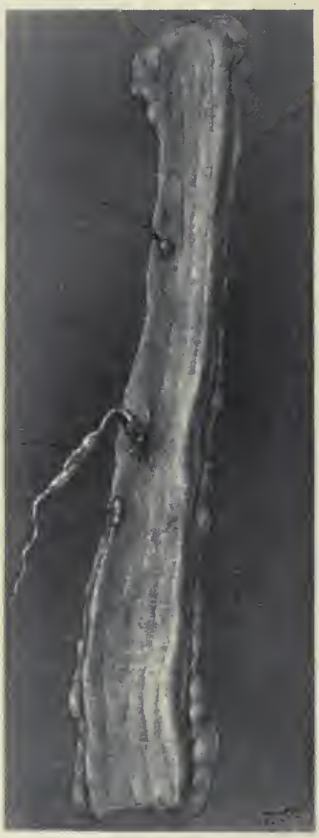

FIG. 1. INNER ASPECT OF OPENED URETER

Arrows pointing to the three small papillary neoplasms

As in neoplasms elsewhere there is no apparent etiology in most instances; however, of the 16 collected instances 3 presented stone intimately associated with the neoplasm, 1 had partial ureteral duplication, 1 ureteral diverticulum at the site of the tumor and 1 had a duodenal renal pelvic fistula presumably

caused by stone. It is seen that just one-third of these presented cases have other local lesions which may be etiological factors. Again the neoplasm may be the primary cause of some of these acquired conditions.

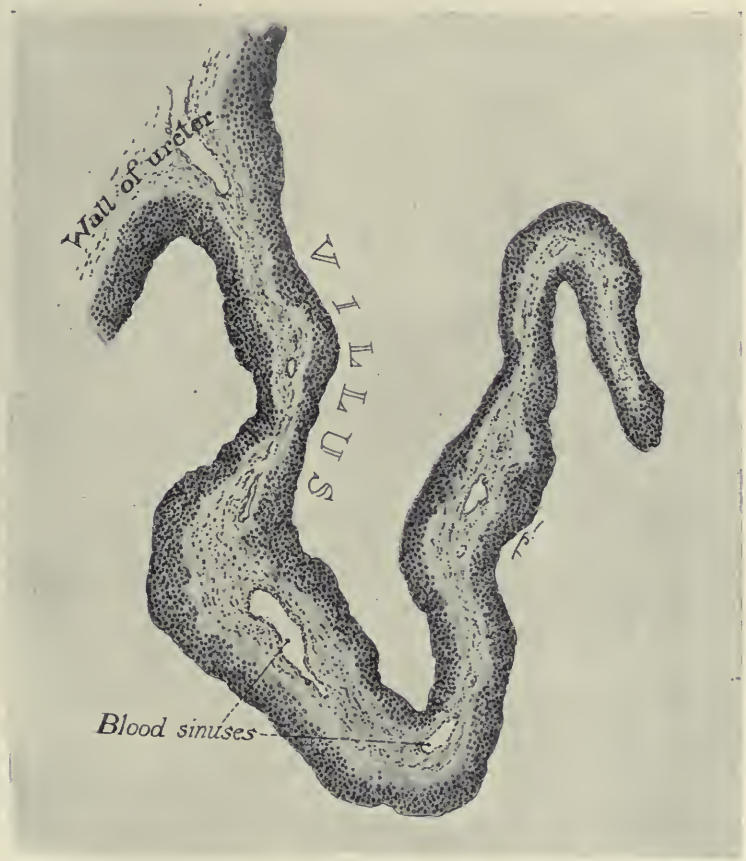

FIG. 2. PARTLY RECONSTRUCTED DRAWING OF A LOW POWER MICROSCOPIC APPEARANCE OF THE LARGEST GROWTH, EMPHASIZING THE RELATION OF ITS VARIOUS TISSUES TO THOSE OF THE URETERAL WALL

The histological structures of these growths is similar to epithelial neoplasms elsewhere along the urinary tract and are multiple in a great majority of cases. As in bladder papillomas the tumor itself may be benign with malignant qualities at the

TABLE 1

An outline of the main features of all the cases of ureteral papilloma reported

REPORTED CASES	SEX	AGE	SYMPTOMS			COEXISTING DISEASE	HOW DIAGNOSED		REMARKS
			Hematuria	Pain	Tumor		Clinically	Autopsy	
Rayer, 1840.....	M		+		+	Ureteral stone	Operation		
Thornton, 1885....	F	32		+	+	Ureteral stone	Operation		
Neelsen, 1888.....	M	57				Partial duplication right ureter		+	Tumor at bifurcation of ureter
Jeben, 1894.....	F	42			+	Pyothorax		+	
Jona, 1894	M					Pneumonia		+	Tumor at edge of ureteral diverticulum
Kaufmann, 1896...	M					Duodeno-pelvic fistula	Operation		
Poll, 1899.....	M	41	+	+	+			+	
Heresco, 1901.....	M	32		+			Cystoscopic		
LaDentu, 1899....	M	33		+	+	Ureteral stone	Operation		
Lancereaux.....	F	64		+	+			+	
Muzio, 1903.....					+			+	
Mackenroth, 1903.					+		Cystoscopically and operation		Ureteral transplant, recovery
Brunet, 1907.....	F	60	+				Cystoscopically and at operation		Ureteral transplant, recovery
Suter, 1910.....	M	56	+				Operation		
Praetorius, 1914...	F	67	+		+		Operation		
Spiess, 1915.....		55	+				Operation		

base, also apparently benign growths of the ureter have resulted in malignant recurrences and metastases as seen in Battles and Pantaloni cases.

The kidney tissue often shows sclerosis and sometimes diffuse nephritis, which lesion was present in my case. These renal lesions occur in both septic and aseptic instances due, it is believed to the elimination of toxins produced by the tumor itself.

Ureteral papilloma have a tendency to be located in one of three manners, (1) the entire ureter involved with the pelvis and bladder, also (2) pelvis and first few centimeters of the ureter involved, (3) lower part of pelvis and upper part of ureter and lower centimeters of ureter with intervening part being normal.

The case here presented belongs to the second class but has no renal pelvic involvement.

Of these 16 collected cases and the case here reported 14 were clinical and 3 were discovered at autopsy. The clinical cases presented essentially three symptoms; tumor, hematuria and pain. Nine of the 17 instances or 53 per cent had definite tumor mass and in 2 cases this was the first sign and the only complaint. Hematuria was present in 7, or 41 per cent of the cases and in 2 cases this was the only complaint. The hematuria is spontaneous, of variable duration and uniformly colors the urine. The bleeding is modified by rest, it is frequently painless but it is not rare to have it accompanied by ureteral collic. In 6 of the 17 clinical cases this occurred when the bleeding was profuse. Pain was present in but 6, or 35 per cent of the cases, and it was the only complaint in 1 case.

The tumor in all instances is that produced by hydronephrosis or hematonephrosis and not due to the presence of the neoplasm itself. The presence of hematonephrosis is considered by some to be of considerable diagnostic importance.

The pain is of two types, the most common type is that associated with intrapelvic pressure and is therefore confined to the region of the kidney and does not radiate. Such was the first pain experienced by my case. The second type of pain is colicky in nature, radiates down the course of the ureter and is caused

by the passage of blood clots; a condition which occurred in my case following nephrectomy.

But two of the clinical cases collected complained of other urinary symptoms and in both instances it was frequency of of urination due to a superimposed urinary infection.

The diagnosis of renal tumor or stone is usually made. It is possible in most instances to rule out renal or ureteral stone, but there is no available method to make an absolute differentiation between renal and ureteral tumor unless there is evidence of ureteral obstruction as determined by the ureteral catheter. Even here the diagnosis may be incomplete as tumor obstruction in the ureter is frequently associated with renal pelvic involvement which may be primary.

Two cases were diagnosed as ureteral tumor by cystoscopy; those of Heresco and Mackenroths but in both instances the tumor mass could be seen protruding from the ureteral orifice. In Brunet's case the bladder was opened to treat a papilloma about the ureteral orifice. Palpation of the lower part of the associated ureter suggested its involvement. In all the other 12 clinical cases the diagnosis was either made at secondary operation which occurred five times or after death either following or during the primary operation. The primary operation was nephrotomy four times and nephrectomy once. Suter's case of primary nephrotomy followed by nephrectomy had to have a third operation of ureterectomy to cure.

As the tendency for all of the neoplasms is to become malignant it is obviously indicated to do as complete a removal as possible. If the tumor is near the bladder, ureteral resection and transplantation can be done as was done in Mackenroth's and Brunet's cases. This method, while apparently successful in these two instances may be very incomplete and call for a second operation of ureterectomy and nephrectomy for tumors above the point of resection. Considering the seriousness of the latter procedure it would appear justifiable to do early ureteral resection and transplantation for ureteral neoplasms near the bladder.

For all tumors above this point—complete nephrectomy and ureterectomy is indicated unless there are serious contraindications.

REFERENCES

Spiess, P.: Centralbl. fur All. Path., 1915, xxvi, 553. Die Primar Epithelialen Tumoren des Nerenbeckens und des Ureters.

Rayer, R.: Traite des Malades des reines, Paris, 1840, Bd. 3, 699.

Thornton: Trans. Path. Soc. of London, 1885, p. 269.

Neelson: Beitrag. z. Path. Anat. w. z. Allg. Path. Zeigler's, 1888, iii, 279.

Jebens, R.: These de Strasbourg, 1894, Concerning Papillary Tumors of Ureter.

Jona, G.: Centfalbl fur Path., 1894, p. 659. Beitrag zu den primaren Tumoren des Ureters.

Poll, B.: Brun's Beitrage fur Chirurgie, 1899, p. 822.

La Dentu: Papillomes de l'ureters. Annales des Malades des Organes Genito urinates, 1899, Bd. 17, 955.

Heresco, P.: Annales des Malades des Organes Genitourinaire, 1901, Bd. 19, p. 656.

Lancereaux: Dictionaire Dechambra, Article Rein 3^{e} Series, iii, 247.

Muzio, G.: Un Caso di Papilloma Multiple Primitivi Dell Uretere. Giornale della R. Accademia diMedicina de Torino, 1903, ix, 193.

Brunet, G.: Uber Zottengeschwulste des Ureters. Gyanekologische Rundschau, 1907, i, 365.

Mackenroth: Demonstration of an Ureteral Papilloma. Centralbl. f. Gyn., 1903, Bd. 27, No. 11, S. 872.

Suter: Beiicht uber einem Fall von Solitarem Ureterpapilla. Correspondenz Blatt fur Sweitz Aertze, 1910, xl, 654.

Kaufman, E.: Lehrbuch d. spez .Patholog. Anatomie, 1909, 6 Auflage, S. 847.

Praetorius: Zeitshcuft fur Urologie, 1914, 415.

Rundle, Henry: Epithelioma of Ureter Causing Hydronephrosio. Trans. Path. Soc. of London, 1896, 47–128.

Stoerk, Oscar: Case of Papilloma of Bladder. Ureter and Renal Pelvis. Beitrag. Path. u. z. Allg Path. (Zeiglers), 1892, 26–367.

Kischensky, D. P.: Primary squamous epithelial tumor of renal calyx with metaplasia of aclyces, pelves and ureters. Beitrag. z. Path. Anat. u. z. Allg. Path. (Zeiglers), 1901, 30–248.

Albarren: Primary neoplasms of the pelvis of the kidney and ureters-mesodermic neoplasma. Annales des Malades des Organes Genito Urinaire, 1900, 18–1129.

Pantaloni: Archives Provinciales Chirrorgie, 1889, p. 2.

Battle: Trans. Clin. Soc., London, 1895, p. 233.

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THE OCCURRENCE OF HEMOLYTIC STREPTOCOCCI ABOUT THE TEETH

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CONTENTS

I. INTRODUCTION

Various investigators have shown that hemolytic streptococci occur in the normal as well as in the pathological oral cavity and upper respiratory tract. The object of the research recorded here was to determine more specifically the frequency in occurrence of various hemolytic streptococci about the teeth and gums in apparently normal, and also in pathological, cases.

Literature

Davis (4, 6) has shown that hemolytic streptococci occur in the crypts of the tonsils practically constantly, and upon the surface of 60 per cent of all tonsils. Maclay (20) found, in two hundred and sixty-eight cases, fourteen strains of hemolytic streptococci in the crypts of tonsils. His examinations show a marked seasonal variation, streptococci occurring less frequently in the summer months than in the winter. Voight (27) found that 30 per cent of all cases of severe infection of the middle ear, ethmoids, and mastoids, are due to hemolytic streptococci and that these organisms occur in 80 per cent of the number of removed tonsils. He has isolated hemolytic streptococci from the urine of patients suffering from acute

tonsillitis. Nichols and Bryan (21) have shown that a high percentage of their tonsil infections is due to these streptococci, and confirm Davis' (5) observations that streptococci may occur in great numbers in the crypts of tonsils, even when surface smears fail to reveal the organisms. Henrici and Hartzell (13) believe that mouth streptococci are the true parasitic organisms of the mouth, because these are the only organisms that can live on the clean mucous membrane before eruption of the teeth. They observed that there is a relative decrease, but an absolute increase, of these streptococci in conditions of oral uncleanliness and dental decay. Henrici and Hartzell also believe that the organisms in question are the cause of dental caries, pulpitis, and pyorrhea alveolaris for the following reasons: (1) Mouth streptococci are the only organisms in these lesions that are pathogenic, as exhibited by their local invasive power, by their ability to invade the blood and lymph streams, and by their tendency to produce metastatic infection. (2) They are the only organisms found in the deep advancing borders of infected tissue. (3) In metastatic abscesses, and in experimental inoculation of animals, these organisms produce subacute and granulomatous lesions similar to the inflammatory reaction found in pyorrhea and chronic apical abscesses. (4) The true mouth streptococcus (Strep. salivarius), which, according to Holman's classification, is of the non-hemolytic variety, invades the dental pulp and peridental tissue. In a later work, Henrici and Hartzell (12) have frequently found hemolytic and non-hemolytic streptococci in dental pulps in cases of both dental caries and pyorrhea.

That streptococci are exciting factors in the etiology of many mouth lesions, and that in the oral cavity they occur as active foci of infection responsible for many systemic conditions, has been further established by Rosenow (23), Babcock (1), Lescohier (19), Eldridge (9), Earl (8), Grieves (11), Potter (22), and others. Rosenow, in his extensive studies on elective localization, lays much stress upon the importance of anaerobic cultures in maintaining the elective-localization property of the bacteria.

European workers differ from the majority of American investigators as to the exciting cause of pyorrhea alveolaris. Euler (10) believes pyorrhea alveolaris is a spirochetosis, caused by the "spiro-

cheta pyorrhica" and influenced in some way by B. fusiformis, which is normally present in the mouth, but is not pathogenic unless abundant. Intensive arsenical (salvarsan) treatment with proper oral hygiene often induces beneficial results. Dufourmentel and Frisson (7) reported three cases of mouth injuries resulting in (a) hypertoxic cellulitis of the neck, (b) phlebitis of systemic cranial facial veins, and (c) septicemia with no anatomic change. They recovered a long anaerobic bacillus, similar to the B. bellonenses of Sacquepee, that produced fatal edematous septicemia in guinea pigs. They apparently did not find hemolytic streptococci in these cases.

From a review of the above mentioned literature, it is noted that no work has been done on the occurrence of hemolytic streptococci about the teeth and gums.

II. EXPERIMENTAL

Collection of material

Cultures were obtained from one hundred and forty-four dispensary and hospital patients in the following manner. At the time of collecting the material from these patients, the presence or absence of pyorrhea and gingivitis, the general condition of the teeth, and, wherever possible, the clinical diagnosis, were noted and recorded.

Wooden applicators were sharpened to a fine point and sterilized with steam. Five-millimeter glass tubing was cut into pieces fourteen centimeters long, and one end sealed. These tubes were then half-filled with ordinary dextrose broth, the wooden applicators inserted and the tubes sealed with cotton plugs. These tubes were then sterilized twice in an autoclave.

The sharpened end of the wooden applicator was then inserted into the space between the tooth and the adjacent free margin of the gum. Here one can, in nearly all cases, obtain a large amount of septic debris that clings to the end of the sharpened point of the applicator. The gums were in no case injured in obtaining such material, as the fine pointed end of the wooden applicator was softened by the broth. For this reason it could be inserted quite deeply into the dento-gingival space; and its use is, therefore, considered more practicable than that of a platinum needle which, when similarly employed, invariably produces pain and bleeding.

After the wooden point had been deeply inserted between the gum and tooth, it was replaced in the glass container half-filled with nutrient broth, and the tube incubated at 37°C. for eighteen hours. Then sub-cultures were made from the broth.

Method of culturing

Serum broth and blood-agar plates were inoculated from the initial broth cultures. Only colonies with hemolytic zones were examined, and from these the hemolytic streptococci were isolated and studied.

The growth of hemolytic streptococci in no instance was very abundant; often there were only two or three colonies upon the plates where a loopful of the initial broth culture was made (18-hr.). Thus, the cultures differ markedly from those made with material from the crypts of tonsils, which in most cases gives an almost pure culture of hemolytic streptococci and often in large numbers.

Of one hundred and forty-four patients examined, twenty-eight (19.4 per cent) were positive for hemolytic streptococci. Twenty-nine strains were isolated, two distinctly different strains having been obtained in one of these patients. One strain fermented mannite but neither lactose nor salicin, while the other fermented lactose but neither mannite nor salicin.

Initial smears were made and studied for the first fifty cases, but this method of examination proved unreliable and unsatisfactory, and hence was discontinued.

Morphology

The twenty-nine strains obtained from one hundred and forty-four patients were isolated in pure culture and studied. After the blood-agar plates had been incubated at 37°C. for twenty-four hours, the colonies of hemolytic streptococci were removed with a wire loop, and blood-agar slants and tubes of serum broth inoculated. Smears were made from the 24-hour serum-broth cultures, and the morphology of the organisms observed. The organisms are round cocci about one micron or less in diameter, arranged in chain formation, and vary slightly in size. Often they occur singly. Frequently the cocci

appear pressed together, especially in blood-agar cultures. Lance-shaped cocci have been seen. These are smaller than the typical pneumococci and without a definite capsule. All strains stain well with the ordinary dyes and are Gram-positive.

Classification by fermentation tests

The twenty-nine strains of hemolytic streptococci (representing twenty-eight positive cases) were classified according to the fermentation reactions advocated by Holman (14). After inoculating infusion broth with B. coli, it was incubated for forty-eight hours, sterilized on two alternate days in an autoclave, and then filtered through cotton. To separate portions of this sugar-free broth, lactose, mannite, and salicin were added, respectively. This broth was then tubed, litmus added and the contents sterilized. Tubes of each kind were inoculated with each of the twenty-nine strains of the organisms and 1 cc. of blood serum added to each.

There were seventeen strains of Streptococcus anginosus. This organism hemolyses blood and ferments lactose, but does not ferment mannite, salicin or inulin. Holman found this organism prevalent in nose and throat infections, especially in scarlatinal and measles throats. The organism has also been found in endocarditis.

Five strains of Streptococcus subacidus were isolated. These are hemolytic but do not ferment any of the carbohydrates mentioned. These, too, are often found in the throat; also in joint and mastoid infections.

Streptococcus hemolyticus-I was present in three cases. This streptococcus induces hemolysis, and ferments lactose and mannite, but not salicin. It is a comparatively rare strain.

There were two strains of Streptococcus pyogenes. This organism hemolyses blood and ferments lactose and salicin. This strain of hemolytic streptococcus is also frequently associated with throat infections. It is the common hemolytic streptococcus found in pyogenic conditions where the streptococcus is the exciting etiological factor.

Streptococcus hemolyticus-II was not found. Streptococcus hemolyticus-III was found in one instance; it is a hemolytic streptococcus

that ferments only mannite. Holman found it in empyema and in the blood of puerperal sepsis.

One strain of Streptococcus infrequens was found. It is a hemolytic streptococcus that ferments lactose, mannite, and salicin. This is also a comparatively rare organism and is usually found in throat conditions. Ruediger (25) isolated it from otitis media, pericardial fluid, suppurative adenitis and conjunctivitis. Holman describes a case of chronic arthritis where this streptococcus was harbored in the tonsils and, after removal of the tonsils, the symptoms improved.

The hemolytic Streptococcus equi was not found among the twenty-nine strains.

Virulence

Generally, the virulence of hemolytic streptococci varies considerably. Passage through animals increases the virulence, while continued growth on artificial media reduces the virulence. The cell substance possesses little toxicity (16). The cells yield a streptolysin that hemolyses blood cells—a true toxin containing a haptophore and a toxophore group, and which, upon injection, produces specific antibodies (24, 2). This hemolytic substance, however, is not the dangerous endotoxin of the streptococci. In 1918, Clark and Felton (3) produced a filterable, non-hemolytic, toxic product from hemolytic streptococci which killed rabbits in doses of 0.5 cc. per 1000 grams of body-weight of the rabbit. This work has not been confirmed.

In my own tests, eleven rabbits were each injected intravenously with 2 cc. of 24-hour serum-broth cultures of the hemolytic streptococci. These were selected at random. Of the eleven strains injected, two proved to be fatal. One of the rabbits died four days after inoculation. The lung was found hemorrhagic; otherwise there was no anatomical change. The organisms were not recovered from the body fluids, a result that is believed to be due to faulty incubation. A second rabbit died ten days after injection of the organisms. There were no swollen joints; the lung was hemorrhagic and the organism was recovered from the heart blood and bile, but not from any joint. Nine of the rabbits remained alive, showing no ill effects with one exception. This one, injected with a strain of Streptococcus pyogenes, had distinctly swollen and painful joints.

Thus, only two strains were fatal to the rabbits. The pathogenicity of hemolytic streptococcus is variable for different animals. Rabbits and mice as a rule are quite susceptible. It is known, however, that strains of streptococcus after being passed through one animal, thus increasing the virulence, may not be so highly virulent for another species (17). Some varieties of hemolytic streptococcus (those often found in mastitis in cows) may be low in virulence for rabbits, although the organisms themselves are markedly hemolytic (15). Animal tests may not, therefore, be a reliable index of the virulence or pathogenicity for man.

III. DISCUSSION

It has frequently been shown that hemolytic streptococci occur in various parts of the body, e.g., upon the tonsils, in the crypts of the tonsils (4), on the hairy parts of the bodies of filthy individuals (26), in the ethmoid and mastoid cells (27), in carious teeth and apical abscess (12, 13), in the appendix (18), and in various other localities without producing acute clinical manifestations. In other words, these localities may be considered as frequent habitats for hemolytic streptococci.

Because of the fact that the space between the gingivae and the teeth is anatomically suitable for the collection of debris, and in the light of the above experimental data, it is reasonable to suppose that the gingival space may occasionally act as a focus of infection. In the one hundred and forty-four cases examined, fewer hemolytic streptococci were found about the teeth and gums to which a toothbrush had been recently applied. The majority of these cases were dispensary and Cook County Hospital patients, most of whom were grossly negligent concerning oral hygiene. Henrici and Hartzell believe, as was previously mentioned, that the "mouth" streptococci produce, in metastatic infections and in experimentally inoculated animals, subacute and granulomatous lesions similar to the inflammatory reactions seen in pyorrhea and chronic apical abscesses. This may account for the fact that the strains of streptococci injected into the series of eleven rabbits referred to above were fatal in only two cases. The virulence of the twenty-nine strains of hemolytic strepto-

cocci recorded here does not appear to be as high as that of the hemolytic streptococci harbored in the crypts of tonsils, in the adenoids, and upon the pharyngeal mucous membrane. The latter are often highly virulent organisms. The possibility that some of the organisms from the crypts of the tonsils, or from the pharyngeal mucous membrane, ordinarily are dislodged and subsequently find their way to the gingival spaces, to reside there merely as transients, must, of course, be considered. If this were true, the decreased virulence might be explained.

In the one hundred and forty-four patients examined, nineteen, or 13.1 per cent, showed distinct clinical manifestations of pyorrhea. Of these, seven, or 36.8 per cent, gave positive cultures for hemolytic streptococci. Three were of the subacidus, and four were of the anginosus, varieties. Many of these individuals had other clinical manifestations, but it should be remembered that the majority were hospital cases.

Because of the great invasive power of the hemolytic streptococci, it is highly probable that they can penetrate not only the fibrous gingivae, but also the alveolar periosteum. Here they may be taken up by the numerous lymph vessels and thence carried into the blood stream, producing metastatic lesions elsewhere. There is certainly a possibility that from this favorable habitat, in deep dento-gingival spaces, the various hemolytic streptococci may act as secondary and terminal invaders.

IV. SUMMARY OF CONCLUSIONS

The method of obtaining material for examination from gingival spaces, as described, is advantageous because it is a clean, painless procedure, and does not cause hemorrhage.

The data presented show that 19.4 per cent of a series of one hundred and forty-four patients gave positive cultures of hemolytic streptococci in material taken from dento-gingival spaces.

Of the patients with well-defined clinical manifestations of pyorrhea, 31.1 per cent gave positive cultures of hemolytic streptococci. Of the latter, three strains were of the S. subacidus, and four strains were of the S. anginosus, groups.

Hemolytic streptococci were found between the teeth and gums in three out of fifteen cases of gingivitis.

Of the various isolated strains of hemolytic streptococci, seventeen were Streptococcus anginosus; five, Streptococcus subacidus; three, S. hemolyticus-I; two, S. pyogenes; one, S. infrequens; and one, S. hemolyticus-III.

Of eleven strains, selected at random from twenty-nine strains of hemolytic streptococci isolated from gingival spaces, two proved to be fatal to rabbits in doses of 2 cc. of a broth culture. Both strains were of the anginosus type.

Because of the occurrence of hemolytic streptococci in deep dento-gingival spaces, these spaces may be considered to be potentially dangerous foci of infection.

There is a possibility that these organisms may, proceeding from the above mentioned site, act as secondary and terminal invaders.

The fact that hemolytic streptococci were found in only a small proportion of the number of cases of pyorrhea examined, and in these not in great numbers, indicates that hemolytic streptococcus is not the primary etiological factor in this condition.

V. REFERENCES TO LITERATURE

(1) Babcock, R. H. 1916 Focal mouth infections, their systemic effects and treatment. *New York Medical Journal*, civ, p. 1084.

(2) Besredka, A. 1901 De l'hemolysine streptococcique. *Annals de l'Institute Pasteur*, xv, p. 880.

(3) Clark, A. H. and Felton, L. D. 1918 A filtrable toxic product of hemolytic streptococcus. *Journal of the American Medical Association*, lxxi, p. 1048.

(4) Davis, D. J. 1912 Bacteriology and pathology of the tonsils, with especial reference to chronic articular, renal, and cardiac lesions. *Journal of Infectious Diseases*, x, p. 118.

(5) Davis, D. J. 1919 Hemolytic streptococci with special reference to their rôle in acute respiratory infections. *Journal of the American Medical Association*, lxxii, p. 319.

(6) Davis, D. J., and Pilot, I. 1919 Hemolytic streptococci in the faucal tonsil and their significance as secondary invaders. *Journal of Infectious Diseases*, xxiv, p. 386.

(7) Dufourmentel, L., and Frisson, L. 1918 Septicème d'origine bucco-dentaire. *Presse Medicale*, xxvi, p. 415.

(8) Earl, George 1919 Periapical abscesses and focal infections. *Lancet* (n. s.), xxxix, p. 79.

(9) Eldridge, Watson W. 1919 The teeth as foci of systemic infection. *Dental Digest*, xxv, p. 12.
(10) Euler, H. 1919 Salvarsan bei Alveolarpyorrhöe. *Therapeutische Monatschrift*, xxxiii, p. 133.
(11) Grieves, Clarence J. 1919 Ulcero-membranous gingivitis: the gingival phase of Vincent's disease. *Dental Cosmos*, lxi, p. 819.
(12) Hartzell, T. B., and Henrici, A. T. 1917 The pathogenicity of mouth streptococci and their rôle in the etiology of dental disease. *Journal of the National Dental Association*, iv, p. 477.
(13) Henrici, A. T., and Hartzell, T. B. 1919 The bacteriology of vital pulps. *Journal of Dental Research*, i, p. 419.
(14) Holman, W. L. 1916 The classification of streptococci. *Journal of Medical Research*, xxxiv, p. 377.
(15) Jones, F. S. 1918 Relation of hemolytic streptococci to udder infections. *Journal of Experimental Medicine*, xxviii, p. 253.
(16) Jordan, Edwin O. 1916 General bacteriology. W. B. Saunders Co., Philadelphia, Pa.
(17) Knorr. 1893 Ueber den Immunisirrungswerth und Heilwerth des Tetannscheil bei weissen Mäusen serums. *Zeitschrift für Hygiene*, xiii, p. 427. (Full name of author not available.)
(18) Kraft, A. 1921 Hemolytic streptococci of appendix vermiformis. *Journal of Infectious Diseases*, xxviii p. 122.
(19) Lescohier, A. W. 1917 Bacterial findings and their relationship to pyorrhea alveolaris and interstitial gingivitis. *Journal of the American Medical Association*, lxviii, p. 414.
(20) Maclay, Otis H. 1918 Bacteriology of tonsil crypts. *Laryngoscope*, xxviii, p. 603.
(21) Nichols, H. J., and Bryan, J. H. 1918 The tonsils as foci of infection in streptococcus hemolyticus carriers. *Journal of the American Medical Association;* lxxi, p. 1813.
(22) Potter, N. B. 1917 The vicious circle in oral sepsis. *New York Medical Journal*, cv, p. 243.
(23) Rosenow, E. C. 1919 Studies on elective localization. *Journal of Dental Research*, i, p. 205; also *Journal of the National Dental Association*, vi, p. 983.
(24) Ruediger, Gustav F. 1903 The production and nature of streptocolysin. *Journal of the American Medical Association*, xli, p. 962.
(25) Ruediger, Gustav F. 1906 The cause of green coloration of bacterial colonies in blood agar plates. *Journal of Infectious Diseases*, iii, p. 663.
(26) Shachter, J. A. 1918 Hemolytic streptococci on body surfaces. *Transactions of the Chicago Pathological Society*, x, p. 301.
(27) Voight, W. C. R. 1917 The bacteriology of focal infection. *Journal of Ophthalmology, Otology and Laryngology*, xxiii, p. 87.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, MAY 24

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CONTAMINATION OF CADAVERS BY SACCHAROMYCES CEREVISIAE

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TWO FIGURES

Recently the cadavers in the anatomical laboratories of the University of Illinois, College of Medicine, became covered by a moist, slimy, slightly elevated growth that has caused no small amount of trouble and annoyance. The growth is dirty gray in color, loosely adherent, and does not penetrate the deeper tissues. It has never been noticed upon the unbroken skin of the cadaver; when the skin is removed, however, the growth begins and spreads with great rapidity, making dissection of the specimen out of the question and causing great waste of material.

A quantity of this grayish substance was taken to the bacteriological laboratory for examination. Smears showed a large number of highly refractive, ovoid cells, measuring about 7μ in diameter. In addition to these, there were large numbers of bacteria, especially staphylococci.

It seemed plain that the slimy growth was largely made up of the above-mentioned ovoid cells, and cultures were therefore made in order to isolate and study them in detail.

After several attempts, pure cultures of the organism in question were obtained.

CULTURAL CHARACTERISTICS

Neutral plain agar. After twenty-four hours' incubation at 37°C. small, round, bluish-gray colonies, about the size of a pinhead were seen. Their margins were smooth and regular. After an additional twenty-four hours' incubation at room temperature

these colonies turned white in color, but did not increase in size or number.

Five per cent dextrose agar. Twenty-four-hour culures showed a growth similar to that on plain agar. After another twenty-four hours at room temperature they were much larger and creamy white in color, becoming confluent in most cases so as to cover the entire surface of the media. The characteristic odor of 'yeast' was noticed.

Plain broth. The growth in plain broth was not profuse. There was a slight flocculent sediment at the end of twenty-four hours. The broth was slightly turbid.

Five per cent dextrose broth. The growth was similar to that in plain broth, but more pronounced; a heavy sediment and the characteristic odor of yeast.

Litmus milk. A marked acid production at the end of forty-eight hours with coagulation; the curd in most cases being completely digested, leaving a whitish turbid whey.

Gelatin stabs. Gelatin-stab cultures showed only a slight growth upon the surface, resembling that on plain agar. No liquefaction.

The organism ferments glucose with the formation of carbon dioxide and alcohol.

STAINING PROPERTIES

The organism stains fairly well with the ordinary dyes and exceptionally well by the Gram method, being strongly Gram-positive (figs. 1 and 2). When stained by Wright's stain, a well-defined blue cell membrane is seen with pale blue mitochondria and numerous vacuoles within.

MORPHOLOGY

The organisms average about 7 μ in diameter and are round to ovoid in form. In a hanging-drop preparation of a forty-eight-hour culture, a highly refractive, non-motile, double-contoured cell is seen in an active state of budding. The budding generally takes place from the long end of the ovoid cells. The younger

cells are small and more rounded in form, while the older cells, from which the budding takes place, are more elongated. There is no tendency to form mycelia.

A pure known culture of Saccharomyces cerevisiae was compared with the organism taken from the cadaver, and it was found that in every way the two resembled each other in morphology, staining properties, and in general cultural characteristics.

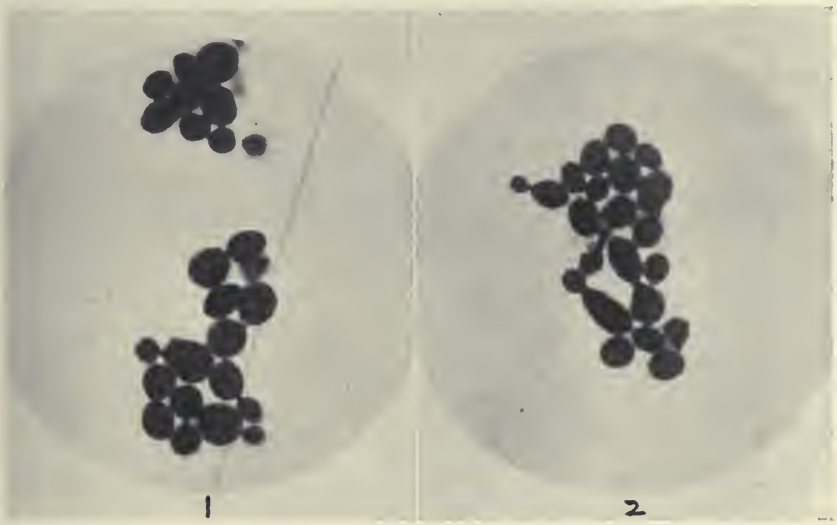

Fig. 1 Strain 'A.' Saccharomyces cerevisiae from cadaver. Gram's stain (×1200).

Fig. 2 Strain 'B.' Known pure culture of Saccharomyces cerevisiae. Gram's stain (×1200).

ANIMAL EXPERIMENTS

White mice, after being inoculated with rather large doses of a normal salt suspension of the organism, showed no ill effects.

An effort was made to reproduce the growth upon animals. Two dead rabbits, with the skin and viscera removed, were immersed in the embalming fluid used for the preparation of the bodies in the anatomical laboratories. This embalming fluid consists of—

Glycerin	300 cc.
Formalin	400 cc.
Alcohol	1000 cc.
Phenol	45 grams
Water	400 cc.

After a period of one week they were removed and a pure culture of the cadaver organism planted upon one and a pure known culture of Saccharomyces cerevisiae planted upon the other. At the end of three days the entire bodies of the two rabbits were similarly covered with a slimy, grayish film. Two days later this growth became a dirty, creamy white and resembled that found upon the cadavers. Thus, it is further evident that the two organisms are alike.

THERMAL DEATH POINT

A series of small test-tubes, each containing 2 cc. of a suspension of the cadaver culture (strain 'A') and a known strain of Saccharomyces cerevisiae (strain 'B') were used. At the different degrees of temperature indicated in the table, tubes of each of the two organisms were placed in a water-bath for a period of ten minutes, allowing one minute for the temperature of the tubes to reach that of the water-bath. The tubes were then removed and 5 per cent dextrose-agar slants inoculated and incubated. The results are given in the table. Both organisms were killed at 58°C. for ten minutes, but not at 56°C. for ten minutes.

Because of the apparent identity of the cultural characteristics and staining properties, as well as the results of the animal experiments with the organisms, it is further evident that the contamination of the cadavers is a strain of Saccharomyces cerevisiae.

I have been able to find nothing in the literature concerning the contamination of cadavers by Saccharomyces cerevisiae. In a personal communication from Dr. Irving Hardesty, of Tulane University, he states that he has had a similar experience with 'molds,' that the mold thrives on formalin-hardened bodies, that alcohol favors its growth, and that carbolic acid will not check it unless the bodies are completely immersed in the carbolic solution.

In order to find some disinfectant for this organism that might be effective in embalming fluids, the following experiments were performed:

The carbolic coefficients for potassium chromate, formalin, and mercuric bichloride were determined according to the method advocated by the U. S. P. H. S. (Hygienic Laboratory Bulletin no. 82) and further described by M. J. Rosenau in his test on "Preventive Medicine and Hygiene." Instead, however, of finding the coefficient with the use of a twenty-four hour culture of typhoid bacillus, forty-eight hour cultures of the two strains of

TABLE 1

Thermal death point

TEMPERATURE (10-MINUTE EXPOSURE)	STRAIN 'A' GROWTH	STRAIN 'B' GROWTH
°C.		
48	Positive	Positive
50	Positive	Positive
52	Positive	Positive
56	Positive	Positive
58	Negative	Negative
62	Negative	Negative
64	Negative	Negative
68	Negative	Negative
70	Negative	Negative
72	Negative	Negative
74	Negative	Negative
78	Negative	Negative

Saccharomyces cerevisiae were used, because the yeast is in its most active state of budding at that time. It was found, by determining the carbolic coefficient, that phenol is the most efficient disinfectant for these yeasts. The action of mercuric bichloride toward these organisms is too inconstant for one to reach any definite conclusion as to its use. Formalin and potassium chromate have too low a coefficient to be of any value.

The prevention of this growth was now attempted by altering the composition of the embalming fluid previously used. A rabbit was embalmed with the following fluid:

Glycerin	300 cc.
Formalin	400 cc.
Alcohol	1000 cc.
Phenol	90 grams
Mercuric bichloride	90 grams
Water	400 cc.

It will be seen that this solution differs from the one previously mentioned in that the phenol is doubled and mercuric bichloride is added. The rabbit was immersed in the same solution for three days, seeded with cultures of both yeasts, and then covered with moist towels. At the end of four days there was no growth. It was considered inadvisable to include mercuric bichloride in the embalming fluid not only because of the extra expense, but because there is a granular coagulation of the blood in the small vessels. This firm, granular coagulum completely obstructs the smaller vessels, thus preventing the thorough penetration of the solution. Other rabbits, embalmed with the same fluid minus the mercuric bichloride, were seeded with both strains of the yeast and incubated for four days. These also showed no growth.

An examination was made of the dust taken from the floor, walls, and tables of the anatomical laboratory. Some of this dust was taken up by means of a sterile cotton swab and 5 per cent dextrose broth and agar inoculated and then incubated for twenty-four hours at room temperature. Many of the samples revealed Saccharomyces cerevisiae.

As a prophylactic measure, cloth was soaked with the following solution:

Glycerin	50 cc.
Phenol	2 grams
Alcohol	50 cc.
Water (q. s. ad)	1000 cc.

and was draped over one-half of the bodies in the laboratory (group A) at the end of each dissection for a period of four months. The other half of the cadavers (group B) served as a control. During these four months none of the bodies of group A was affected, while six of the bodies of group B became covered with the growth.

By applying the above solution upon the embalmed bodies, the specimens are not only protected from the yeast but the glycerin keeps the exposed muscles more soft and pliable.

CONCLUSIONS

Because of the apparent identity of the cultural characteristics, morphology, staining properties, and of the animal experiments mentioned, it is concluded that the organism in question is a saprophytic strain of Saccharomyces cerevisiae.

The growth of Saccharomyces cerevisiae upon anatomical specimens renders them useless, thereby causing great waste of material.

Phenol is the most efficient disinfectant for this particular strain of yeast.

The contamination can be prevented by using the embalming fluids and the prophylactic measures mentioned.

The use of mercuric bichloride in embalming fluids is not practical; first, because it forms a firm granular coagulum of blood in the vessels, thus preventing the complete penetration of the fluid, and, second, because of the expense of the chemical. The prophylactic measures indicated not only protect the cadavers from the Saccharomyces cerevisiae, but prevent rapid drying and hardening of the exposed muscles.

Hemolytic Streptococci of the Appendix Vermiformis

ADOLPH KRAFT

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HEMOLYTIC STREPTOCOCCI OF THE APPENDIX VERMIFORMIS

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The significance of hemolytic streptococci in the etiology of appendicitis is not well known. Osler [1] says that the Streptococcus pyogenes is present in a large number of cases. Rose and Carless [2] appear to agree with Osler in this respect. Rosenow and Dunlap [3] reported an epidemic of streptococcus appendicititis at Camp Culver, and according to their report the cause was a hemolytic streptococcus. Takaki [4] and Ungerman [5] reported the isolation of Streptococcus pyogenes from the vermiform appendix. Others have reported the occurrence of appendicitis due to streptococci, but it is not clear whether the organisms were hemolytic or nonhemolytic. In view of the foregoing facts, I undertook an investigation of this lesion with the object of determining the frequency with which hemolytic streptococci are found in the normal appendix and in acute and chronic appendicitis and, if possible, the rôle they play as a primary etiologic factor in appendicitis.

TECHNIC

In all, 175 appendixes were examined; the first 50 were used in a preliminary way in order to develop the technic. The appendixes were gathered from various clinics and I am indebted to Dr. Meyer and Dr. Stangl of Cook County Hospital, Chicago; Dr. Ochsner and Dr. Nuzum of Augustana Hospital, Chicago; and to others for material used. Immediately after removal and while still free from external contamination, the appendix was placed in sterile cheese cloth several layers thick, the whole wrapped in clean waxed paper and placed in the icebox. No appendix was used that had been in the icebox more than 24-36 hours; the gross examination, smears of contents and of the mucosa, and bacterial cultures of the mucosa and wall were made as soon as possible. The instruments used in dissecting the material, which was done on a sterile porcelain plate, were thoroughly sterilized before used. Cultures of the mucosa and wall were made by scraping the mucosa and muscular layers with a sterile knife, and using some of the finely divided tissue

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1 Principles and Practice of Medicine, 1918.
2 Manual of Surgery, 1919.
3 Jour. Infect. Dis., 1916, 18, p. 383.
4 Sei-i-Kwai, Med. Jour., 1915, 34, p. 21.
5 Centralbl. f. Bakteriol., 1909, 50. p. 513.

for broth and poured blood-agar plates. The plates and broth cultures were incubated for 24 hours at 37 C. and the colonies and growth in broth were then examined by methylene blue and Gram's stains. The plates were examined with special reference to hemolysis, and to the size, shape, color, elevation, pigmentation, moisture, predominance, and variety of any colonies present. Only those colonies that gave a distinct hemolysis about a small pinpoint grayish slightly elevated growth, and that on staining showed gram-positive cocci growing in chains or as diplococci, were tabulated as hemolytic streptococci. Although these tests are practically conclusive, later tests were also made and the organisms grouped in accordance with Holman's [6] scheme. All nonhemolytic streptococci producing a green halo were classed tentatively as Streptococcus viridans without further attempt at classification. After cultures and smears had been made, the specimen was again placed in the icebox so as to be available for reexamination should uncertain results appear.

In this series, 48 were normal and 77 pathologic. The criteria for this differentiation will be discussed later. Two strains of hemolytic streptococci were isolated from the 48 apparently normal appendixes; these were strains 18 and 54 and belong to the type Streptococcus infrequens (Holman). Four strains of hemolytic streptococci were found in the 77 pathologic appendixes, namely, strains 37, 39, 71 and 104, all belonging to the type Streptococcus infrequens, except strain 104, which belonged to the type Streptococcus hemolyticus II. From this it appears that hemolytic streptococci occurred in this series in normal appendixes in 4.17 % and in the pathologic in 5.2 %. No hemolytic streptococci were isolated from 25 appendixes presenting evidence of chronic inflammation. The instances of acute appendicitis yielding hemolytic streptococci presented either ulcerative or gangrenous appendixes.

Other findings were: 108 strains of nonhemolytic colon bacilli, of which 45 strains were found in 48 normal appendixes and 63 strains in 77 pathologic appendixes; 51 strains of hemolytic colon bacillus, of which 19 were found in 48 normal appendixes; 32 strains of Streptococcus viridans were isolated from 48 normal appendixes. Two probable pneumococcus strains, one case of pinworm and many large unidentified bacilli, which were, no doubt, nonpathogenic, were the other results.

In the normal appendix, hemolytic streptococci, when found, occurred only in small numbers. One loopful of the macerated mucosa and wall when added to blood agar, plated and incubated for 24 hours gave 6 to 10 colonies. Streptococci in chains of 4 to 12 were found

[6] Jour. Med. Res., 1916, 34, p. 377.

in smears from the walls and contents, but these no doubt were practically all viridans as indicated by the blood-agar plates. Leukocytes were seen only occasionally in the normal appendix. Hemolytic streptococci when isolated from pathologic appendixes were present in large numbers; furthermore, they were in almost pure culture. One loopful of the contents or macerated walls of the appendix when placed in 5 c c of blood agar, plated and incubated for 24 hours gave innumerable typical hemolytic colonies. The third dilution was usually necessary for the isolation of individual colonies. Smears from the contents and walls showed an almost pure culture of streptococci, which were gram-positive and in chains of 4 to 15. One case gave practically a pure culture of diplococci, which on growth in beef broth developed chains of 6 to 18. The smears, furthermore, showed an enormous amount of polymorphonuclear leukocytic infiltration; many pus cells and cells with ingested bacteria were present. Blood cells were present, too, but most of these were already disintegrated.

The hemolytic streptococci in this series were pathogenic for rabbits. The strains were incubated in 5 c c of plain beef broth at 37 C. for 18 to 24 hours; 3 c c were injected intravenously into the lateral vein of the ear of young healthy rabbits weighing 1,000 to 1,200 gm. Two rabbits were similarly injected with the sterile beef broth for controls. Strains 37, 39, 71 and 104 killed the rabbits in 48 to 72 hours. The organisms were recovered in pure cultures from the heart blood, 10 drops of the blood when plated giving 20 to 30 colonies. Strains 18 and 54 killed rabbits in 5 days when similarly injected. The organism was recovered from the grayish pus in the joints. No other gross lesions were noted.

As stated, of the 125 appendixes of which a record was kept, 77 were pathologic and 48 normal. This classification was made on the basis of gross appearances and clinical diagnosis. It is to be emphasized that at times it becomes extremely difficult to determine whether an appendix is normal or slightly pathologic. The statement has been made by pathologists and surgeons that in adults an absolutely normal appendix does not exist.

A word should be said in regard to the possible avenues by which hemolytic streptococci reach the appendix. As elsewhere, three routes are usually considered: contiguity, progression and hematogenous or lymphogenous channels. There was no periappendiceal involvement in any case in which the hemolytic streptococcus was found, and for

that reason, one may, with reasonable certainty, say that the mode of entrance was either by progression along the gastro-intestinal tract or hematogenous. Rosenow [7] and others [8] have laid stress on the hematogenous route, and name as the primary source the tonsils in a majority of the cases. Rosenow produced appendicitis experimentally by intravenous injection of streptococci and colon bacilli. A number of investigators have emphasized the selective action of certain bacteria for the appendix.

Hemolytic streptococci, as a rule, do not frequent the gastro-intestinal tract. The countless numbers of these bacteria that pass down the esophagus encounter their fate in the stomach because the ferments and acid there show a decided antagonistic action toward them. They may, however, enter the intestinal tract in a lump or mass of food. An achylia may permit them to pass into the bowel where a more favorable medium is afforded, although not the optimum. Davis [10] fed hemolytic streptococci to rabbits every day for a month and only occasionally recovered them in the stools. Holman [11] isolated 4 strains from feces; Oppenheim 5 strains from 15 stools; [12] and Broadhurst [13] 9 from 31 stools. This is of interest here because 5 out of the 6 strains isolated by me from normal and pathologic appendixes were of the same type.

In 1890 Kruse and Pasquale [14] found streptococci in large numbers in feces of patients with acute dysentery. They were probably nonhemolytic. Beck,[15] in 1892, isolated a streptococcus from the stools of cholera nostras and concluded that this organism was the causative agent, but he does not state whether the organism isolated by him was hemolytic or nonhemolytic. Lameris and Harrevelt [16] obtained streptococci from stools of patients suffering from diarrhea following the use of contaminated milk, but they were not pathogenic for animals.

SUMMARY AND CONCLUSIONS

Hemolytic streptococci were found in two of 48 normal appendixes (4.17 %) and in 4 of 77 pathologic appendixes (5.2 %).

[7] Jour. Infect. Dis., 1915, 16, p. 240.
[8] McCoy, Lancet-Clin., 1916, 116, p. 49.
[9] Pilot and Davis, Jour. Infect. Dis., 1919, 24, p. 386
[10] Davis, Jour. Am. Med. Assn., 1919, 72, p. 323.
[11] Jour. Am. Med. Assn., 1919, 72, p. 319.
[12] Jour. Med. Res., 1916, 34, p. 377.
[13] Jour. Infec. Dis., 1920, 26, p. 117.
[14] Jour. Infect. Dis., 1915, 17, p. 277; Ztschr. f. Hyg. u. Infektionskr., 1893, 16, p. 1.
[15] Centralbl. f. Bakteriol., 1892, 12, p. 632.
[16] Ztschr. f. Fleisch. u. Milch Hyg., 1901, 11, p. 114.

In the pathologic series they were found in acute cases only. They were not recovered from the chronically inflamed appendixes.

When hemolytic streptococci occurred in pathologic appendixes they were present in large numbers and in practically pure culture; while when found in the normal appendixes they were few in number.

Two types were found, namely, Streptococcus infrequens (5 strains) and Streptococcus hemolyticus II (1 strain).

Hemolytic streptococci apparently do not play an important rôle in the production of appendicitis; however, when they occur in the pathologic appendix, they usually predominate and appear to be the principal etiologic agent.

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THE ACTION OF DRUGS IN INFECTION

I. THE INFLUENCE OF MORPHINE IN EXPERIMENTAL SEPTICEMIA

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Because of its extensive use, the therapeutic value of opium and its derivatives, in infectious diseases, should be thoroughly understood by the internist and surgeon. The influence of morphine on infection is apparently not generally understood, although as early as 1898 Cantacuzene (1) called attention to the fact that phagocytosis is inhibited by its administration. Several years later, Reynolds (2) verified the above experiment and investigated the problem further. He found that the phagocytic property of leucocytes towards staphylococcus aureus was markedly inhibited. Later (1913) the influence of drugs on infection was studied by Arkin (3) who noticed that phagocytosis was hindered by morphine solutions in vitro. Cantacuzene (4) found that animals given various derivatives of opium succumbed more readily to anthrax and other infectious diseases than did normal animals. Crothers (5) states that addicts of morphinism are very often subjects of and succumb to, pneumonia, nephritis, and other diseases.

The purpose of the present investigation was to observe the course of septicemia produced by the Streptococcus hemolyticus as influenced by morphine and to compare the course of the infection in morphinized, and non-morphinized animals.

METHODS

Twelve healthy rabbits, weighing about 2.5 to 3 kilos each were used. The strain of streptococcus used was isolated from a case of human septicemia terminating fatally. Two of the twelve rabbits were used as controls: Each of these were injected subcutaneously with 0.030 gram (0.5 grain) of morphine sulphate in 1 cc. water, per kilo of weight. Five of the remaining rabbits were likewise injected with an equal amount of morphine sulphate per kilo of weight and also with a twenty-four hour blood-agar culture of Streptococcus hemolyticus suspended in normal saline. The remaining five rabbits were injected with an equal amount of streptococcus culture per kilo of weight, as those above; however, they were given no morphine.

A second similar experiment to the above was done with a series of ten rabbits. Two animals received morphine only; three received streptococci only; and five received both morphine and streptococci. The strain of hemolytic streptococcus used in this series was isolated from a case of tonsillitis. Thinking that a larger dose of morphine might prove fatal to normal rabbits, the two controls (I and XI) were given 0.03 gram (0.5 grain) at the beginning of the experiment; a second dose fifteen hours later, and a third dose forty-five hours later. Numbers IV, V, VI, VII, VIII, those receiving both streptococci and morphine, were likewise injected with morphine as the control animals.

Observations of the first series were recorded every six hours (see table 1). Observations of the second series were recorded every three hours (see table 2).

We also observed that the temperature of the rabbits, receiving the morphine, was lowered from 0.5° to 1.5° and progressively came back to normal gradually as the effects of the morphine disappeared (10). This is probably due to the quieting influence of the drug as well as a central antipyretic action. The temperature was likewise lowered in those rabbits receiving both morphine and intravenous injection of streptococci; however, in these the temperature began to rise before the effects of the morphine disappeared.

Post mortem examinations were made on all animals succumbing to the infection. The organisms were recovered from the heart blood in every instance; this gave evidence that infection was present.

TABLE 1

		8.00 A. M.	2.00 P. M.	8.00 P. M.	2.00 A. M.	8.00 A. M.	2.00 P. M.	8.00 P. M.	2.00 A. M.
Controls: 0.03 gram morphine sulphate per kilo									
V	Injected	+++	++	++	+	−	−	−	
VI	Injected	+++	++	+	+	−	−	−	
Morphine as above plus streptococci									
I	Injected	+++	+++	++	+++	Dead			
IV	Injected	+++	+++	++	+++	Dead			
VII	Injected	+++	+++	++	+++	Dead			
VIII	Injected	+++	+++	++	+++	Dead			
IX	Injected	+++	+++	++	+++	Dead			
Streptococci as above, no morphine									
II	Injected	+	+	++	++	+	+	−	
III	Injected	+	+	++	++	+	+	−	
X	Injected	−	+	++	+	+	−	−	
XI	Injected	+	++	++	++	+	+	+	
XII	Injected	+	+	++	++	+	−	−	

Note: The signs "− and +" indicate the condition of the animal with respect to activity, attitude and reaction toward stimuli. (−) signifies that the animal seems as if it had never been touched. (+) signifies the variation from normal.

DISCUSSION

In the administration of morphine at least two viewpoints should be considered; viz., the value of morphine as a sedative, and the effect it has in lowering the defense of the body against infection. That morphine usually produces a depression of the senses, insensibility to pain, and is often followed by comfort and rest can hardly be questioned, especially when it is administered in proper dosage. Its value in this respect is excellent and is, for this reason appreciated by the patient and those giving the drug. One is undoubtedly often justified in its use

TABLE 2

		STREPTOCOCCUS-MORPHINE					STREPTOCOCCUS			MORPHINE	
		IV	V	VI	VII	VIII	III	IX	X	I	XI
4 p.m.	1-22	*	*	*	*	*	*	*	*	*	*
7 p.m.		+++	+++	+++	+++	+++	−	−	−	+++	+++
10 p.m.		+++	+++	+++	+++	+++	−	−	−	+++	+++
1 a.m.	1-23	++	++	+	++	++	−	−	−	++	++
4 a.m.		+	++	++	+++	++	+	−	−	++	+
7 a.m.		+++	+++	+++	+++	+++	+	+	−	+++	+++
10 a.m.		+++	+++	+++	+++	+++	+	+	+	++	++
1 p.m.		++	Dead	+++	+++	+++	+	+	+	+	++
4 p.m.		++		+++	Dead	++	+	+	+	+	+
7 p.m.		++		+++		++	++	+	+	+	+
10 p.m.		++		Dead		++	++	+	+	−	+
1 a.m.	1-24	++				++	++	++	+	−	−
4 a.m.		++				++	++	++	+	−	−
7 a.m.		++				++	++	++	+	−	−
10 a.m.		++				++	++	++	+	−	−
1 p.m.		+++				+++	++	++	++	+++	+++
4 p.m.		+++				+++	++	++	++	++	++
7 p.m.		+++				+++	+++	++	++	+	+
10 p.m.		+++				+++	+++	++	++	−	−
1 a.m.	1-25	++				+++	+++	++	++	−	−
4 a.m.		++				+++	+++	++	++	−	−
7 a.m.		++				+++	+++	++	++	−	−
10 a.m.		+++				+++	+++	++	++	−	−
1 p.m.		+++				+++	+++	++	++	−	−
4 p.m.		+++				Dead	+++	++	++	−	−
7 p.m.		+++					+++	++	++	−	−
10 p.m.		+++					+++	++	++	−	−
1 a.m.	1-26	+++					++	++	++	−	−
4 a.m.		+++					++	++	++	−	−
7 a.m.		+++					++	++	+++	−	−
10 a.m.		+++					+++	++	+++	−	−
1 p.m.		Dead					+++	+++	+++	−	−
4 p.m.							+++	+++	++	−	−
7 p.m.							+++	+++	++	−	−
10 p.m.							Dead	+++	+	−	−
1 a.m.	1-27							+++	+	−	−
4 a.m.								Dead	−	−	−
7 a.m.									−	−	−

* Animals were injected at this time. All injections were proportionate to weight.

whenever infection is not involved. Morphine is also valuable in inhibiting excessive bowel movements; but when it is administered in the presence of infection, the greatest precaution should be taken. One should know exactly why it is given, its effect on the patient, and of equal importance, its effect on the course of the infection. Only after the nature, severity of the infection, its ultimate outcome, and the symptoms of the patient, are considered, can a proper decision be made regarding the value of morphine. Many surgeons object to the use of morphine, stating that its effects modify or entirely obscure important symptoms. They are apparently justified in making such an assertion. For instance, in acute appendicitis, many of the diagnostic symptoms are either greatly modified or entirely eliminated. In this and other cases morphine should be withheld until the diagnosis is made. However, just as important a factor to be considered is the effect of morphine on the course of infection. It is in all probability true that some of the fatal terminations of infection where morphine has been used would not have resulted had the drug been withheld.

Crothers (6), after a thorough study of morphinism, states that few morphine habitués live longer than ten to fifteen years after the beginning of the addiction; and, most of them die in about ten years. He also states that the continuous use of morphine without break or change makes the case a fatal one, death following from exhaustion and acute intercurrent diseases.

Elsner (7), in writing on prognosis of morphinism says, "Most of my cases of morphinism have fallen into wretched states, have developed intercurrent disease—often infection." He also says that pyemia, tuberculosis, and pneumonia are complicating conditions. Sollmann (8) states that opiophagic diabetics die sooner than others.

The lowering of temperature in the morphinized animals may have been due either to the quiet condition produced, or a central action or to both. In doses large enough to produce narcosis, there undoubtedly is a depression of the bodily metabolism, with consequent depressions of oxidation and heat production. Cushny (9) states that morphine frequently causes a fall in the

temperature which may be explained by the less active movements and the dilatation of the cutaneous vessels; the temperature regulating center is also affected.

Gottlieb (10) observed that a rise in temperature due to heat puncture of brain could be lowered by the administration of morphine as well as by antipyretics. This he accomplished by small doses (0.01 to 0.02 gram for rabbits weighing about 1900 grams; in a few instances he repeated, making a total of 0.03 gram in four hours). In our work, 0.03 gram per kilo, was used —a somewhat greater dose than that used by Gottlieb.

From the results obtained by us in this experiment, it is clear that morphine in itself was not fatal for the animals, in as much as about one-tenth of the fatal dose of morphine sulphate for rabbits was given (according to Sollmann (11)). Those animals receiving both morphine and streptococcus (hemolytic) injections, did not come out of the stupor at all, but soon succumbed. Those animals receiving Streptococcus hemolyticus injections only, gradually showed increased symptoms of the septicemia and then the symptoms of the immediate infection declined. After ten to fifteen days, however, these animals acquired complications, such as arthritis, snuffles, or other diseased condition which caused their death.

It is natural for the body to protect itself against infection, and to rid itself of the infection after it has once become invaded by micro-organisms. This it does in various ways, amongst which are phagocytosis, elimination, bodily nutrition, elevation of temperature, and specific antibodies. Then, since the various factors mentioned above are true, a depression, elimination of any one or several of the factors will allow infection to proceed more rapidly and extensively. As mentioned above, morphine in certain concentrations hinders phagocytosis; and produces intestinal stasis, which hinders elimination and allows the organisms of the gastro-intestinal tract to produce toxic products that are taken into the general system, thus adding poisonous material to that which the par-enteral infection produces.

Arkin (12) states that morphine should be used cautiously in bacterial disease. Hewlett (13) states that, from the results

of studies upon animals we must conclude that a moderately high temperature, even though maintained for a long time, is not in itself a dangerous manifestation. On the whole, an increased temperature causes artificial infections in animals to run a more favorable course and it increases the speed with which protective antibodies are formed. According to Rolly (14) the favorable effect of higher body temperatures on the formation of antibodies exists up to temperatures of 103.2° to 104° F.

The recent work of Emil Diehl (15) on collapse poisons (amylen hydrate, antifebrine, dysentery toxin, etc.) has some bearing on this problem. He points out that the difference between antipyretics and collapse poisons is mainly one of degree. The toxic dose of collapse poisons being very close to any therapeutic effect they might have. Very large doses of antipyretics also may have a collapse effect. In collapse he found that the regulatory mechanism which prevented undercooling of the body *is lost* while the protection against over-heating remains. The collapse animal is very much like a poikilothermal animal. Consequently when exposed to cold temperature the animal readily succumbs. Among his collapse poisons was dysentery toxin. One might suppose that the action of hemolytic streptococcus toxin is similar to this toxin, and that the morphine aided in the collapse. The amount of morphine used however was not per se sufficient to cause collapse, yet the room temperature (about 20°) may have had some influence on the last stages of the infection, since in some cases Diehl found that the lowest temperature adjustments of the body to cooling external temperature could take place within a very narrow range not below 30° to 34°C. This point however is of little bearing on our present problem since we were working with normal external temperatures, such as would prevail in a home or hospital. The conclusion of Diehl that the action is mainly central, we think would hold also for hemolytic streptococcus, though future work may somewhat modify this opinion.

Since alcohol acts at times somewhat as an antipyretic, it is perhaps proper here to refer to Laitinen (16) who observed that alcohol diminishes the resistance to bacterial infection.

Furthermore, while the animal is under the influence of morphine, it does not eat normally, if at all, and metabolism in

general is at a standstill or is depressed; and in consequence of this, interpretation of experimental or practical observations deserve careful consideration. Further observations are being recorded and will appear in other papers of the series.

CONCLUSIONS

1. Morphine sulphate given in 0.03 gram (0.5 grain) doses which is about one-sixth to one-tenth fatal dose lowers the resistance of rabbits toward septicemia produced by the Streptococcus hemolyticus.

2. Morphine sulphate, given as above, lowers the temperature of rabbits.

3. In the administration of morphine at least two effects should be considered: First, the sedative action of morphine; second, the influence of morphine on the course of infection.

4. The harmful influence of morphine is probably due to a number of factors, such as inhibition of phagocytosis, increase in intestinal stasis, with the increased production of toxins, and a general depression of the body temperature, of metabolism and the body defense.

REFERENCES

(1) Cantacuzene: Annales de l'Institut Pasteur, 1898, xli, 273.
(2) Reynolds: Lancet, 1910, i, 569.
(3) Arkin: Jour. Inf. Diseases, 1913, xiii, 408.
(4) Cantacuzene: Annales de l'Institut. Pasteur, 1898, xli, 273.
(5) Crothers: Text—1902, W. B. Saunders & Co., Philadelphia.
(6) Crothers: Text, Morphinism and Narcomania from other drugs. 1902, 128 et passim. W. B. Saunders & Co., Phila.
(7) Elsner: Text, Monographic Medicine, 1916, vi, 1186. D. Appleton & Co., New York and London.
(8) Sollmann: Text, A Manual of Pharmacology, 1917, 239.
(9) Cushny: Text, Pharmacology and Therapeutics of the Action of Drugs. 1915, 245. Lea and Febiger, Philadelphia and New York.
(10) Gottlieb: Archiv fur Experimentelle Pathologie, 1889, xxvi, 419–453
(11) Sollmann: Text, A Laboratory Guide in Pharmacology. 1917, 330. W. B. Saunders & Co., Philadelphia and London.
(12) Arkin: Jour. Inf. Diseases, 1913, xiii, 408–424.
(13) Hewlett: Text, Monographic Medicine, 1916, i, 487. D. Appleton & Co., New York and London.
(14) Rolly: Experimentelle Untersuchungen uber den Stoffwechsel im Fieber und in der Rekonvalescenz. Deutsch. Arch. f. Klin. Med., 1911, ciii, 93.
(15) Diehl: Archiv f. Experimentelle Pathologie and Pharmakologie, 1920, lxxxvii, Heft. 3 and 4, 206.
(16) Laitinen: Ztsch. f. Hygiene u. Infektionskrankh., 1900, xxxiv, no. 2.

THE VITALITY AND VIABILITY OF HEMOLYTIC STREPTOCOCCI IN WATER.

By GEORGE S. LIVINGSTON, M.S.

THE VITALITY AND VIABILITY OF HEMOLYTIC STREPTOCOCCI IN WATER.*

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Introduction.

The subject of the viability and vitality of bacteria in water has been thoroughly investigated, mainly with reference to the bacterial content of natural waters to determine their potability. More especially has attention been centered upon *Bacillus coli* and its more dangerous relatives in the field of hygiene and public health, to the end of preventing and controlling the endemic and epidemic water-borne diseases. The scope of this work was extended to the streptococci, and the investigations here reported were conducted to ascertain their behavior and significance in water.

The early workers in this field reported on the mere presence of streptococci, with little attempt at classification. More recently the literature contains references to specific types which appear always to be of intestinal origin, and are usually non-hemolytic.

The object of the present work was to obtain data on the vitality and viability of hemolytic streptococci in water, and to discuss the possible rôle which various waters containing these organisms might play in the spread of disease.

The earliest reference to streptococci in water is that of Roscoe and Lunt (1) in 1891, who found them in sewage. Laws and Andrews (2), in 1894, found them in sewage from St. Bartholomew's Hospital. Houston (3) in 1898 reported the first of an extensive series of studies on streptococci in water. Classification on the basis of hemolysis was not used at that time, but it may be judged from the source and nature of the organisms, that they were of non-hemolytic varieties. In the United States the first reference to streptococci in water was made in 1902, by Winslow and Hunnewell (4) who found them in sewage, in septic tanks, and in river water polluted by sewage.

It appears, then, that streptococci have been found in water con-

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taminated by excretions, and that where there is human fecal contamination the organism most commonly found is *Streptococcus fecalis,* a non-hemolytic variety. No reference can be found to hemolytic streptococci in water, but their occasional presence in feces suggests the possibility of finding them in water polluted by sewage. Holman (5) states that of fifty-three strains of streptococci from feces, thirteen were hemolytic, and that of thirty-eight strains from urine and the urethra, eleven were hemolytic. Oppenheim (5) found five hemolytic strains out of fifteen isolated from feces, and Broadhurst (7) reports that nine out of thirty-one fecal strains were hemolytic. Davis (8), however, found no hemolytic streptococci in the stools in fifty-three cases, many of whom harbored these organisms in their throats; he also found them absent from the stools of four scarlet fever patients. Moody and Irons (9) found them in the stools in thirty per cent. of cases of scarlet fever. It appears, therefore, that hemolytic streptococci are rare and inconstant inhabitants of the excretions, and that the probability of their reaching water by this route is slight. That disease may be spread by water containing streptococci is suggested, however, by Wilson (10), who reports an epidemic of streptococcus pneumonia with intestinal complications, in a war camp. All of the patients gave a history of having drunk shell-hole water, the diarrheal symptoms ensuing from one to five hours after. Bacteriological examination of the water showed that twenty-eight out of forty-two samples contained streptococci in large numbers. Hemolytic activity was variable, but it appears that some of the strains isolated were hemolytic.

Hemolytic streptococci appear not to have a natural habitat outside the animal body. Broadhurst (7) examined eighteen samples of soil and water from wood edges, moist road-banks, and brooks, of which one sample of water, from a country roadside overflow, showed short-chained micrococci; these, however, were not hemolytic.

Experimental work.

The experimental work of the problem comprised a series of tests to determine the vitality and viability of hemolytic streptococci in water, under various conditions. In these tests the general method employed was to place the water in a suitable vessel in measured amount, and seed it with a suspension of hemolytic streptococci. These were grown in pure culture on slants of blood-agar for forty-eight hours, at 37.5° C. The growths were then removed by scraping the

surfaces of the slants with a wire loop, and transferred to a tube of sterile distilled water. This gave a concentrated suspension of the bacteria in water, without admixture of any nutrient material from the culture medium. The suspension was shaken until it was of uniform opacity, and contained no large particles, and was then added to the water in Erlenmeyer flasks of Pyrex glass, to give the desired concentration. In those tests in which enumeration of the bacteria was not necessary, a qualitative examination was made at the time of seeding, and periodically thereafter, once a day, once in two days, or twice a week, as the conditions of the experiment required. For this purpose plates of blood-agar were used, inoculated with the water, and incubated at 37.5° for twenty-four hours. The plates were examined for the presence of hemolytic streptococcus colonies, and if found, the water was recorded as containing living hemolytic streptococci on the day on which the sample was taken. Negative plates were incubated for twenty-four hours longer, before recording them as such.

In certain experiments it was necessary to keep a daily record of the quantitative streptococcal content of the water. This was done by pouring blood-agar plates inoculated with accurately measured amounts of the water by the method of decimal dilutions. After the usual incubation, colonies were counted in the most suitable plate, and the number of hemolytic streptococci per cubic centimeter of water was calculated.

I. *Longevity of various strains of hemolytic streptococci in sterile distilled water.*

Eleven strains of hemolytic streptococci and one of *Streptococcus viridans* were selected for comparative study. All the strains were of human origin, and all were virulent at the time of isolation. All but two of the strains had caused the death of the individual from whom they had been isolated, and these two were associated with acute infectious processes. The strains varied in age from one month to ten years. They were obtained from the following sources:

1. Abscess (autopsy).
8. Acute ulcerated appendix.
12. Lung; influenzal pneumonia (autopsy).
39. Lung; pneumonia (autopsy).
40. Tonsils; acute tonsillitis.
84. Trachea; pneumonia (autopsy).
90. Heart blood; septicemia (autopsy).

104. Pleura; pneumonia (autopsy).
113. Lungs; pneumonia (autopsy).
208. Cerebrospinal fluid; streptococcus meningitis.
211. Fatal septic sore throat; during milk epidemic.
49. Streptococcus viridans; endocarditis (autopsy).

All the strains grew in chains of varying length, in liquid media; all were Gram positive; all grew in characteristic colonies on blood-agar plates, with a clear wide zone of hemolysis around the colony (except 49); none of them liquefied gelatin, and none fermented inulin. Each strain was added to 100 c.c. of sterile distilled water, in a cotton-plugged flask, by the method previously outlined, and the flasks were kept in the laboratory, at room temperature, in subdued light. The presence of hemolytic streptococci was determined qualitatively only, by plating on blood-agar. Plates were poured at the time the experiment was begun, and twice a week thereafter, until negative plates appeared. Negative plates on three consecutive days, using one cubic centimeter of water, were taken as evidence that all streptococci in a given flask of water were dead.

TABLE I.

Longevity of various strains of hemolytic streptococci, in sterile distilled water.

Days.	104	12	113	49	40	39	90	84	211	8	208	1
Start	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	−
10	+	+	+	+	+	+	+	+	+	+	+	
14	+	+	+	+	+	+	+	+	+	+	+	
17	+	+	+	+	+	+	+	+	+	+	+	
21	+	+	+	+	+	+	+	+	+	+	+	
24	+	+	+	+	+	+	+	+	+	+	−	
28	+	+	+	+	+	+	+	+	+	−		
31	+	+	+	+	+	+	+	+	−			
35	+	+	+	+	+	+	+	−				
38	+	+	+	+	+	+	−					
42	+	+	+	+	+	−						
45	+	+	+	+	+							
49	+	+	+	+	−							
52	+	+	+	−								
56	+	+	−									
59	+	+										
63	+	−										
66	+											
70	+											
73	+											
77	+											
80	+											
84	+											
87	+											
91	−											

The results of this experiment show that the length of life of these organisms in water, under these conditions varies within wide limits. It is, in effect, a test of the resistance of each strain to starvation, since no nutrient material was present in the water. Strain 1 was the first to succumb, no colonies appearing in plates after the third day. Strain 104 survived the longest, viz., eighty-seven days. The average length of life of all the hemolytic strains was thirty-eight days. The green-producing streptococcus, 49, lived for fifty days. The average for all strains was thirty-nine days. In Table I the strains are arranged in the order of their longevity.

II. *Variation of longevity of a single strain of hemolytic streptococci, in different concentrations, in sterile distilled water.*

A heavy suspension was made of strain 104, from a number of blood-agar slants. This was adjusted to contain approximately one billion streptococci per cubic centimeter. The suspension was added to flasks of sterile distilled water, in quantities to give a final content of 100 c. c. of water for each flask, the bacterial content varying as follows:

1. 100,000,000 per c.c.
2. 10,000,000 per c.c.
3. 1,000,000 per c.c.
4. 100,000 per c.c.
5. 10,000 per c.c.
6. 1,000 per c.c.

These flasks were kept in the laboratory, at room temperature, in subdued light. Quantitative estimations of the bacterial content were made at the start of the experiment, and at two day intervals thereafter. Absence of colonies in plates, poured from 1 c. c. of water, on three successive days, was taken as evidence that all streptococci in a given flask were dead. Table II gives the detailed record of this experiment. The flasks are arranged in the order of their longevity. The bacteria in Flask I survived for fifty-two days, in Flask 2 for thirty days, in Flask 3 for twenty days, in Flask 4 for twelve days, in Flask 5 for six days, and in Flask 6 for four days. The results were quite uniform and significant. The greatest number of organisms died during the first week. After that the rate of decrease was less rapid. The greater the concentration of streptococci in a given flask of water, the longer they survived. The curve below

shows the relation between the initial concentration of streptococci in water and their longevity.

TABLE II.

Variation in longevity of a single strain of hemolytic streptococci, in different concentrations, in sterile distilled water.

Days.	1	2	3	4	5	6
	No. per c.c.	No. per c.c.	No. per c.c.	No. per c.c.	No. per c.c.	No. per c.c.
Start	100,000,000	10,000,000	1,000,000	100,000	10,000	1,000
2	40,000,000	6,000,000	910,000	6,300	451	29
4	9,000,000	1,500,000	250,000	945	77	3
6	800,000	500,000	72,000	124	8	0
8	300,000	95,000	13,700	38	0	
10	150,000	36,000	2,150	14		
12	90,000	8,500	890	6		
14	35,000	2,300	315	0		
16	22,000	650	84			
18	12,000	435	27			
20	4,500	210	6			
22	1,700	62	0			
24	1,100	18				
26	845	4				
28	670	7				
30	595	3				
32	230	0				
34	180					
36	195					
38	65					
40	23					
42	14					
44	3					
46	4					
48	5					
50	4					
52	2					
54	0					

III. *Longevity of a single strain of hemolytic streptococci in various waters.*

Samples of water were collected from various sources, and a suspension of strain 104 was added to each sample, in a flask. 100 c. c. of each kind of water were used, and the streptococcal content was adjusted to approximately 100,000 per c. c. The flasks were kept in the laboratory, at room temperature, in subdued light. The tests for viability were qualitative only. Blood-agar plates were poured daily. The routine technic was used. In addition, tubes of 1 per cent. dextrose bouillon were inoculated, and smears made after incubation for twenty-four hours. These were stained by Gram's method, and examined for streptococci. This was useful in those cases

where the blood-agar plates were doubtful, owing to excessive contamination by other organisms. The sources of the various waters and the longevity of the streptococci, in each, are listed below.

1.	Sterile distilled water	15 days
2.	Sterile physiological salt solution	12 "
3.	Water from deep well	11 "
4.	Water from surface well	8 "
5.	Water from Lake Michigan	7 "
6.	Street water, sterilized in autoclave	6 "
7.	River water, Desplaines River	6 "
8.	Water from country road-side ditch	5 "
9.	Street water, not sterile	4 "
10.	Water from park lagoon	4 "
11.	Tap water	3 "
12.	River water, Chicago River	2 "

The average survival in all waters was seven days.

It is to be noted that these waters can be roughly divided into two groups, viz.:

A. The "clean" group, comprising those waters in which vitality was retained for the average length of time or longer, and

B. The "dirty" group, comprising those waters in which the streptococci died out in less than seven days. An exception is No. 11, tap water, in which all organisms were dead after three days. The experiment was later repeated, with sterile distilled water, as a control. The results were substantially the same, the streptococci dying sooner in tap water, in each case. This is attributed to the fact that the tap water used was quite heavily chlorinated. In all of the dirty waters other bacteria, the natural contaminators, were present in large numbers, after all streptococci were dead. It would seem that this was the main factor in determining the length of their vitality, they having been crowded out and overgrown by the saprophytes present. In Table III the water specimens are arranged in the order in which the streptococci survived.

IV. *Effect of temperature on rate of decrease.*

In this experiment three flasks, each containing 300 c.c. of sterile distilled water were seeded with a suspension of Streptococcus hemolyticus, strain 211, adjusted to approximately 100,000 per c.c. One flask was kept in the incubator at 37.5° C., another at room temperature, in subdued light, the average being 27° C., and the third

TABLE III.

Longevity of a single strain of hemolytic streptococci in various waters.

Days.	1	2	3	4	5	6	7	8	9	10	11	12
Start....	+	+	+	+	+	+	+	+	+	+	+	+
1.....	+	+	+	+	+	+	+	+	+	+	+	+
2.....	+	+	+	+	+	+	+	+	+	+	+	+
3.....	+	+	+	+	+	+	+	+	+	+	+	−
4.....	+	+	+	+	+	+	+	+	+	+	−	
5.....	+	+	+	+	+	+	+	+	−	−		
6.....	+	+	+	+	+	+	+	−				
7.....	+	+	+	+	+	−	−					
8.....	+	+	+	+	−							
9.....	+	+	+	−								
10.....	+	+	+									
11.....	+	+	+									
12.....	+	+	−									
13.....	+	−										
14.....	+											
15.....	+											
16.....	−											

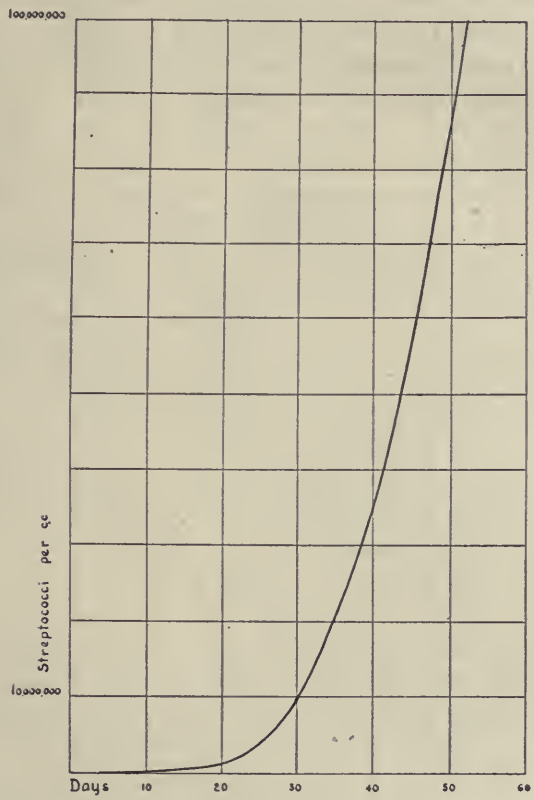
was kept in the refrigerator, at an average temperature of 1° C. Quantitative plates were poured daily, and the number of surviving streptococci, per cubic centimeter, was calculated. Strain 211 was one isolated during a milk epidemic from a fatal case of streptococcus sore throat. It was an old laboratory strain having been isolated ten years previously. In the incubator the streptococci re-

TABLE IV.

Effect of temperature on rate of decrease.

Days.	37.5° C.	27° C.	1° C.
	No. per c.c.	No. per c.c.	No. per c.c.
Start.....	100,000	100,000	100,000
1.....	23,000	18,500	37,000
2.....	3,400	7,200	15,700
3.....	655	1,170	4,200
4.....	48	415	1,740
5.....	9	167	885
6.....	2	38	390
7.....	0	6	187
8.....		15	93
9.....		4	43
10.....		0	21
11.....			27
12.....			16
13.....			4
14.....			7
15.....			3
16.....			0

tained their vitality, in water, for six days, at room temperature for nine days, and in the refrigerator for fifteen days. It appears, then, that survival is favored by low temperature, and is hindered by high temperatures. Too much importance, however, must not be attached to these figures, since they are not widely separated, and the differences are not sufficiently pronounced to be of much significance. Table IV gives the detailed record of these tests. It is to be noted that



Curve showing relation of the concentration of streptococci to their longevity in water.

during the first week there was a rapid decrease in all three flasks. The rate of decrease is much less for subsequent days. The rapid decrease in the first week is quite constant, and appears to be characteristic.

V. *Comparison of longevity of virulent and avirulent strains of hemolytic streptococci, in sterile distilled water.*

A number of strains of hemolytic streptococci was selected for this study, some from old laboratory cultures, others, recently isolated, from various human sources. Rabbits were inoculated with these strains to determine their virulence. 1000 gram rabbits were used. Each was given three cubic centimeters of a twenty-four hour broth culture of the strain used, injecting into the ear vein. Ten avirulent strains were chosen, viz., such as produced no change in the animals in two weeks; and ten virulent strains, each of which had produced arthritis and caused death in the rabbit inoculated. Each strain was

TABLE V.

Comparison of longevity of virulent and avirulent strains of hemolytic streptococci, in sterile distilled water.

Days.	Avirulent strains.										Virulent strains.									
	7	6	11	4	3	5	10	8	2	12	20	23	28	21	22	24	25	26	27	31
Start...	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.....	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
2.....	+	+	+	+	+	+	+	+	+	+	+	+	+							
3.....	+	+	+	+	+	+	+	+	+	+	+	−	−							
4.....	+	+	+	+	+	+	+	+	+	+	+									
5.....	+	+	+	+	+	+	+	+	+	+	+									
6.....	+	+	+	+	+	+	+	+	+	+	+									
7.....	+	+	+	+	+	+	+	+	+	+	−									
8.....	+	+	+	+	+	+	+	+	+	+										
9.....	+	+	+	+	+	+	+	+	+	+										
10.....	+	+	+	+	+	+	+	+	+	+										
11.....	+	+	+	+	+	+	+	+	+	−										
12.....	+	+	+	+	+	+	+	+	+											
13.....	+	+	+	+	+	+	+	+	−											
14.....	+	+	+	+	+	+	+	−												
15.....	+	+	+	+	+	+	−													
16.....	+	+	+	+	+	+														
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28.....	+	+																		
29.....	+	+																		
30.....	+	−																		
31.....	+																			
32.....	+																			
33.....	−																			

seeded in 100 c. c. of sterile distilled water, as in the previous tests, and kept at room temperature in the laboratory. Qualitative determinations were made daily. In Table V the various strains are arranged, in the order of their longevity. The avirulent strains varied in their survival from ten to thirty-two days, averaging over eighteen days. Of the virulent strains one survived for six days, two for two days, and all the rest succumbed within a day of the start.

Discussion.

In reviewing the results of the series of experiments set forth above, one is struck by the wide variations and the inconstancy in the behavior of hemolytic streptococci in water. Any conclusions which are drawn from this work must, therefore, be very general.

It is seen that hemolytic streptococci are capable of retaining their vitality when kept in water. In Experiment I the average period of survival was thirty-eight days, but one strain, 104, was able after being kept for eighty-seven days under these conditions, to multiply rapidly, when placed in a favorable environment. That the long period of survival was due in part to the enormous numbers originally placed in the water, is shown by the behavior of the same strain in various concentrations in Experiment II. Of a small number of streptococci in water, all will die out rapidly, but of a larger number a few individuals retain their vitality for a considerable length of time. Houston (3) tested a large number of streptococci in sterile tap water and sterile salt solution. Many died out in a few days, and those, that survived for forty or fifty days, showed a great decrease in their number.

Under more natural conditions the element of interference by other organisms becomes an important factor. Thus in waters of the "dirty" group, in Experiment III, hemolytic streptococci failed to survive for more than a week. Savage and Wood (11) found that streptococci of the intestinal variety die rapidly in sewage, and that most of them are dead at the end of two weeks.

In Experiment IV it was found that the rate of death, in water, is higher at body temperature than at room temperatures, and is lowest at 1° C. The differences in the periods of survival at the various temperatures were not marked enough to be of great significance. Hinds (12), working with colon and typhoid bacilli, in natural and distilled water, found that the rate of death increased with the temperature.

The greatest diminution in numbers was found in the first week. Those organisms which survived longer were eliminated more gradually. This agrees with the findings of Savage and Wood (11) with fecal streptococci. They conclude that the finding of streptococci in water is evidence of recent pollution.

Hemolytic streptococci, stored in water, always progressively decrease in their numbers, as shown by several of the quantitative tests. Savage and Wood found the same to be true of fecal streptococci, but noted that colon bacilli increased in number for a considerable length of time.

The effect of virulence is striking. In Experiment V none of the virulent strains survived as long as the least viable of the avirulent strains. Most of them died out over night. The evidence is quite definite that virulent hemolytic streptococci, recently isolated from lesions in the human body, are poorly adapted to storage in water, while old strains, long accustomed to artificial media, survive much longer.

Summary.

Hemolytic streptococci, when placed in water, remain alive for a variable length of time, depending upon their number, upon the temperature, upon the presence of other organisms, and upon virulence. They are capable under special conditions, of retaining their vitality for a long time, but under natural conditions, if placed in water, they will succumb quite rapidly, especially if recently isolated.

LITERATURE.

1. Quoted from Winslow and Hunnewell (4).
2. Ibid.
3. Ibid.
4. Winslow, C.-E. A. and Hunnewell, M. P
   1902. Streptococci characteristic of sewage and sewage-polluted waters apparently not hitherto reported in America. Science, N. S., XV, 827.
5. Holman, W. L.
   1916. The classification of streptococci. Journ. Med. Res., XXXIV, 377.
6. Oppenheim, C. J.
   1920. The human fecal streptococci. Journ. Inf. Dis., XXVI, 117.
7. Broadhurst, Jean.
   1915. Environmental studies of streptococci. Journ. Inf. Dis., XVII, 277.
8. Davis, D. J.
   1919. Hemolytic Streptococci with special reference to their rôle in acute respiratory infections. Journ. A. M. A., LXXII, 319.

9. Moody, W., and Irons, E. E.
1920. On the occurrence of hemolytic streptococci in the stools of scarlet fever. Journ. Inf. Dis., XXVII, 363.

10. Wilson, J. A.
1918. Streptococcus infections of the respiratory tract. War Medicine, II, 556.

11. Savage, W. G. and Wood, D. R.
1917. The vitality and viability of streptococci in water. Journ. Hyg., XVI, 227.

12. Hinds, M. E.
1917. University of Illinois Bull. 14, No. 5, and Water Survey Series, 1917, No. 13, 225.

THE INFLUENCE OF X-RAY ORGAN STIMULATION ON THE COAGULATION MECHANISM

BY

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THE INFLUENCE OF X-RAY ORGAN STIMULATION ON THE COAGULATION MECHANISM

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DURING the experimental observations on the effect of the *x*-ray exposure of the hepatic, splenic and intestinal areas for varying periods of time as reported in the preceding paper, evidence of a change in the coagulation time of the blood was obtained. A number of recent investigators, observing the reduction of the coagulation time following the raying of the splenic area have suggested that measure for therapeutic use in cases of severe hemorrhage. Stephan [1] for instance reports a case of purpura fulminans in a man of forty-five in which a refractory hemorrhagic diathesis was successfully combated early in 1919 by means of deep roentgenotherapy applied to the spleen. Investigations that he undertook in connection with this result led him to state that roentgen radiation applied to the spleen rapidly decreases the coagulation time of the blood *in vitro,* and increases likewise to a considerable extent the amount of coagulating ferment in the blood serum. Radiation seems to have the same effect on the organism as extensive loss of blood. The essential factor that arrested and cured the hemorrhagic tendency was the increase in the quantity of the coagulating ferment, and this was realized by the action of the roentgen rays on the spleen. He thinks they exert a specific functional stimulus on the elements of the spleen other than the lymph follicles. The blood platelet count does not seem to be modified.

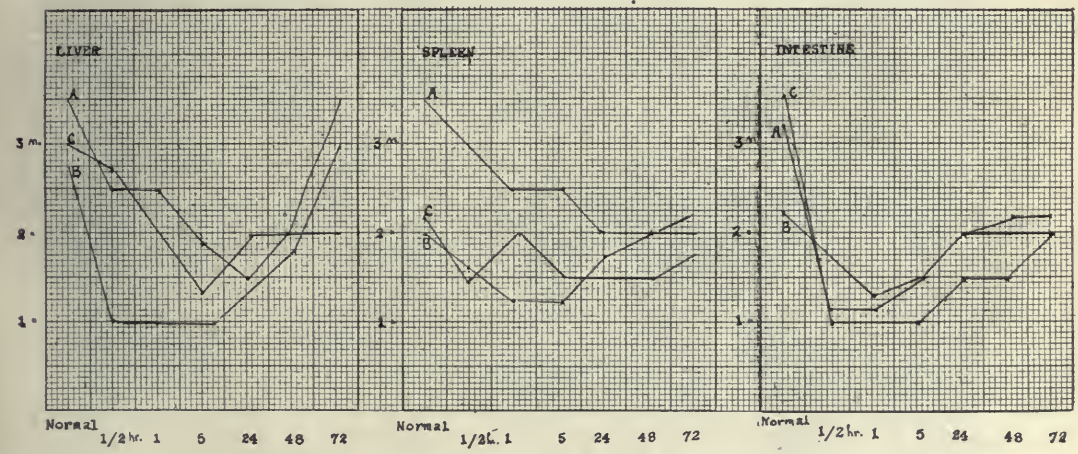

CHART I. COAGULATION TIME OF THE BLOOD AFTER *X*-RAY EXPOSURE FOR FIVE, TEN AND TWENTY MINUTES.

The coagulation time was shortened sometimes to one fourth even in normal subjects by raying the spleen; the maximum effect was apparent between the second and fourth hours, and then gradually subsided. His clinical and experimental research demonstrated, he believes, that stimulating the functioning of the spleen by roentgen radiant energy must be regarded as theoretically a true physiologic method of arresting venous and parenchymatous hemorrhages. In numerous cases it proved extraordinarily effectual in practice, far surpassing the effect of any medical hemostatics.

Jurasz [2] considers this observation of con-

siderable practical importance in surgery, and recommends that before operative procedures the coagulation time of the patient be determined, and if it is found delayed, that the patient be rayed from fifteen to twenty hours before the operation in order that the coagulation time be brought within normal limits.

As a matter of fact it is probable that any stimulation of the spleen results in this same effect on coagulation. Thus Nonnenbruch and Szyszka [3] found that simple diathermy of the splenic area would appreciably increase the coagulability of the blood in from one to two hours. The effect was, of course, not quite so pronounced as following radiation. Szenes [4] found that the increase in coagulation occurred not only after raying the spleen, but after raying lymphatic tissues in general.

In a more recent publication Stephan [5] takes exception to the work of Szenes, however, for the reason that his observations were limited wholly to the measuring of the coagulation time, not to a study of the individual factors in the coagulation balance. Thus a lowering of the antithrombin content, or an increase in the platelet count might simulate the results obtained when there is an actual increase in the thrombin.

In view of the practical importance of the subject we have made a detailed study of the alterations that occur in the coagulation mechanism following raying of the hepatic intestinal and splenic area. The observations included the following: clotting time; prothrombin; antithrombin; fibrinogen; blood platelets.

Prothrombin and antithrombin determinations were performed according to the method of Minot.[6] Thrombin was prepared according to Howell.[7] Fibrinogen determinations were recorded according to Wohlgemuth.[8] Blood platelet counts were observed by the Wright-Kinnicut method.

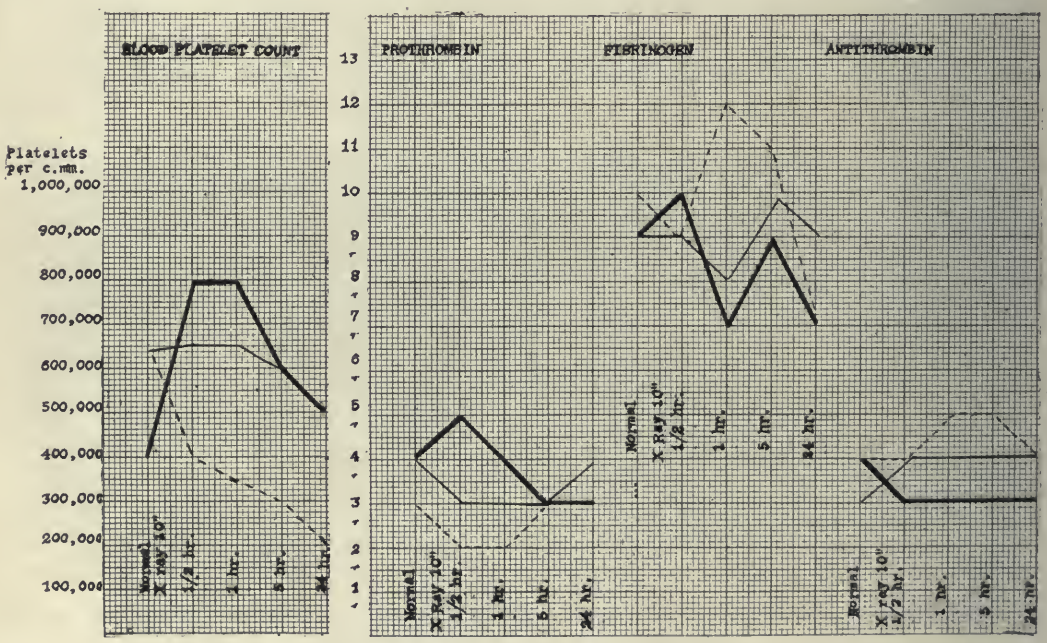

Chart II. Heavy Line—Titer after raying hepatic area; Dotted Line—Intestinal area; Light Line—Splenic area.

Dogs were exposed for 10 minutes (Coolidge tube, screened by a 4 mm. aluminum screen, at 10 inch distance, 8 ma. and 5 inch back-spark) over the liver, intestinal and splenic areas. The exposures were made in the morning (serum samples being obtained before) one-half, one, five and twenty-four hours after exposure.

Blood Coagulation.—In studying the coagulation time, capillary tubes drawn out to a uniform diameter were used, the clotting

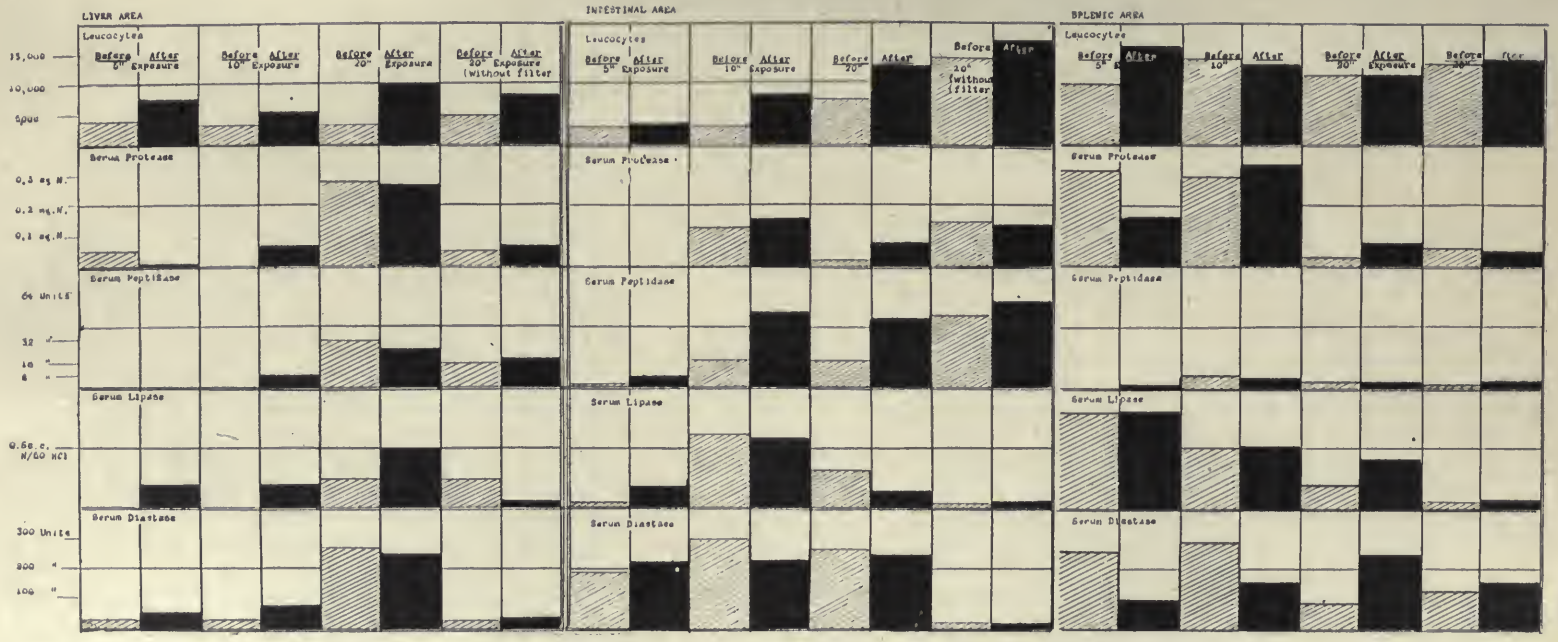

CHART III. Illustrating leucocytic and enzymatic reaction following raying of liver, intestinal and splenic areas in dogs for five, ten and twenty minute periods. The shaded blocks represent the titer before the period of raying, the black blocks the average for the determinations following the raying (serum samples taken ½, 1, 5, 24, 48 and 72 hours after the exposures).

time recorded when on breaking the tube a firm coagulum could be drawn out. In our experiments the most prompt effect on the clotting time seemed to follow raying of the lower intestinal tract; the effect on the clotting time following exposure of the splenic area was more delayed. In the later case, however, the effect on the coagulation especially after longer raying periods was more prolonged. In Chart I the average coagulation time is shown.

In view of the rather decided changes in the coagulation time above noted we anticipated marked alterations in the titers of the coagulating factors in the blood, corresponding to the observations of Stephan. In this we were disappointed. As will be observed in the next chart (Chart II) fluctuations in the various elements of the balance did occur but not to the extent demanded by the marked lowering of the actual coagulation time.

Blood Platelets.—Raying of the hepatic area gave a maximum increase of blood platelets during the one half to one hour period, gradually returning to normal. Raying of the splenic and intestinal areas gave a gradual diminution in the platelet count during the ensuing time periods.

Prothrombin.—Exposure of the liver showed a slight decrease up to the one-half hour sera, with gradual increase in the remaining sera, while the spleen and intestinal exposures showed an increase with a subsequent return to normal. (The curve, Chart II, in which the serum dilutions are charted, represents the inverse of the actual titer of the prothrombin present in the serum. Thus the actual amount following hepatic raying after a slight diminution, was increased, as was also the case after raying the splenic and intestinal areas.)

Antithrombin.—Raying the intestines showed a maximum increase during the one to five hour periods; splenic raying showed a slight increase through to the twenty-four hour sample, while raying of the hepatic area showed a miximum increase during the one

Fibrinogen.—The maximum increase in fibrinogen from intestinal raying was noted in the one and five hour period, diminishing to below normal in twenty-four hours. The splenic raying was followed by a decrease with a maximum increase at the five hour period, returning to normal at the twenty-four hour period. Liver raying showed transitory increase with fluctuating decrease and increase to below normal as noted in Chart II.

CONCLUSIONS

1. Raying of the splenic area in dogs is followed by a diminution in the clotting time of blood determined by the capillary tube method.

2. Raying of other areas (hepatic and intestinal) is also followed by similar changes in the clotting time. The mechanism of the alterations in clotting time may differ following various regional exposure. Thus raying of the splenic area was followed by an increase in prothrombin, some increase in antithrombin, a rather delayed increase in fibrinogen, with little alteration of the platelet count. Raying of the hepatic area was followed by a rather considerable increase in platelet count; raying of the intestinal area by an increase in the amount of fibrinogen.

3. Inasmuch as the effect of the *x*-ray exposure is quite prompt the use of this measure in surgical cases as well as in the management of medical cases associated with a hemorrhagic diathesis seems a feasible procedure. The clinical success of the *x*-ray in the treatment of uterine hemorrhage may depend in part on the general effect on the coagulation mechanism.

BIBLIOGRAPHY

1 Stephan, R. *München. med. Wchnschr.*, 1920, lxvii, 309.
2. Jurasz, T. A. *Zentralbl. f. Chirg.*, 1920, xlvii, 824.
3. Nonnenbruch, N., and Szyszka, W. *München. med. Wchrschr.*, 1920, lxvii, 1064.
4. Szenes, A. *München. med. Wchnschr.*, 1920, lxvii, 992.
5. Stephan, R. *München. med. Wchnschr.*, 1920 lxvii, 992.
6. Minot, Denny and Davis. *Arch. Int. Med.*, 1916, xvii, 101.
7. Howell. *Am. J. Physiol.*, 1913; xxxii, 264.
8. Wohlgemuth. Grundriss der Fermentmethoden, Berlin, 1913.

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THE CORRELATION OF RABBIT PNEUMONIA AND HUMAN INFLUENZAL PNEUMONIA

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In this paper I wish to report certain studies on pneumonia in rabbits and incidently to compare the lesions with the lesions in human influenzal pneumonia.

An epidemic of rabbit influenza occurred among the laboratory animals in the fall and winter of 1918 and the spring and summer of 1919. While contemporaneous with the epidemic of influenza, there was no reason whatever to surmise a common etiology. It had occurred many times before in the laboratory.

The duration of the disease was variable; most animals succumbed at the end of from 5 to 7 days. Early there was loss of appetite and a thin, watery nasal discharge, accompanied by frequent sneezing. The discharge descended from the nares to the breast and anterior extremities and, as the disease progressed, became mucoid and purulent in character. Animals used for experimental purposes (infections, vaccine injections, etc.) appeared more susceptible and succumbed 6 to 48 hours earlier as a rule.

The exciting cause is the B. bipolaris, an organism classed in the hemorrhagic septicemia group. This organism has been reported under various names: e. g., B. bronchisepticus, B. bovisepticus, Bacillus of pleuropneumonia, Bacillus of rabbit septicemia, etc. In this article I use the term B. bipolaris. It was recovered from the following sources: nasal discharge, nasopharynx, pleural fluid, pericardial fluid and heart blood. Koch's postulates were fulfilled. The bacillus is short, about 1-3 microns in length, staining intensely at the poles and only slightly in the middle, and at times is pleomorphic. It is nonmotile, nonspore-forming, aerobic and facultative anaerobic. It stains with the ordinary aniline dyes and is negative to the Gram stain. In fluid medium the organism is often coccoid while on solid medium it tends to retain its characteristic form. It has appeared in cultures as a coccoid bacillus, a diplobacillus, a streptobacillus and at times in threadlike

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forms similar to B. influenzae. After cultivation on artificial medium for a few generations, it tends to lose its characteristic bipolar staining.

In the literature are reports of a number of varieties of bacilli isolated from rabbits dying of lung involvement. Beck[1] mentions a small gram-negative, nonmotile bacillus, pathogenic for rabbits, guinea-pigs and mice, having a marked tendency to form threads, as the cause of "Breustseuche" in rabbits. Laven[2] described a bacillus pathogenic for rabbits and guinea-pigs; small, gram-negative, variable in size and a tendency to grow in thread and chain forms. It is strictly hemolytic and gives a peculiar sperm-like odor on blood agar. Kurita[3] described a small gram-negative polar staining bacillus which killed animals when injected by producing "Breustseuche." Undoubtedly the organism isolated from this series of cases belong to the same general group as those above described. It produces a confluent lobular pneumonia on intratracheal insufflation.

Experimental production of the disease was effected as follows: a 24-hour agar slant of bacteria was emulsified in 5 c.c. sterile normal salt solution and sprayed into the nose and nasopharynx of 5 healthy animals. All succumbed to the disease in the acute stages. To prevent the possibility of contamination at the institution, 2 animals, obtained from a source other than that from which they were usually obtained and kept at a distance of some miles from the laboratory, readily contracted and succumbed to the disease 8 days after inoculation.

A series of 17 animals was collected, of which 12 succumbed to the disease acquired in the natural manner and in 5 the disease was produced experimentally. Necropsies were performed within 1 to 14 hours after death. Sections for microscopic study were taken from the lungs, trachea, heart, liver, kidney and spleen, fixed in formalin or Zenker's fluid, sectioned in paraffin and stained with hematoxylin and eosin. In certain instances, special staining methods were also used.

Macroscopically the lungs presented the typical picture of a confluent bronchopneumonia. They did not completely collapse on opening the chest and the pleural surfaces were frequently mottled with patchy areas of dark, bluish red color; often in the acute stage the pleura was covered with a thin layer of fibrin. The pleural cavities usually contained some fluid. Small consolidated masses varying in size from that of a pinpoint to that of a pea, hard and firm, with crepitating lung tissue surrounding them could be felt, usually more prominent in the lower lobes and especially of the right lung. The distribution of the bronchopneumonic areas was as follows: upper lobe, left 12%, right 14%; middle lobe, right 18%; lower lobe, left 26%; right 30%.

On cut section the lungs were moist and edematous, with a red, frothy liquid, sometimes purulent in character, exuding from the cut ends of the bronchioles. On scraping, in some instances, plugs of necrotic material were removed. The distribution of the consolidated areas was variable; in the more

[1] Kolle-Wassermann: Handbuch der path. Mikroorg., 1903, 3, p. 405.
[2] Centralbl. f. Bakteriol., 1, O., 1910, 54, p. 97.
[3] Ibid., 1909, 49, p. 508.

acute cases it was usually in small patches near the periphery of the lung, in the more chronic it often became confluent in character and was situated around a central bronchiole. The consolidated areas were usually surrounded by a zone of hyperemia. At times the centers of these consolidated areas appeared necrotic. Engorgement of all the pulmonary vessels was particularly evident.

Microscopically, the picture varied with the stage of the disease. In the acute stage, perivascular edema and leukocytic infiltration were the most prominent features. In some instances, a clear, edematous exudate containing few or no cells was prominent. In rabbit 6, there was a proliferation of cells beneath the intimal coat of the blood vessels. As the stage of the disease became more advanced, it tended to resemble red hepatization. The alveolar epithelial cells were swollen, edematous and degenerative. In some instances, complete desquamation had taken place. The interstitial tissue was edematous and swollen, the vessels hyperemic and distended, with perivascular infiltration, as a rule. The alveoli were packed with red cells and desquamated alveolar cells together with strands of fibrin interspersed. In the advanced stage, the invasion of large numbers of white cells occurred, similar to gray hepatization. The fibrin increased in amount and later at times became organized. The outlines of the alveoli were indistinct and in some areas imperceptible, the process having become confluent with subsequent obliteration of the individual alveoli. Occasionally the pneumonic process was complicated by a tendency to small abscess formation. In the small consolidated areas, central necrosis, quite intense in some instances, was noted. Not infrequently this process involved over one half of the consolidated area. In many instances the bronchioles contained an exudate, the constituents of which depended on the stage of the process. All stages of cellular degeneration could be observed. Phagocytosis was often seen; the phagocytized structures being red cells, polymorphonuclears and cellular débris. Definite focal hemorrhages were occasionally seen, often involving large areas.

In the acute cases, less alteration was noticed, a slight transudation of serum and a few leukocytes plus slight hyperemia being the chief manifestations. In the more subacute and protracted cases, the picture varied from degenerative changes of the epithelial lining to complete desquamation and necrosis of the underlying tissue. The larger bronchi were less involved, this varying from slight to intense necrosis and sloughing. In most instances the peribronchial lymphatic spaces were distended and infiltrated with leukocytes.

The trachea contained a slimy, mucoid or frothy, slightly blood tinged fluid, especially near the bifurcation. On removal of this material, intense hyperemia of the mucosa was evident. In some instances, on opening the trachea, the affected side revealed more of the frothy, blood tinged fluid showing a fairly sharp line separating the diseased from the normal side. Microscopically an acute tracheitis was present, with edema and marked degenerative changes in the epithelial lining in some instances; in others only a moderate hyperemia was present.

Desquamative bronchiolitis was frequent in places with complete or nearly total destruction of the mucosa. Strands of fibrin and polymorphonuclear cells were found abundantly in some bronchioles; others appeared quite normal.

The heart was little altered. In most cases, a few c.c. of clear, straw-colored fluid was contained within the pericardial sac. In no animal in this series was there evidence of a fibrinous pericarditis. Occasionally the right heart was dilated. The valves were normal in every case except one in which a small vegetation occurred on the mitral valve and endocardium, from which

a mixed culture of hemolytic streptococci and a gram-negative bacillus was obtained. The muscle was reddish brown and without evidence of myocarditis. Microscopically there was no noteworthy change.

The kidneys appeared little altered excepting slight congestion. Microscopically, the glomeruli and tubules showed some evidence of degeneration in many of the animals. In a few, foci of hemorrhages were present. The liver parenchyma was in some instances slightly fatty. No areas of focal necrosis were found. The spleen was acutely swollen, without other noteworthy change.

I wish now to correlate certain features of the rabbit disease with those in the human influenzal lesions. This is done because of the general similarity of the two diseases in their symptomatology and epidemiology and possibly also in their etiology. With reference to etiology it is quite certain that B. bipolaris is the cause of the rabbit influenza. A similar organism, the Pfeiffer bacillus, is often associated with human influenza but presumably is only a common secondary invader and not the primary cause. It no doubt often plays a rôle in the causation of influenzal pneumonia and being somewhat similar to B. bipolaris it was thought a comparison especially of the pulmonary lesions in these two conditions would be of value.

A comparison of B. bipolaris and B. influenzae (Pfeiffer bacillus) has already been made by Davis.[4] While similar in many ways certain distinguishing features exist, symbiosis and the hemophilic property being the most important; some other points of difference exist which need not be detailed here. It will be sufficient to state that these organisms cannot be considered identical or even very closely related.

Rabbit bronchopneumonia and human influenzal bronchopneumonia reveal somewhat similar gross alterations if approximately the same stages of the disease are taken. In the former, on opening the pleural cavities, the lungs do not completely collapse and the pleural cavities usually contain a moderate amount of fluid, seldom blood tinged and containing fibrin. The picture in human influenzal bronchopneumonia is an excessive amount of blood tinged fluid, usually remarkably free from fibrin. On the pleural surfaces in both diseases are seen frequently small petechial and confluent hemorrhages. In rabbits the distribution of the consolidated areas is variable; in the acute cases they are for the most part situated in small patches near the periphery of the lung; in the more chronic ones they tend to become confluent and may be located more centrally. In several specimens the centers of these consolidated areas appeared to be necrotic and were studded with

[4] Jour. Infect. Dis., 1913, 12, p. 42.

little yellow pin point size foci which on scraping often yielded plugs of necrotic material. While human influenzal pneumonia presents in the main a similar pathological picture one obvious difference is the lack of the central necrosis in the small consolidated areas and a greater tendency to become confluent, resulting in the massive confluent pseudolobar pneumonia, socalled.

Microscopically the rabbit bronchopneumonia shows a more marked perivascular edema and leukocytic infiltration than does the human type. The central necrosis above mentioned is much in evidence in the rabbit lung while little if any mention is made of it in the human disease. The exudative material is perhaps richer in cell content than is the case in human influenzal bronchopneumonia. Fibrin is not especially abundant, simulating therefore the human influenzal bronchopneumonia. Small miliary abscesses are not infrequently found. Focal necrosis of the pulmonary blood vessels was not evident in the rabbits as described by LeCount [5] in human influenzal bronchopneumonia.

In order to compare the pathogenesis of these two infections it will be necessary to discuss experimental pneumonia. There are two principal theories with respect to the initial mode of pneumonic infection, namely, the hematogenous which has received little experimental support, having consistently failed in the hands of Wadsworth,[6] Rasquin [7] and Armstrong,[8] and the bronchiogenic, which has received a certain amount of confirmation in the experimental production of pneumonia in animals by various methods of intratracheal or intrabronchial insufflation.

Müller [9] undertook a study of the pathogenesis of aspiration pneumonia experimentally produced in rabbits by vagotomy. From his observations he inferred that the bacteria gained entrance into the pulmonary tissue at the point where the cuboidal epithelium of the terminal bronchiole gave place to the flattened epithelium of the alveolar duct and atrium and that the invasion was facilitated by the mechanical injury produced by aspirated foreign material. He established the fact that further spread of the infection was by way of the interstitial tissue of the lung framework and by way of the alveolar walls.

[5] Jour. Amer. Med. Assn., 1919, 72, p. 1519.
[6] Am. J. Med. Sc., 1904, 127, p. 851.
[7] Arch. med. exper. et d'anat. path., 1910, 22, p. 804.
[8] Brit. Med. J., 1914, 2, suppl. 57.
[9] Arch. klin. Med., 1902, 74, p. 80.

Blake and Cecil [10] state that pneumonia was consistently produced in normal monkeys by intratracheal injections of pneumococci and showed that the pneumonia produced ran a clinical course identical with that of man. They furthermore state that attempts to produce pneumonia by subcutaneous or intravenous inoculations have consistently failed and therefore conclude that pneumonia is a bronchiogenic and not a hematogenous affair.

They, however, obtained their experimental results by intratracheal inoculation of the animals through needle puncture. Winternitz, Smith and Robinson [11] have pointed out that in such inoculations, the needle, though sterile on entry, is unquestionably infected when it is withdrawn and consequently a possible path of infection to the lung may be found elsewhere than through the lumen of the trachea. They demonstrated that the submucosa of the trachea and bronchi furnishes a pathway of infection to the lung. It contains a rich plexus of lymphatics prominent everywhere, devoid of valves. There is a continuity throughout this lymphatic system so that bacteria which once find their way into it may easily spread.

In this rabbit bronchopneumonias produced by intratracheal injections through a soft rubber catheter, the process was apparently not bronchiogenic in character, for the patchy, confluent consolidations were not situated near the large bronchi or the hilum of the lung; the larger bronchi in the greater proportion of cases were uninvolved and no evidence of bacterial passage through the mucosa was discernible. The consolidations were situated indiscretely over the lung surface, both near the periphery and the center and often numbering as high as 15 to 20 to a lobe. Microscopically the lymphatics, especially the perivascular, were dilated and infiltrated with leukocytes. Bacteria were not seen in them. From this it seems reasonable to assume that the rabbit bronchopneumonia is a hematogenous or lymphogenous affair in contradistinction to human influenzal bronchopneumonia. This view is in harmony with the fact that this infection in rabbits often manifests itself as a septicemia, indeed is often called rabbit septicemia.

No direct evidence was obtainable as to the site of the primary invasion. That the bacteria passed through the mucosa somewhere near the hilum of the lung and entered the lymphatic system seems probable for two reasons: first, the catheter was inserted to the

[10] J. Exp. Med., 1920, 31, p. 445.
[11] Bull. Johns Hopkins Hosp., 1920, 31, p. 63.

bifurcation of the trachea and second, the peribronchial lymphatics were involved in most instances.

In no instance have observations been recorded of the tendency to multiple abscess formation in human influenzal bronchopneumonia comparable to the striking process seen in rabbit pneumonia in which the consolidated areas undergo an intense central necrosis without delimitation of the process by capsule formation. Large regional softenings as seen at times in human lungs following pneumonia were not observed in the rabbits.

SUMMARY

Rabbit bronchopneumonia may be caused by B. bipolaris which belongs in the hemorrhagic septicemia group. The disease may be produced experimentally.

Under natural and experimental conditions a bronchopneumonia appears which is usually distributed throughout all portions of the lung, both peripherally and centrally, the microscopic picture being dependent on the stage of the disease. Peribronchial and perivascular infiltration of the lymph spaces and regions of central necrosis were the most constant lesions.

A comparison of rabbit pneumonia and influenzal pneumonia as described by various writers indicates certain points of similarity, but also certain differences. Grossly and microscopically both are bronchopneumonic processes, often confluent in type. They differ, however, in that in rabbit pneumonia the perivascular and peribronchial leukocytic infiltration of the lymph spaces and the regions of necrosis in the consolidated portions are more constant and striking features. The latter appears to be especially distinctive of rabbit pneumonia.

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MULTIPLE BRAIN ABSCESSES SECONDARY TO BRONCHIECTASIS AND KYPHOSCOLIOSIS[1]

BY CLARENCE C. SAELHOF

The etiology of brain abscess is, in approximately 60 per cent. of the recorded cases, due to a suppurative condition of the middle ear. Carious processes of the bones of the skull, chiefly the ethmoid, sphenoid, mastoid, frontal, etc., give rise to about 15 per cent. Brain abscess secondary to orbital injury, nasal and pharyngeal infections, traumatisms, foreign bodies, etc., are the causative agents in approximately 15 per cent.

The type of abscess with which this article deals is secondary to bronchiectasis. It may result from other respiratory diseases. Coyley,[2] Williamson,[3] Porot,[4] Rivet,[5] Grunberger,[6] Schorstein,[7] Bramwell,[8] and others have reported cerebral abscesses of hematogenous origin from a suppurating bronchitis or bronchiectasis. Fruhwald,[9] Ghon,[10] and Dick[11] report brain abscesses caused by *B. fusiformis;* the two former authors record cases in which this organism caused meningitis, with subsequent involvement of the brain. Dick is the only one reporting a case of cerebral abscess caused directly by the *B. fusiformis* without subsequent involvement of the meninges. It is stated by Stengel that brain abscess secondary to lung infections are transmitted either by the blood stream or through the retropharyngeal lymphatics. The case which I herewith present is undoubtedly hematogenous in character, having been transmitted from the bronchiectatic part of the lung to the terminal blood vessels of the brain.

W. C., aged 30, male, colored, U. S. A., admitted with a diagnosis of

[1] From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine, Chicago.
[2] Trans. Path. Soc., London, 1883–4, XXXV, p. 12.
[3] Ibid., 1893–4, XIX, p. 379.
[4] Bull. et mem. Soc. anat. de Par., 1905, LXXX, p. 897.
[5] Bull. Soc. med. d. hop. d. Lyon, 1904, III, p. 299.
[6] Prog. med. Wchnshr., 1907, XXXIII, p. 171.
[7] Lancet, London, 1909, II, p. 843,
[8] Rev. Neurol. and Psychiat., Edinb., 1910, VIII, p. 77.
[9] Monatschr. f. Ohren., Berl. & Wien., 1913, XLVIII, p. 1021.
[10] Cent. f. Bact., Vol. 81, p. 243.
[11] Trans. Chi. Path. Soc., 1914, IX, p. 95.

Pott's disease and tuberculous meningitis. Patient complains of rigidity of neck and severe pain in the head.

Present Illness: Patient took sick five days previously with headache, followed by chill and was bedridden afterwards. After the second day his neck became stiff and this stiffness gradually increased. There was spasticity of the right leg with loss of control of the same. No vomiting. Had an initial chill; sweating was constant. Patient lost eight pounds in the last two months. There have been no convulsions. On admission to the hospital, the respiration was shallow and thoracic; the pulse regular and of fair volume.

No history of tuberculosis or cancer in his family. He had smallpox ten years ago, gonorrhea two months ago, and a hard chancre a few years ago.

Accidents: Patient attributes his present trouble to an accident occurring fourteen years previous to admittance, having been caught in an elevator at that time and claims that his back was broken. He remained, however, in the hospital for only thirty days.

Physical examination: On admittance, increased reflexes, rigidity of the neck, and moderate spasticity of the right lower and right upper limbs associated with a stuporous position were evident. Patient could move head only from side to side, being unable to move it forward to the slightest degree. Eyes were negative. Kyphosis extended from the tenth dorsal vertebra to the second lumbar vertebra, with a swelling to the right of the spine in this region about the size of an orange. On examination of the extremities, the right arm gave slight spasticity, the right leg gave moderate spasticity.

Reflexes	Right	Left
Patellar	+++++	++++
Babinski	0	0
Ankle clonus	+	+
Gordon	0	0
Chadwick	0	0
Oppenheim	+	+
Kernig	+	+
Brudzinski	+	0
Brudzinskibilateral	+	+
Cremasteric	+	+
Abdominal	+	+
Triceps	+	+
Supinator	+	+

Laboratory examinations: Wassermann reaction of the blood serum was negative three times, as was likewise the spinal fluid. Spinal fluid contained albumin (++) by the Ross-Jones method, with no sugar and 104 lymphocytes per c. mm. Urine was negative. Blood examination gave a Hb. estimation of 80, a red cell count of 4,100,000 and a white count of 8,200 cells.

A tentative diagnosis of poliomyelitis was made. A week later, the patient gradually improved. Two days later, the patient became stead-

ily worse, with spasticity of both arms, temperature ranging from 98 degrees to 103.8 degrees, and died in a comatose condition nineteen days after admission. Patient had had no convulsions since entering the hospital.

A *necropsy* was performed and the anatomical diagnosis was as follows:

1. Bilateral brain abscesses.
2. Pus in the right lateral ventricle.
3. Bronchiectasis of the right lung.
4. Fibrosis of the right lower lobe of the right lung.
5. Bilateral adhesive pleuritis.
6. Left broncho-pneumonia.
7. Kyphoscoliosis.
8. Fetal lobations of both kidneys.
9. Chronic localized fibrous peritonitis.
10. Alopecia.
11. Sacral decubital ulcers.
12. Hemorrhoids.

Little need be said about the appearances of the different organs grossly, as they appeared practically normal.

The cardiac musculature microscopically shows a segmentation and fragmentation which have been described as being associated with acute infections or sudden deaths. Voluntary muscle appears normal.

In the kidney there is faint outline of the former cells and the cytoplasm has become granular. Some of the cells are loose in the lumen of the tubules. The glomeruli are swollen, completely filling the capsule of Bowman. The capillaries are extremely distended.

The spleen shows hyperplasia of connective tissue in the capsule, trabeculæ and arterial walls; otherwise it is normal in appearance.

Liver shows no change except slight fatty infiltration.

Kyphoscoliosis was present from the tenth dorsal vertebra to the second lumbar vertebra, and appeared as an extremely marked deviation to the left. This curvature of the spinal column formed a large, hollow cavity on the right side, of a size much larger than a person's two closed fists. Into this was wedged the right pleura and lung, firmly bound down by extremely dense, fibrous adhesions to the spinal column and chest wall. There was marked fibrosis of the lower lobe of the right lung. The pleura of the lungs were covered by fibrous tags, which firmly bound them to the parietal pleura. Crepitus was present throughout all portions of the upper lobes of both lungs. On section, the lungs presented a moist edematous appearance, with prominent outstanding bronchioles. In the left lung were small patches of broncho-pneumonia. The right lower lobe showed wide, outstanding dilated bronchi surrounded with masses of dense fibrous adhesions.

Microscopical sections of the left lung showed dilated bronchioles filled with a homogeneous staining substance and surrounded by a red

staining consolidation largely made up of white cells and erythrocytes. The air cells are densely packed with cellular exudate near the bronchioles, while near the periphery of the area, the alveolar exudation becomes less. Other alveoli are distended and quite empty. The consolidations radiate into the adjacent tissue and presents a typical picture of broncho-pneumonia.

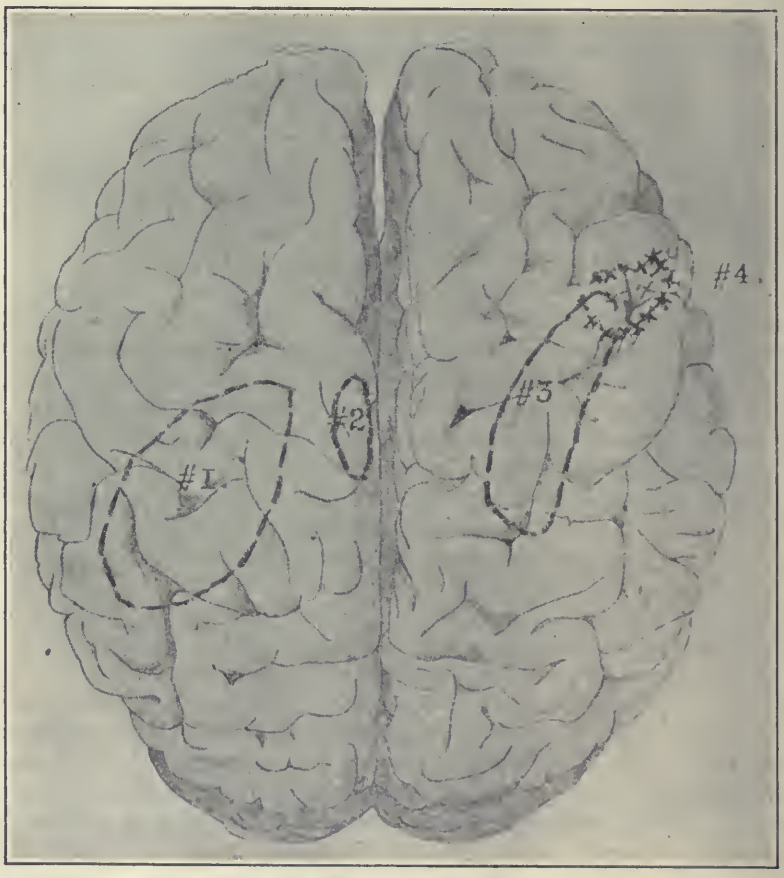

Fig. 1.

On opening the cranial cavity, there was noted a slight increase in the amount of cerebrospinal fluid and slight hyperemia of the meninges. On section of the brain, four abscesses were found, two in each hemisphere. The outline and extent of the cavities are illustrated in Fig. 1.

Cavity No. 1 measured 45 mm. in length and at its posterior end was 7 mm. from the mesial surface of the brain. The diameter of the abscess in its posterior portion was 20 mm. with a capsule of connective tissue

completely lining it, 1 mm. in thickness. It continued forward and downward so that the anterior end of the abscess was 30 mm. from the dorsal surface of the brain whereas the posterior end was 20 mm. from the dorsum of the brain. In this abscess, only the white matter of the brain was involved.

Cavity No. 2 was much smaller, measuring 6 mm. in length, with a dorso-ventral diameter of 9 mm. and the medio-lateral diameter of 5 mm. giving, on cross-section, a sort of diamond shaped cavity. A distinct capsule 1 mm. thick can be seen lining the cavity throughout its entire periphery. This abscess involves both the white and gray matter, lying at the anterior aspect of the gyrus temporalis medius, between the gyrus temporalis superior and the gyrus temporalis inferior.

Cavity No. 3 measured 57 mm. in length, and had collapsed following accidental evacuation of pus during removal. It measured 41 mm. in the dorso-ventral diameter, a width of 1 mm. (due to its collapsed conditions) and surrounded throughout its entire course with a capsule of 1.5 mm. in thickness. The posterior tip of this cavity involves the white matter of the brain only; as it progresses forward, it extends ventrally till it has a small outlet to the exterior surface of the brain on the mesial side just above the corpus callosum. This opening, which was at its anterior end, in the region of the gyrus centralis anterior, was caused mechanically in removal of the brain; there was no evidence of meningitis. (Undoubtedly this part of the cavity involves the motor area controlling the neck, arm and leg, hence the spasticity in these parts.)

Cavity No. 4 is situated at the anterior end of cavity No. 3 and slightly mesial to it and is small in comparison with the other abscess. It is 9 mm. in length and 8 mm. in width. A capsule .5 mm. thick encloses the whole abscess. It is located 15 mm. from the dorsal surface and 10 mm. from the medial surface of the brain, and involves only the white substance.

Microscopical sections of the walls of these abscesses stained with hematoxylin and eosin varied according to the localization of pus foci, areas of necrosis, hemorrhages, etc.

The polymorphonuclear cells were the most prominent type of cell seen throughout the inflammatory area. They were in all stages of degeneration; some had as high as five nuclei, while the predominating number in all sections were two or three nuclei. Many disintegrated cells were evident, only the nuclei being visible and showing fragments of nuclear material.

Plasma cells, variable in size and with the large type predominating, were found in small numbers. Nuclei were generally single, although some cells with double nuclei and a few with triple nuclei could be distinguished. Disintegration was evident in some of the nuclei by the protrusion of granules; in places, free nuclei were found. The plasma cells seemed to be located near the periphery of the inflammatory area and only a few were found near the pus zone. Large, round, vacuolated

cells, with a round or oval nucleus, placed at one end of the cell, were discernible both within and without the pus zone, in greater numbers, however, outside the pus area. These appear to be large mononuclear

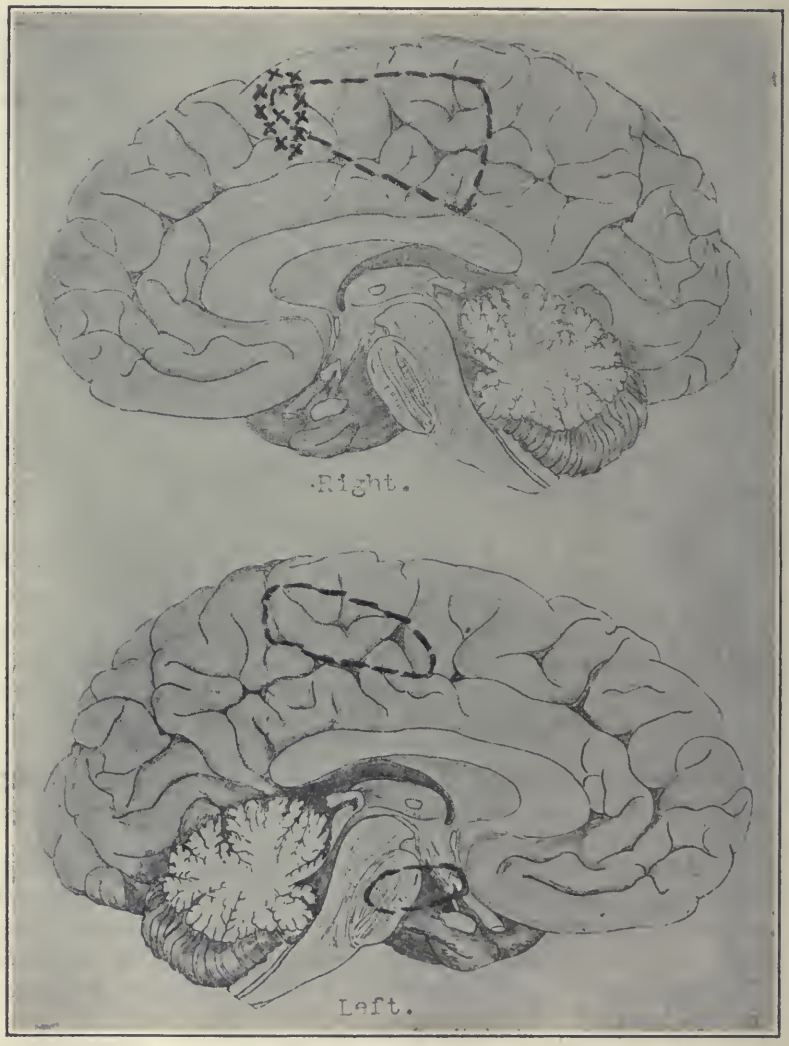

FIG. 2.

wandering cells. Fibroblasts could be seen in all parts of the inflammatory area.

An extremely thick exudate, composed chiefly of fibrin, was seen in sections of all the abscesses. It was laid down in such compact masses

that it was hardly possible to recognize it as fibrin. It appeared in long, wide strands, at right angles to the wall of the abscess, devoid of any cellular structure, and simulating the appearance of a homogeneous, collagenous material. In certain small areas, the characteristics of fibrin could not be made out, and it was only by careful study that this collagenous appearing material was determined to be that of fibrin. In some sections, particularly those of abscess No. 3 and No. 4, small round or oval masses were seen deposited in the brain tissue just at the edge

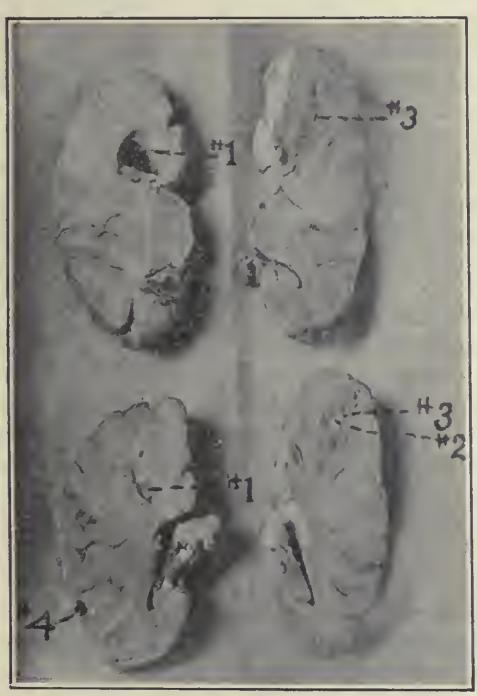

FIG. 3.

of the inflammatory area, completely surrounded by a small capsule of connective tissue.

Necrosis is evident in two regions. Here are completely disintegrated cells, and many pycnotic nuclei. Perivascular infiltration was noticeable throughout with many of the vessels hyperemic. In some portions, extensive foci of hemorrhage were evident.

Few ganglion cells appear in the inflammatory zone. Outside this area, these cells have lost most of their cytoplasm, only the nucleus remaining. These nuclei take the stain very poorly, due to the lack of chromatin material.

Most of the abscesses involved only the white substance of the brain,

comprising the association fibers, commissural fibers and the projection fibers. Only two abscesses involved the gray matter to any degree: abscess No. 3, which had eroded away a portion of the gray matter at its anterior end in the region of the gyrus centralis anterior. This eroded portion is located in the motor area controlling the movements of the neck, arms and lower limbs, and undoubtedly was the cause of the spasticity of these portions of the body. Abscess No. 2 involved a portion of the gray matter in the gyrus temporalis medius and probably involved a portion of the auditory center, although no record of defective hearing is recorded in the clinical history. The rest of the involved tracts were the association, commissural and projection fibers. The striking thing is that more definite symptoms of derangement of the paths of the brain due to the large size of the abscesses and their locations did not occur.

On section of the brain, a large quantity of green, foul-smelling pus exuded and, on smear preparations, stained with a Gram stain showed Gram positive cocci in diplo- and short-chain forms, and a Gram negative, curved, beaded, often long, segmented bacilli with sharp pointed ends (*B. fusiformis*). A growth on blood agar under anaërobic conditions gave Gram staining cocci and long Gram negative bacilli. Under aerobic conditions, neither cocci nor bacilli were found; only a few large, white colonies, which were, without doubt, contaminations.

From the bronchiectatic cavities a bloody, mucoid exudate was obtained. Smears stained in the same manner gave Gram positive cocci in short-chain formation, and Gram negative bacilli, curved and sharp pointed. Cultures under anaërobic conditions gave a Gram positive cocci, with long Gram negative bacilli (*B. fusiformis*). Under aërobic conditions, the tubes were overgrown with putrefactive aërobic organisms.

Kyphoscoliosis was present from the tenth dorsal vertebra to the second lumbar vertebra, and formed an extensively marked deviation to the left. The etiology of the kyphoscoliosis was apparently due to the "broken back" which the patient had had fourteen years previous. This curvature of the spinal column caused the large, hollow cavity on the right side described above and into this aperture was wedged the right pleura and lung. It was in this recess or pocket that the bronchiectasis developed and here the anaërobes, *B. fusiformis* and anaërobic streptococci, grew, there being little chance for drainage. Presumably thence by the hematogenous route, the organisms localized in the brain, causing the multiple abscesses. I have been unable to find any record or report in the literature of a brain abscess occurring secondary to kyphoscoliosis.

The organisms isolated from the pus of the brain and the bronchiectatic cavity were, without doubt, the same; long, spindle-shaped bacilli with their greatest diameter in the middle and tapering out to narrow ends, taking the Gram stain both positive or negative, depending on the

degree of washing with alcohol. Dick[11] found the actinomycosis-like bodies in the pus of the abscess, and from the pus isolated pure cultures of *B. fusiformis* similar to those found in tonsils. Such bodies were not seen in our case.

As to pathogenesis one can readily see how with the lung packed away in a pocket formed by the kyphoscoliosis of the spinal column, with poor drainage, a bronchiectasis would furnish conditions for the growth of the fusiform bacillus; thence through the blood stream, the infection localized in the brain, causing multiple abscesses. Most authors agree that commonly various organisms are transmitted through the blood stream or through the lymphatics to the brain. As the most frequent invaders may be mentioned the pneumococcus, tubercle bacillus, staphylococci and the streptococci; fusiform bacilli are rarer invaders. Fusiform bacilli are found occurring in the mouth, beneath the gums, around carious teeth, in tonsils both normal and diseased, in Vincent's angina, noma, etc. Davis[12] demonstrated the occurrence of *B. fusiformis* living in symbiosis with streptococci and spirilla in the actinomyces-like granules found in normal and diseased tonsils.

It would seem reasonable that the fusiform bacilli and cocci, found in these various localities, pass down the trachea and bronchi into the bronchiectatic cavities where they may cause suppuration and later by the hematogenous route localize in the brain causing brain abscess.

Summary

A case of multiple bilateral brain abscesses, secondary to bronchiectasis caused by the wedging of the lower lobe of the right lung into a pocket formed by kyphoscoliosis is described.

The causative agents isolated and cultivated from both the abscesses and the suppurating lung were *B. fusiformis* and anaërobic streptococci.

The most probable route by which the infection travelled from its primary focus was the blood stream.

[12] Jour. Infect. Dis., Vol. 14, no. 1, January, 1914, p. 144.

RECOVERY OF STREPTOCOCCUS HEMOLYTICUS FROM RESTAURANT TABLEWARE

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"Dangers of dirty dishes" this paper might be termed. It is a quantitative presentation of just what these dangers are in public restaurants. They constitute a vexing problem to every health officer, and he needs the plain facts for the education of his public. With these, his people will back him in efforts to improve such places.

THE following experiments were performed to determine how commonly hemolytic streptococci could be isolated from the supposedly clean eating utensils obtained in a group of restaurants and cafés in Chicago.

Cummings (Am. Jour. Pub. Health, Vol. IX, No. 6, June, 1917, p. 424) reports that of 23 sets of tableware he recovered hemolytic streptococci in 91%; pneumococcus from 17% of nine sets of tableware; diphtheria from 2% of 26 sets of tableware; and *Streptococcus viridans* from two sets of tableware (100%). Lynch and Cummings (Am. Jour. Public Health, Vol. IX, January, 1919, p. 25) record that the average bacterial count in 54 specimens of water used to wash eating utensils was 4,000,000 per cc.

Our procedure for taking cultures was as follows: A large number of throat swabs were made and sterilized in test tubes in the hot air oven at 160° C. each day before being used. They were then moistened under aseptic conditions, in sterile, distilled water in order that the organisms might adhere to the swab.

The different articles, such as spoon, knife, rim of the water glass, surface of the plate, etc., were swabbed as they were placed before us on the restaurant tables. The swabs were brought back to the laboratory, and smeared over plain agar plates (made neutral by means of the colorimetric method, using bromthymol-blue as an indicator) to which 7 drops of fresh blood had been added. These plates were incubated for 24 hours at 37° C. and at the end of that time we made macroscopic and microscopic examinations of the different types of colonies.

In making a macroscopic examination of the different types of colonies, the size, shape, margin, texture and color were noted under the hand lens so as to determine the type of organism as far as possible. In this manner, such colonies as *Streptococcus hemolyticus, Bacillus subtilis, Staphylococcus aureus* and S. albus were easily determined. A subsequent microscopic examination of the same colonies was made to verify the macroscopic findings, using ordinary and, when necessary, special stains. Sub-cultures of all suspicious colonies were made for further identification and upon special media, such as the sugars, gelatin, etc. whenever necessary. For animal experiments broth cultures were used.

The articles from which we obtained organisms were as follows: spoon, knife, fork, butter-dish, glass and plate. They were from nine different restaurants. An extra swab as a control was exposed to the air for approximately the

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same length of time as was taken in swabbing the tableware. The data obtained are presented in Table I.

The groups of organisms encountered were not numerous. We were chiefly concerned with the hemolytic streptococcus. We found this organism in the percentages given in Table 2. Three different strains of hemolytic staphylococci were found; we did not test their pathogenicity on animals. Most of the strains of staphylococci isolated were *S. albus;* three strains isolated were *S. aureus.* The pneumococcus was typical of Type III, having the mucoid and slimy growth, a distinct capsule and fermenting mannit and inulin. The strain of *B. coli* was identified by its odor, the shiny white colonies with dentate edges, its reaction with dextrose and lactose and by its morphology. Other organisms were encountered but were not identified.

The *Streptococcus hemolyticus* colonies were of the typical pin-point variety, having a sharp, clearly defined zone of hemolysis about them, and, on inoculation in broth, produced long chains of six, eight, and ten cocci as a rule; one strain producing chains of 16 to 20 cocci.

Each of the four strains of hemolytic streptococci, grown in broth culture, was inoculated in 1.5 cc. doses intravenously in rabbits, weight approximately 1200 grams. There occurred in all a loss of weight, and swelling of the joints. Death occurred in 24 to 60 hours. Post-mortems of these animals showed congestion of the internal viscera and lesions of the joints and endocardium. Cultures made from the joints and the heart's blood in each rabbit gave organisms identical with those injected.

TABLE I.*

Restaurant	Character of place	Spoon	Knife	Fork	Butterdish	Glass	Plate	Control
1	Fairly clean	B. subtilis	Strep. hemolyticus. Staph. albus	B. subtilis	Staph. albus B. subtilis	Staph. albus	Strep. hemolyticus	.....
2	Clean	Staph. albus	B. subtilis	B. subtilis	B. subtilis...	Strep. hemolyticus	B. subtilis	.....
3	Very dirty	Staph. albus	Staph. albus	Staph. albus	Staph. albus	Staph. albus	Staph. albus	.....
4	Reasonably clean	Staph. albus	B. subtilis	B. coli	Staph. aureus	Staph. albus	Staph. albus	.....
5	Fairly clean	Staph. albus	Staph. albus B. subtilis	Staph. albus B. subtilis	Staph. albus	Staph. albus B. subtilis	Staph. albus	.....
6	Dirty	B. subtilis	Pneumococcus Type III	Staph. aureus	.....	B. subtilis	B. subtilis Staph. hemo.	B.subtilis.
7	Fairly clean	Staph. albus B. subtilis	Strep. hemolyticus B. subtilis	B. subtilis	Staph. albus	Staph. albus	Strep. hemolyticus	.....
8	Fairly clean	B. subtilis	Staph. albus	Staph. albus	Staph. albus B. subtilis	Staph. albus	Staph. albus B. subtilis	....
9	Dirty	B. subtilis	Staph. albus	No growth	.....	Staph. albus	B. subtilis	.....

*Only the predominating organisms were classified.

TABLE II.

Bacteria	No. of Examinations	Number Positive	Percentage Positive
Streptococcus hemolyticus	63	4	6.35%
Pneumococcus	63	1	1.60%
B. coli	63	1	1.60%
Staphylococcus aureus	63	2	3.20%
Staphylococcus albus	63	31	50.81%
B. subtilis	63	23	36.50%

Discussion

It is evident from these results that the dishes and eating utensils are not sufficiently cleaned and washed to render them sterile. The dishes after being washed and drained usually are stacked on an open shelf exposed to dust and droplets from the sneezing, coughing, and spitting of both the employees and customers who may be carriers of virulent bacteria.

Direct contamination of eating utensils from the mouths of persons harboring virulent bacteria of various kinds naturally must occur. Many persons have sore throats, colds, influenza, and other contagious diseases and go about their business regularly and eat in public places. This source of dangerous bacteria is probably the most important one in contaminating eating utensils. Lynch and Cummings (Am. Jour. Pub. Health, Vol. IX, No. 1, January, 1919, pp. 24-38) isolated 12,000,000 organisms, many of which were streptococci, from the ladle of a spoon used by a streptococcus carrier. Hands, clothes, handkerchiefs, etc. of the customers harbor germs and no doubt further aid in carrying infection.

Another source is from indirect contamination. Cummings. (Am. Jour. Pub. Health, January, 1919, Vol. IX, No. 6, pp. 415) found that 80% of the cases of influenza from 22,084 troops epidemiologically investigated occurred among troops using mess kits. The men invariably used their hands as mops to clean kits. He states that the distribution of influenza in this manner is by indirect contact and chiefly by the hand to mouth route of travel. Our findings corroborate his experiments. There is also the possibility of infection by the aerial route.

It is easily seen that pneumonia, influenza, diphtheria and other throat and lung infections might readily be disseminated through the medium of the dirty and greasy plate. During a pandemic such as we have just experienced, it is reasonable to suppose that a certain percentage of the cases were contracted from the eating utensils. Less attention has been paid to this possible mode of dissemination than it deserves.

People working about restaurants should take special care to avoid contamination of hands or clothes. All dishes and eating utensils should be thoroughly cleaned in hot water made alkaline with strong soap. It is not sufficient merely to pass the greasy dishes through hot water. The alkalinity is an effective germicide. Strong soapy water will do a great deal towards killing and removing pathogenic organisms. The surfaces of all dishes should then be subjected to live steam for at least five minutes. It is absolutely necessary that each dish be subjected to this procedure. If the plates, etc., are stacked in piles the steam does not reach the entire surface. Mannheimer and Yhavez (Am. Jour. Pub. Health, 1917, Vol. VII, pp. 614-618) suggest subjecting eating utensils to a temperature of 80° C. for one minute. We, however, think that the utensils should be subjected in a hot air oven to a temperature of at least 100° C., for 30 minutes.

The following suggestions seem pertinent.

(1) All dishes should be thoroughly scoured and washed and subjected to live steam at least five minutes and then dried in a hot oven.

(2) All dishes and utensils should be stacked in a covered oven or box and not in the open air.

(3) The floor should be washed down once a day and the fixtures at least once a week with a strong germicide. Lysol is probably as efficacious and as easy to use as any; its odor is objectionable to some people.

(4) The employees of restaurants should be examined by a health officer at regular intervals in order that carriers may be detected.

Summary

(1) Hemolytic streptococci were iso-

lated from the "washed" dishes and tableware, in small restaurants and cafes.

(2) 6.35 percent of the articles examined yielded this organism.

(3) The strains of *Streptococcus hemolyticus* were virulent for rabbits. They correspond to the human type.

(4) For the protection of the public a better system of washing dishes is needed in the small eating place.

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THE BACTERIAL CONTENT OF TELEPHONES WITH SPECIAL REFERENCE TO RESPIRATORY PATHOGENS.*

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Bissell (1) sets forth the question as to whether the public telephone transmits disease. A limited amount of work was performed with special reference to the *B. diphtheriae,* the conclusions of which were that it was impossible to demonstrate this organism on the transmitter. Other work has been done on the bacterial flora of telephones, particularly by Allan (2) who isolated *B. tuberculosis* from public call office booths, and by Tomarkin (3) who worked with pathogenic organisms (*B. diphtheriae, B. typhosus,* etc.) with special reference to their viability under varying conditions of exposure, disinfection, etc.; however, none of the articles discusses completely the bacterial content of the transmitter or receiver.

That the public telephone might be a causative factor in the transmission of certain respiratory diseases, for example, influenza, pneumonia, etc., either by direct contact (mouth of the individual placed immediately in the transmitter, or the receiver approximated directly to the ear), or by some other mode of transmission, led me to investigate the bacterial content of a series of telephones in Chicago.

Cultures from the transmitters and receivers of 94 telephones were taken at various periods as follows: 21 before the influenza epidemic (during the summer of 1919); 28 during the epidemic (the fall and winter of 1919–20); 24 following the epidemic (the first week of February, 1920), and 21 in a second post-epidemic group (the first week of March, 1920).

The telephones selected were all public, located in booths, usually in drug stores, cigar stores, etc., and were used by a large number of people each day. They were not taken from an isolated territory, but included large districts, both residential and factory. The telephones were divided roughly into three classes; "clean," in which the mouth-

* From the Department of Pathology and Bacteriology, University of Illinois College of Medicine, Chicago.

piece and booth seemed fairly clean; "filthy," in which a residue of dirt, grease, etc., was present in the transmitter and the booth none too clean; and "very filthy," in which the transmitted and receiver were covered by a thick layer of dirt, buccal and nasal secretions, etc.

The manner of obtaining the organisms was as follows: Sterile throat swabs were dipped into sterile salt solution and replaced into too clean; and "very filthy," in which the transmitter and receiver were swabbed by encircling them three or four times. These swabs were returned to the laboratory, applied first to Loeffler's blood serum for the detection of the diphtheria bacillus, next to "chocolate blood agar" for the detection of the influenza bacillus, and lastly inserted into plain veal broth (pH 7.0, using phenol red as indicator), and incubated at 37° C. for ten hours. At the end of eight hours, the Loeffler blood serum tubes were examined for the diphtheria organism. The "chocolate blood agar" was allowed to incubate for forty-eight hours, being examined for all suspicious colonies in the meantime. One loopful of the ten hour growth in broth was inoculated into blood agar (12 drops of fresh rabbit's blood to every 5 c.c. of agar); plates were poured and then incubated for twenty-four hours.

Macroscopic with subsequent microscopic examinations of all colonies were made and, in suspicious cases, transplants made to obtain pure cultures.

The organisms encountered were not numerous. I was chiefly interested in those pathogenic for man. The types and percentages of organisms found during the different periods are listed in the accompanying Table.

TABLE I.

Percentage recovery of various bacteria during different periods.

Bacteria.	Preceding influenza epidemic. 21 telephones examined.	During influenza epidemic. 28 telephones examined.	Post-influenza epidemic, Feb. 1, 1920. 24 telephones examined.	Post-influenza epidemic, Mar. 1, 1920. 21 telephones examined.
Streptococcus hemolyticus	7.69%	4.00%	1.47%	2.75%
Staphylococcus hemolyticus	11.53%	9.00%	5.88%	6.25%
Staphylococcus aureus	3.85%	6.00%	5.88%	2.50%
Staphylococcus albus	32.69%	36.00%	41.17%	34.75%
Bacillus diphtheriae	0	2.00%	0	0
Diphtheroid bacillus	0	1.00%	0	0
Pneumococcus	0	1.00%	0	0
Bacillus coli	11.53%	6.00%	16.17%	12.50%
Bacillus subtilis	32.69%	35.00%	29.41%	41.25%

In all, 11 strains of hemolytic streptococci (Beta type) were recovered. On blood agar, they appeared as a sharp, pin point colony with a clear, distinct zone of hemolysis. Ten strains were virulent for rabbits. One strain was non-virulent, the animal surviving 33 days after an injection of 5 c.c. of a twenty-four-hour broth culture.

To determine virulence, broth suspensions of a twenty-four hour culture of the organism were injected into the marginal vein of the ear of young rabbits, weighing about 1000 grams. Arthritis and death usually occurred in from thirty-six to ninety-eight hours. Recovery of the organisms from the heart's blood and joints was usually possible. No attempt was made at differentiation by fermentation reactions.

Of the staphylococci encountered, a large number were hemolytic in character and retained that property throughout many transfers on artificial media. *Staphylococcus albus* was common. The colonies appeared large, white, moist and glistening, measuring 0.5 to 4 mm. in diameter; many were surrounded by a clear zone of hemolysis, in some cases distinct, in others, tending to fade out at the margins. Pathogenicity was not determined. *Staphylococcus aureus* was present in moderate numbers. They were hemolytic, but on artificial media soon tended to lose that property. One strain retained it for two months.

Two strains of diphtheria bacilli were isolated, both during the height of the influenza epidemic. Strain 7 was of the granular type, while Strain 10 was barred. .2 c.c. of broth suspensions of each were inoculated into guinea pigs. The animals died within fifty hours, necropsy revealing fibrinous exudation of fluid into the body cavities, with intense congestion of the viscera, especially the adrenals. Control animals were protected by antitoxin.

One strain of a diphtheroid bacillus was recovered. It resembled closely the true diphtheria organism, but was nonpathogenic for guinea pigs. According to Hamilton's (4) classification, it belongs to Group 1.

One strain of pneumococcus was found on a transmitter immediately after a person had finished conversation over the telephone. The organism was bile soluble, fermented inulin and possessed a distinct capsule.

Colon bacilli were present commonly in large numbers. They were identified by fermentation reactions. Bacillus subtilis was regarded as a contamination.

No influenza bacilli were isolated on the "chocolate blood agar."

Experimentally, the viability of the different types of organisms isolated was tried on transmitters under varying conditions. Ten mouthpieces were cleaned with soap and water until all debris had been removed, washed with alcohol and ether to remove the fatty material, and then immersed in a 1/1000 bichloride solution for three days. Ten other mouthpieces, filthy and more or less covered with mouth excretions, were taken directly from booths and swabbed in the manner described previously. The clean and filthy mouthpieces were set up side by side and tenacious, mucoid sputum, containing the types of organisms recovered, was planted on both and allowed to dry. Daily swabs were taken and plated on suitable media. The hemolytic streptococcus was recoverable after the longest period of time (four days) with the staphylococcus and pneumococcus in subsequent order. The organisms lived two to three days longer on the filthy transmitters than on the clean ones. The infectivity of a mouthpiece would therefore vary inversely with its cleanliness. This is in accord with facts already established.

In comparison with the transmitter, the receiver showed very few pathogenic organisms. This is as one might expect. Organisms may be implanted on the receiver from individuals suffering from a discharging ear. By intimate contact with the ear, as the receiver is during conversation, organisms could localize in the external auditory canal and under suitable conditions possibly set up an infection. An even greater danger might be the general dissemination of virulent organisms from running ears through contact of the receiver with the hand.

The telephones during the influenza epidemic contained more pathogenic organisms than during the interepidemic periods. However, hemolytic streptococci were more numerous preceding the epidemic than during or following it.

Numerous devices, as special mouthpieces, ear devices, etc., have been and are on the market to promote cleanliness. However, the following procedure appears to be of more practical value than any device now upon the market. The transmitter and receiver should be cleaned first by the use of good soap and warm water to remove the debris, and then sterilized in 1/1000 bichloride of mercury, lysol or some other disinfectant solution for a period of ten minutes; this to be done once or twice a week, with the daily removal of dust and secretions by means of cloths moistened in bichloride of mercury or some other antiseptic. The booths should be swept out at least once a week and disinfectants applied with subsequent proper ventilation.

From the above it is not to be concluded that the telephone plays even an important rôle in the transmission of infectious diseases. Indeed the data presented are of such character that no conclusions regarding frequency of transmission can be drawn therefrom. It is probable that the danger of infection from this source is slight. However the point may be emphasized that the telephone is an instrument upon which dangerous bacteria are commonly deposited and there continue to live for some time. This source of infected material should be known and as a possible danger under certain conditions should be given proper consideration.

Summary.

1. Various pathogenic bacteria are present and can be isolated from the transmitters and receivers of telephones.

2. Hemolytic streptococci were isolated in 15.9 per cent., the diphtheria bacillus in 2 per cent., and the pneumococcus in 1 per cent., from the transmitters and receivers of 94 telephones.

3. 90.9 per cent. of 11 strains of hemolytic streptococci isolated were virulent for rabbits.

4. Sterilization of telephones should be practised to prevent the spread of virulent organisms. Cleansing with soap and warm water and subsequent sterilization in bichloride of mercury, lysol, etc., for a period of 10 minutes, is recommended.

5. In speaking, the mouth should not come in direct contact with the transmitter. The public should be taught how to use the telephone hygienically.

BIBLIOGRAPHY.

1. Bissell, W. G.
1902–3. Does the public telephone transmit disease? Buffalo Med. Journ. N. S. XLII, 408.

2. Allan, F. J.
1908. The public telephone call office as a factor in the spread of disease. Lancet, London, I, 1862.

3. Tomarkin, E.
1906. Über d. Gefahren d. Ubertragung von Infektionsstoffen durch d. Telephon u. ihre Verhütung. Münch. med. Woch., LIII, 2435.

4. Hamilton, A.
1904. The question of virulence among the so-called pseudo-diphtheria bacilli. Journ. Inf. Dis., I, 690.