MELLON LECTURE

(UNDER THE AUSPICES OF THE SOCIETY FOR BIOLOGICAL RESEARCH)

UNIVERSITY OF PITTSBURGH

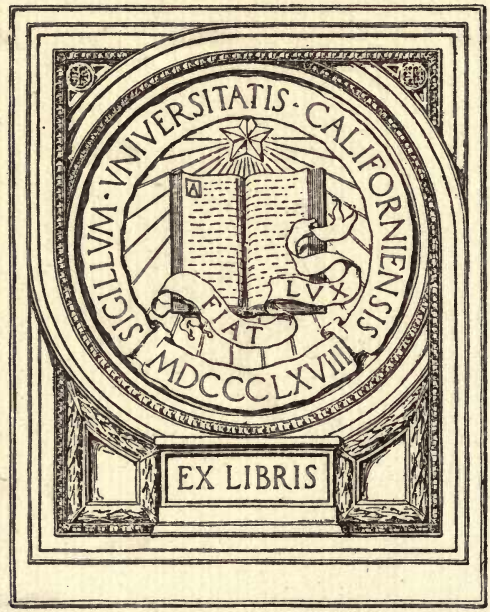
THIRD LECTURE

RECENT BIOCHEMICAL INVESTIGATIONS ON BLOOD AND URINE; THEIR BEARING ON CLINICAL AND EXPERIMENTAL MEDICINE

BY

OTTO FOLIN

1917



RECENT BIOCHEMICAL INVESTIGATIONS ON BLOOD AND URINE; THEIR BEARING ON CLINICAL AND EXPERIMENTAL MEDICINE

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It is generally recognized that progress in the development of any experimental science is more often due to the discovery of new methods than to new and more ingenious applications of old ones.

An unending procession of new remedies and new procedures moves across the field of clinical medicine. The wide attention bestowed on Abderhalden's pregnancy test may be cited as a specific illustration of the eagerness with which the medical profession is waiting for new methods. Brugsch's new nitric acid test for syphilis is another illustration. This test will doubtless prove even more alluring to those who have opportunity to try it; for, whereas the Abderhalden test was complicated and admittedly full of pitfalls, this new test for syphilis seems almost ideal in its simplicity. Its lack of "rhyme or reason" will not deter many from trying it.

In the science of biochemistry, too, we have had a constant succession of new methods, and a very important phase of the biochemical research of today is still the development of analytic technic. In the last few years there have been very great changes in the methods used for the analysis of urine, while the advancement in the field of blood analysis has been ever more remarkable.

The foremost characteristic of the successful modern analytic method is speed. With reference to the value of time, the biochemical investigator of today is no less keen than are the leaders in modern industry. Many of the older methods in use, say fifteen years ago, as, for example, Salkowski's method for uric acid, the Mörner-Sjöquist method for urea, or the Schlösing method for ammonia, were accurate enough, but they were slow, and they did not encourage the application of chemical methods to the study of clinical problems.

When a physician had to wait four days before he could learn how much ammonia a given urine contained, he did not bother himself very often about determinations of ammonia. And, as a matter of fact, this good old method for the determination of ammonia was so little used that investigators gradually forgot how the method must be employed in order to yield correct results.

Today any analytic method in urine analysis which cannot be finished within less than two hours stands in need of further revision. Most of the common determinations in urine and blood will become from fifteen to thirty minute methods for single determinations, and also several determinations will be made with little extra expenditure of time.

Almost every material reduction in the time and labor required for a given determination is followed by an increase in the number of investigators who make researches based on that determination. But it is only when we come down to the very shortest of biochemical methods that they begin to appeal to clinicians, and it is, I think, no small triumph for the modern technic that clinicians have begun to compete with laboratory specialists in the fields of research suggested by these new methods.

A considerable and constantly growing number of American physicians now possess laboratories in which chemical determinations are made. It is difficult as yet to appraise the probable value of this kind of work contributed by clinicians. A large proportion are busy practitioners who have considered their own time too valuable to permit them to acquire any personal mastery over the methods. Hence they hire technicians to do the work without themselves being competent to judge of the quality of the work. The net gain to science from such work must be distinctly problematic.

There is a legitimate and important use for technical assistants in the innumerable little laboratories springing up in connection with private and with hospital practice of medicine, but it seems to me extremely important that this use should conform to some reasonably honest standard of responsibility. The physician should know at least as well as his technician each and every kind of determination that the technician makes for him. Every method goes wrong now and then, and the physician who is as helpless as is the

technician when something does seem to be wrong has but meager qualification for the work he is trying to do.

The current misuse of technicians does impair somewhat the credibility of laboratory observations recorded by clinicians, but this represents presumably only a passing phase, and it does not obscure the fact that many clinicians are keen and alert for every new chemical method which is sufficiently practical for their needs. It is unfortunate that the laboratory expert and the competent clinician cannot be united in one person. It is unfortunate that methods must be simple and easy, as well as quick, before they can find any very widespread and sound application within the medical profession. The modern methods are wonders in quickness in comparison with the old ones, and they are not very complicated; but no man knows them who does not know how to check up his own results by working against theoretical figures.

A second characteristic of modern analytic technic, as distinguished from the old, is the tendency to make the methods microchemical. Formerly a full twenty-four hour quantity of urine was scarcely large enough to permit a complete analysis. Today 10 c.c. of urine is abundant for a determination of all the more common nitrogenous constituents. We still demand twenty-four hour samples of urine, and have gone on the assumption that we must have them to secure a satisfactory picture of the waste products; but we do not need them for the analyses. Indeed, many modern researches are based on analyses of urine representing periods much shorter than twenty-four hours.

The importance of being able to make quantitative studies on the basis of less than twenty-four hour urines is shown in the recent communications by Dr. Denis on creatin. We still do not know for certain some things which we must find out about the origin of this product. We know that the muscles contain creatin, or its precursor, and that in the urine we find normally (except in children) a great deal of creatinin, but only traces of creatin. It now turns out that these traces are not excreted, as is creatinin, at a substantially uniform rate from hour to hour. On the contrary, the creatin excretion is confined to a short period after each meal.

The time honored twenty-four hour system of urine analysis has been and will remain important; indeed,

it will remain indispensable in some kinds of metabolism work. The twenty-four hour system is, nevertheless, largely a relic of what might be called the nitrogen equilibrium period in the history of the science of nutrition. But nitrogen equilibrium has largely ceased to be a factor to which we must pay attention in metabolism studies on human beings. For we know perfectly well that it is only by means of carefully selected diets that we can give a person enough food to meet his energy requirements without at the same time giving him enough protein for the maintenance of a normal level of nitrogen equilibrium. We may often enough wish to determine the general level of nitrogen elimination, but it is rare indeed that we need to know or actually do determine any little discrepancy that may occur between the intake and the outgo of nitrogen. Nor can such discrepancies be determined except on the basis of experiments a great deal longer than one twenty-four hour period. Twenty-four hour urines are no longer needed even for very comprehensive analyses, and I am convinced that for many purposes, and particularly for clinical studies, we shall find that urine analysis based on definite three hour, or at most four hour periods of urine collection, will yield considerable new information and will greatly simplify the application of quantitative methods to the study of metabolism problems. The change will increase enormously the possibilities for doing quantitative metabolism work on patients, very few of whom can be relied on to collect twenty-four hour urines. The use of standard three hour urines will reduce to a minimum the extent to which the work can be ruined by carelessness or incompetence on the part of nurses; in fact, the nurse's part of the work will be almost eliminated. Private patients, even office patients, and outpatients in the hospitals, can be included in our metabolism investigations if we once get our metabolism period reduced from twenty-four hours to three hours.

For such work we shall need a large series of normal figures corresponding to the twenty-four hour figures which have been used in the past. I hope shortly to furnish such figures for the first three hour morning period and for a second period representing the effect of a standard test meal.

The practical advantages to urine analysis derived from the speedy microchemical colorimetric methods

are less fundamental than are the advantages gained from the application of the same principles to the analysis of blood. Before the introduction of these methods one could hardly say that there was such a thing as quantitative blood analysis. There were no definitely recognized methods, and in the scattered analyses attempted each investigator usually improvised some sort of procedure according to his own light and without reference to any general need for some system of blood analysis. For a qualitative test for uric acid in blood, from 100 to 200 c.c. were required, and even then positive results were obtained only with blood abnormally rich in uric acid. Today we can make quantitative uric acid determinations on any kind of human blood, and do not need more than 10 c.c. for the determination. If necessary we can make the determination with 5 c.c. or less.

The merit of the newer microchemical system of blood analysis was convincingly demonstrated in connection with studies on the problem of protein absorption. For more than a generation different investigators had tried over and over again to demonstrate the nitrogenous digestion products in the blood stream, but had failed, and we had nothing but unproved hypotheses with which to explain the absorption of albuminous food. This problem became acute when it was shown by Kutscher, at the beginning of the present century, that the pancreatic digestion is capable of completely destroying the protein character of albuminous materials. The earlier investigators worked with anywhere from 100 c.c. up to several liters of blood in attempts to solve this problem, and failed. Working with from 2 to 5 c.c. of blood by the micromethods, it was shown with absolute certainty that the old hypotheses were false, that the simple digestion products elucidated by Kutscher were readily and rapidly absorbed. The products could be traced through different parts of the circulation and even into the various tissues of the body.

The methods were equally applicable to the determination of the waste products produced within the tissues. In studying these phenomena, the results obtained were as clear as if we had actually seen the digestion products (the amino-acids) pass into the blood and into the tissues, there to linger awhile, and finally break down and yield urea. To any one who has actually followed this process, as Dr. Denis and I followed it in cats, there can be no doubt as to the

essential features of protein metabolism, and the ability of muscular tissues to form urea. Nor can there be any doubt about the important fact that metabolism products pass with great speed and readiness from the blood into the tissues, and vice versa. There is every reason to believe that when we determine the urea content of the blood we are at the same time determining approximately the urea concentration of the muscles.

The speed with which a condition of approximate equilibrium is established between the blood and the tissues in the case of ordinary soluble products has never received the consideration and study that this phenomenon deserves. The literature abounds in explanations of clinical or metabolism problems based on the assumption that the blood is almost a closed system with reference to the general tissues of the body. I doubt not but that there is such a thing as selective affinity between this or that product and this or that tissue. There must be some specific cause, however, for every case of such selective accumulation, for each case must be regarded as more or less of an exception to a general rule.

As an interesting erroneous explanation of an important metabolism problem based on a misconception as to the equilibrium phenomenon between the blood and the tissues may be mentioned the old hypothesis that creatin is the intermediate product in the breakdown of body protein into urea.

The muscles are relatively loaded with this simple crystallizable product, creatin, and it was indeed plausible to assume that this highly nitrogenous extractive, a hundred times as abundant in the muscles as in the blood, must be the connecting link between the broken down tissue protein and the urea. Feeding experiments with creatin did not yield urea; but to this Bunge retorted that creatin, when fed as such, does not have time to get into the tissues and is eliminated before there has been a chance to accomplish the conversion into urea. Both the original hypothesis and Bunge's auxiliary hypothesis advanced in its defense are thus clearly seen to assume the absence of the equilibrium phenomenon referred to. The fallacy in Bunge's argument has never been pointed out so far as I know, and it was not until three or four years ago that it was shown by microchemical analysis that

Bunge's argument was false; that, in point of fact, creatin is readily absorbed from the blood by the tissues.

While the view that creatin is the chief precursor of urea was long ago abandoned, many still believe that a part of the urea is so produced. The important point, however, is that creatin is not to be regarded as essentially a precursor of urea, and that we accordingly must find some other explanation of why the muscles contain such large amounts of creatin. This is a problem of normal metabolsim rather than of clinical or experimental medicine. Whether out of it will grow any clinical problems we cannot yet tell. Human beings do not lend themselves to tissue analysis.

Before I go any further it is perhaps well that I should indicate a little more definitely the character and scope of the microchemical methods already in use for the analysis of blood. We have then, first of all, the colorimetric methods for the determination of each of the chief nitrogenous substances found in urine, that is to say, the total nonprotein nitrogen, the urea, the ammonia, the uric acid, the creatin and the creatinin. In addition we have Bloor's micromethods for the lipoids-fat, lecithin, and cholesterol; Marriott's nephelometric methods for the acetone bodies; Benedict's method for the blood sugar, and finally Lyman's very recent method for calcium. The remarkable thing about this series of methods is that they are devised with reference to a single instrument, the colorimeter, which can also be used as a nephelometer. With the combined instrument we simply measure the amount of color which the substance to be determined can be made to give with some suitable reagent, or we measure the cloudiness, if the characteristic reaction used results in a precipitation instead of a color. In principle these methods are somewhat similar to the clinical method of determining the hemoglobin in blood. In the analytic methods of earlier times the investigators proceeded on the principle that the amount of material taken for each analysis must be inversely proportional to its concentration of the substance to be determined, and this principle, for obvious reasons, virtually excluded human blood or blood from small laboratory animals. In the microchemical system of analysis, on the other hand, the guiding principle is to overcome the lack of concen-

tration and the limited supply of blood by the application of correspondingly intense and sensitive reactions. The amount of blood used for each determination in the series mentioned above varies from a fraction of 1 c.c. up to 10 c.c. To be strictly practical, a blood method must not require more than 10 c.c. of blood, and its value is greatly increased if it can be applied to 5 c.c. or less. On the other hand, when one attempts to work with too insignificant quantities, that is, with drops of blood, the technic again ceases to be practical, because this can be done only by a complicated system of weighing instead of measuring the blood taken. This modern system of blood analysis has all been developed within the past six or seven years, and it is still not complete.

I have made no reference to other recent useful analytic procedures, such as Van Slyke's methods for amino-acid nitrogen and for chlorids.

We are still in the early stages of a pioneer period of research opened by means of all this new technic, but it is clear that much research lies ahead of us with reference both to physiologic and to clinical problems. As yet there is not always sufficient agreement as to the values which should be accepted as strictly normal. Take the nonprotein nitrogen and urea, for example. There is no doubt, I think, that in case of strictly normal persons such as we find among medical students (or outside of hospitals), the nonprotein nitrogen content and urea content of blood are low. The nonprotein nitrogen will not exceed 28, or at the most 30 mg. per hundred c.c. of blood, and the urea nitrogen will be almost exactly one half of the nonprotein nitrogen. Nor are these levels materially affected by reasonable variations in the nitrogen content of the food. To me it seems a matter of considerable significance that we have to accept as normal considerably higher levels just as soon as we begin to work on hospital patients. Here values lying between 30 and 40 are quite as common as values under 30. There can be no doubt about the fact that efficiency of the kidneys is the chief factor which determines the level of waste products which any individual carries in his blood and tissues. The higher levels of waste products found among hospital patients must therefore indicate that at least one half of these persons have kidneys which are no longer perfect, kidneys which at one time or another and in one way

or another have been damaged. Is this a clinical problem? As yet there has, of course, not been time enough to find out whether the less efficient kidneys are in a stationary condition or whether they are in a slow process of deterioration. In time the records of physicians and of hospitals ought to throw light on this problem.

One interesting aspect of the nonprotein nitrogen and urea problem is the question as to the effect of the food protein on the level maintained. In the case of strictly normal persons it makes practically no difference whether the diet is rich or poor in nitrogen. In persons having clinically damaged kidneys, the protein content of the food makes a great deal of difference. In many such cases (but not in all) it is possible by means of low nitrogen diets to reduce the nitrogen level of the blood to nearly or quite the normal level. The further elucidation of this point is, I should say, a clinical problem. In this connection one would naturally ask whether it makes any practical difference, or any difference, to the well-being of patients whether they carry a normal or high level of waste products in their blood. This is an obscure problem. As the level of nonprotein nitrogen in blood is raised, the percentage of the urea fraction of that nitrogen increases. When the nitrogen is very high, as in certain cases of threatened uremia, by far the greater proportion of the blood nitrogen (nonprotein nitrogen) is represented by urea.

Urea is believed to be harmless, and it is certainly true that a good many nephritic patients can go about feeling well, and yet carry as much as 100 mg. of urea nitrogen in their blood, while others go into so-called uremic coma without carrying any unduly high levels of nonprotein nitrogen or urea. Uremic attacks with low levels of blood nitrogen happen perhaps most frequently in cases of so-called toxic pregnancy. All this has been known for a long time.

We have lately taken up the study of blood in pregnancy partly for the purpose of verifying the findings previously reported by others, and partly with the hope of being able to advance the subject a little further.

The results which we have obtained have proved rather surprising, and are at least interesting. Our subjects are obtained from the Boston Lying-In Hospital, and we are indebted to Dr. Newell for the privi-

lege. Indeed, the research is essentially a cooperative one between Dr. Newell, Mr. Foster and myself.

We have thus far analyzed the blood of about 100 pregnant women, most of them clinically normal. As yet we have paid attention only to the nonprotein nitrogen and urea. From the class of patients to which these women naturally belong we should expect to find substantially the same rather high level of nonprotein nitrogen and urea as we find in other hospital patients.

Such is not the case. Very few pregnant women, except the toxemic ones, give a nonprotein nitrogen over 30 mg. per hundred c.c. The more interesting fact, however, is that the urea of blood obtained from normal pregnant women is practically without exception very much smaller than the amounts of urea found in other normal human blood. Such other human blood does not contain less than 11 or 12 mg. of urea nitrogen per hundred c.c. The bloods of pregnant women, on the other hand, run between 5 and 9 mg., and very few indeed run as high as 9 mg. of urea nitrogen for 100 c.c. of blood. Whereas in other normal persons the urea nitrogen represents 50 per cent. or more of the total nonprotein nitrogen, in these bloods it represents only from 20 to 35 per cent. of the total.

Several investigators have recently published some observations on blood in pregnancy, and I am at a loss to explain their results. They have, to be sure, found a few low urea figures, but these have been exceptional, and they have thus failed to find this peculiar characteristic of pregnancy.

I would hesitate as yet to try to explain this remarkable phenomenon. The problem is complicated by the fact that we do not possess full information as to the percentage distribution of the nonprotein nitrogen of normal blood. We have been going on the assumption that this nitrogen, so far as it is not accounted for by the ordinary waste products, must be largely amino-acids, that is, valuable food products. This assumption is probably not quite correct, for the figures obtained by Van Slyke's method for amino-acids leave a considerable proportion of the nonprotein nitrogen of blood unaccounted for. Two thoughts naturally suggest themselves in connection with our peculiar analytic findings. A low proportion of urea should leave a higher proportion of amino-acids and other

similar products (possibly peptids), and it is conceivable that this is the result of a mechanism for providing a more abundant and constant supply of the kind of nitrogenous food materials needed by the growing fetus. The other thought is this: The pregnant organism may be more susceptible than others to the toxic effects of certain waste products, and in self defense may be compelled to keep these waste products, including urea, at a subnormal level. This thought is rather attractive, for it contains the added hint that the blood in toxemic pregnancies may be abnormally rich in toxic products, even when the total amount of nonprotein blood nitrogen is not very high; from 35 to 40 mg. of nonprotein nitrogen in such blood may be an entirely different proposition from the same amount in other subjects. Whatever the correct explanation may be, the fact itself is, I think, decidedly interesting.

I have already referred once to Abderhalden's pregnancy test. The thought has, of course, occurred to us that urea determinations in the blood may have some diagnostic value with reference to pregnancy. But as yet we have had onlv a few cases representing the third month of pregnancy and no earlier ones. I therefore make absolutely no claims in this direction.

Before leaving the subject of the nonprotein nitrogen and urea, I ought perhaps to refer briefly once more to the use and value of these determinations as means of estimating the renal efficiency, and to the "refinement" represented by the so-called Ambard coefficient, which is simply a combination of urea determinations in blood and in the urine. The underlying idea of this combination is to eliminate any confusion which might arise because of changes in the blood concentration (in urea) due to the level of the general protein metabolism. In normal persons, as I have already indicated, there is no material change in the urea content of the blood because of changes in the level of the nitrogen metabolism. In nephritics, considerable variations can be produced by changes in the diet; but these changes are produced very slowly so that it usually requires several days of low protein feeding to produce a marked alteration in the urea content of the blood. Yet nephritics, like normal persons, adapt themselves promptly to changes in the protein content of the food, and, like normal persons, tend to remain in a condition of nitrogen equilibrium.

The complicated mathematical formulas introduced in connection with the Ambard coefficient do not tend to increase one's confidence in that coefficient. It is difficult to see how square roots and cube roots can help to elucidate such a simple metabolism proposition.

Work along the lines of the Ambard coefficient is one of the researches I had in mind in stating that many metabolism investigations based on metabolism periods shorter than twenty-four hours are now being made. The Ambard period, seventy-two minutes, seems to me, however, to be too short. I believe that a more suitable condition for studying the effects of the metabolism level on the urea retention will be found in connection with the three hour metabolism period to which I have already referred.

Determinations of nonprotein nitrogen and urea in blood have up to date proved the most popular in the study of blood. This is natural enough, at least so far as it concerns clinicians, because these determinations stand for concepts which to them are perfectly clear.

A theoretically equally interesting determination in connection with certain clinical problems should be that of the ammonia. Ammonia determinations in urine have become important in connection with the study of acidosis. Very few, however, have ventured to undertake the determination of ammonia in blood, notwithstanding the fact that it has figured very extensively in attempts to make ammonia responsible both for uremic and for diabetic coma. It is not difficult to see why this field is being neglected. No one recognizes more clearly than I do that the method for the determination of this substance in blood is far from easy. Moreover, the amount of ammonia present in blood is so small that it is difficult to see how these traces can be of much clinical significance. It is an interesting and remarkable fact that even in diabetic acidosis, when the daily urine may contain several grams of ammonia, the concentration of ammonia does apparently remain at an extraordinarily low level in the blood. If any one should here attempt to apply an "Ambard coefficient," he would doubtless be led into the very highest fields of mathematics before a satisfactory formula could be obtained. I sometimes suspect that there is something wrong about our ammonia determinations in blood. Dr. Denis and I have repeatedly returned to the investigation of the subject; we have spent several weeks on it again this year, but as yet have found nothing to indicate that our

earlier work is not substantially correct. Henriques, in Denmark, has recently published an apparently very thorough research on the same topic, and has in the main verified our findings. For the present, therefore, the problem of the ammonia in blood remains as before, theoretically interesting, but practically unfruitful.

For several years the determination of creatinin in urine has been used and accepted as an indispensable feature of every metabolism investigation involving urine analysis. In hospital work this determination is absolutely necessary as a check on the work of the nurses. It is the only means we have for detecting gross errors in the collection of the urine. Every other urinary constituent may vary up and down, but the creatinin remains practically constant, so that when a patient's creatinin output begins to show remarkable variations it is time to give up the experiment or begin all over again.

The creatinin in the blood is normally not large, 1 or 2 mg. per hundred c.c. of blood, but it is normally constant and easily determined. I say this advisedly, though I recognize that one investigator has lately published results purporting to prove that the values as ordinarily obtained are several times as great as the true creatinin content of blood.

The creatinin is one of the last waste products to accumulate in the blood as a result of kidney insufficiency. It is apparently only in rather advanced uremic conditions that this product begins to increase in the blood. A great many more observations are needed, however, on this point, and as the determination is easy and simple when a standard creatinin solution has once been obtained, it is a determination which should be taken up by clinicians. The only reason why clinical workers have not already stepped into this field is the fact that pure creatinin, or a pure creatinin salt, is needed for the standard solution which must be used in connection with the colorimetric determinations.

It seems strange that no chemical manufacturer has yet undertaken to prepare creatinin zinc chlorid to meet the demand for this salt.

Pure creatinin can, as a matter of fact, be dispensed with in connection with this determination. It takes only a few minutes' work to determine colorimetrically the creatinin content of normal urine by the help of my

older potassium bichromate method. By appropriate dilution of such urine with half normal hydrochloric acid a perfectly serviceable standard creatinin solution is obtained, and this solution will keep for weeks, if not indefinitely, so far as the creatinin content is concerned. The changes which do occur, darkening in color, precipitation of uric acid, etc., do not destroy the value of the solution as a creatinin standard.

Among the waste products of the animal metabolism there is none more interesting, alike to the laboratory worker and to the clinician, than uric acid. From the standpoint of normal metabolism it is generally believed that the uric acid problem is very nearly settled. I am less sure on the subject now that I would have been two or three years ago. The remarkable results reported by S. R. Benedict on the presence of extraordinary quantities of latent uric acid in beef blood, his demonstration of a synthesis of uric acid in Dalmatian dogs, and his findings indicating that the allantoin in dog urine does not represent decomposed uric acid, all indicate that we may yet have to revise in radical fashion views which but a short time ago seemed firmly established. The many vague clinical hypotheses which used to be associated with uric acid have, however, been swept aside. The uric acid crank has all but disappeared from among the medical profession. As a problem of comparative physiology, uric acid is exceedingly interesting, and from the standpoint of both clinical and experimental medicine it is yet destined to be the subject of many investigations.

The human organism has the almost unique distinction among mammals of not being able to destroy any of the uric acid which it produces. The human kidney is also less competent to get rid of this waste product than we could wish. In consequence of this combination of circumstances, the quantitative determination of uric acid in the blood is one of great promise and importance. The method for this determination is of such recent origin that the modern literature on the uric acid in blood is not large, and yet there is already considerable diversity of opinion concerning both the uric acid content of normal blood and the pathologic fluctuations which may occur. How far these differences may be due to imperfections in the analytic method or to lack of skill in the use of the method I am not prepared to say.

It is absolutely certain, however, that some kinds of human blood carry abnormally large amounts of uric acid and at the same time substantially normal amounts of nonprotein nitrogen and urea. It is equally certain that other kinds of blood carry substantially normal amounts of uric acid, yet exceedingly large amounts of total nonprotein nitrogen and urea. The former condition, as was to be expected, is most frequently found in gout, the latter in nephritis. The high uric acid in the blood of the gouty, like the high urea in the blood of nephritics, is due to lack of excretory power on the part of the kidneys and not to increased production of uric acid (or of urea).

With reference to the origin of the uric acid produced within the body, we cannot yet say how large a proportion comes from the muscles and how much from glandular organs. That the glands, at least in proportion to their size, are by far the most important centers of uric acid production follows as a matter of course from our present day teachings and beliefs to the effect that uric acid is formed in or from the nuclei of cells. The validity of this view can be experimentally demonstrated. Even in laboratory animals whose blood and muscles contain very minute traces of uric acid, we find in the spleen, liver, etc., as much as from 10 to 14 mg. per hundred gm. of organ, that is, fully twenty times as much as we find in the blood or the muscles.

I have confined this discussion to a consideration of only a few familiar waste products. As we get into the field of blood and tissue analysis, as we get into experimental touch with the chemical processes going on within the body, we must also begin to pay more attention to products which never find their way into the urine. I must not close without having called attention to the very interesting and promising line of research in the field of the lipoids—a field opened by the application of micromethods worked out by Dr. Bloor. Already Bloor has established the normal relationship existing between these lipoids—fat, lecithin, cholesterol—and the results obtained seem to furnish a basis for a more detailed knowledge concerning the intermediate processes involved in the metabolism of the fats. The extraordinarily practical character of the analytic procedures developed for this work should insure the attention of the clinician, for the fats, no less than the proteins, are associated with

many metabolism disorders; I need but refer to malnutrition in infants, pathologic obesity, lipemia, and diabetes.

Before closing I wish also to make just one brief reference to another class of products in the study of which a beginning has been made on the basis of microchemical methods. I mean the phenols. As yet we have not gone beyond the urine, however, in the study of these substances (which, as you know, are chiefly the products of intestinal putrefaction, and at least some of which are distinctly poisonous). I had hoped before this to have definite methods for the determination of phenols in blood, for it is their concentration in the blood, not the amount in the urine, which is significant in relation to diseases. We have run into these products both in connection with studies on epinephrin, which is a phenol, and in connection with uric acid, which in chemical constitution and in many of its reactions is also similar to the phenols. These very similarities are the difficulties against which we have to contend when endeavoring to determine the ordinary phenols in blood. It is a difficult problem, but in time it will be solved.

It will seem to you that I have discussed little else than analytic methods—a subject which cannot be made very interesting outside of the laboratory. I am convinced, however, that both the biochemist and the clinician must pay more and more attention to this least interesting but most important aspect of research. Scientists of earlier generations discovered a great many important facts in the realm of metabolism and of medicine. It will be found on scrutiny, however, that their discoveries were accidental or of such a character that they were bound to be made by one or another reasonably keen observer. That pioneer stage is over. The surface problems have been solved.

It is now only by means of finer and ever finer technic that progress can be made toward the solution of the many metabolism problems which must be solved by us and those who folow us, in order to secure an increasingly better basis for clinical, experimental, and, above all, preventive medicine.

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