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RESEARCHES ON ALBUMEN; WITH SPECIAL REFERENCE TO ALBUMINURIA.

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In the course of an investigation of certain properties of albumen, I was led to examine the comparative delicacy of our tests for it in urine. Some account of this will form the best introduction to what I shall have to lay before you on the general reactions of proteids. Considerable dissatisfaction has been expressed by various authors with regard to those tests. Dr. Roberts, of Manchester, more especially, has proved that even the nitric acid test in the cold is deficient in delicacy, although he considers it the best we possess for those cases in which it is applicable. He found that when he diluted an albuminous urine with water, he could trace the albumen further than when he diluted with normal urine.* Repeating this experiment, I found the same difference in the delicacy of the test in the two cases, and that it was frequently very considerable. Dr. Roberts attributes this to the presence of the solid ingredients of the urine, and no doubt this is so; but it appeared that there were some of these more especially concerned in this result than others. Passing over this in the

* *Urinary and Renal Diseases*, pp. 123, 124.

meantime, however, I ascertained, on further investigating this subject, the following facts:—

1. When an albuminous urine is treated with alkali, the albumen becomes more coagulable by acids, and by and bye can be precipitated in the cold by hydrochloric and even acetic acid.

2. The degree in which this effect is produced is in proportion to the amount of alkali used, the highness of the temperature, and the time during which the alkali is allowed to act. Thus, a quantity which will produce a given effect at once at a boiling temperature, will take a considerable time to do so at lower temperatures, and the effect of the boiling continues after the liquid has cooled down to any temperature, however low. In these experiments I never found alkali albumen to be produced, that is, an albumen yielding a precipitate on neutralisation which dissolves in dilute acid. On the contrary, the acid required to be added in excess of the alkali to produce coagulation. In this respect, it will be observed, the reaction resembled that of caseine, or of alkali albumen in the presence of alkaline phosphates, which is not precipitated till the solution is acid from the presence of free acid. While the albumen was thus rendered coagulable by acetic acid at ordinary temperatures, it was found that nitric acid had also a greater power to coagulate it than before, and that its delicacy as a test was much improved. Soda was found the best alkali for the purpose, as it threw down, along with the earthy phosphates, any free or loosely combined uric acid in the form of the insoluble urate of soda, and thus left a clear field in which the test produced very little reaction except with the albumen. On this principle is founded the following test, which will be found more delicate than the usual nitric acid test, and applicable to all conditions of the urine.

Alkali and Acid Test for Albumen in Urine.—Add to half an ounce (14·2 c.c.) of the urine, 5 to 10 drops of a saturated solution of caustic soda; boil and allow to cool, and the precipitate of phosphates and urates to settle down; pour off some of the clear supernatant liquor and test it in the usual way with nitric acid; namely, by allowing the acid to trickle carefully down the side of the tube, held apart from the perpendicular, till it forms a separate layer at the bottom of the urine. Instead of pouring off the supernatant liquid, we may filter, either before boiling, or after boiling and cooling. The liquid may be cooled rapidly by immersing the tube in water, or by directing a stream of water on it from a water

pipe. It is convenient to have half an ounce of urine in a rather long test tube, so that the upper part may be easily poured off free from any of the precipitate. The soda solution, of which 5 to 10 drops (small drops like those from a burette), were found necessary, was nearly equivalent to $\frac{1}{3}$ its volume of hydrochloric acid. Much less than this will make the urine alkaline, but this quantity seems necessary to produce the reaction. On the other hand, too much must not be used, as in that case the nitrate of soda, formed where the two liquids meet, would not be all kept in solution, and would of itself give a precipitate. If now an albuminous urine be diluted either with water or normal urine till the albumen shows but as a trace with nitric acid, taking, let us suppose, five minutes to come into view, it will be found after boiling it with the alkali that the albuminous reaction appears instantly. It will even be found that hydrochloric acid, used in the same way as nitric, is as delicate a test as the latter acid was previously. If the dilution be now carried twice, or even three times as far, the albumen will still be clearly detected. I should state that in a watery dilution less soda is necessary, and potash or ammonia will do equally well. Before diluting with normal urine, it is well to observe the effect of the test on the latter alone. The soda makes the urine of a deeper yellow colour; in a few cases it becomes of a reddish-brown. The nitric acid, after the soda, gives little reaction with colouring matters, and generally little with urates. It is evident there must be a formation of nitrate of soda above the acid, and this is shown by a zone of a slightly different colour from the clear acid below or the yellow urine above. This has a sort of grey colour, but when too much soda is not used there is no precipitate, and no appearance which could be taken for albumen. After the soda the albuminous zone of reaction forms a broader band than before, and there is generally a slight ring above, showing a trace of remaining urates. This sometimes lies so high as to be quite separate, but sometimes looks like the upper part of the albuminous zone, to which it then gives a double outline. On account of the tendency of the albuminous opacity to diffuse upwards after the soda, the acid should be poured in very gently.

With hydrochloric acid there is also an upper zone of urates, which rises higher than in the case of nitric acid. Sometimes also there is a violet reaction with colouring matters, and when such is the case the acid is a much less delicate test, a proof that the reaction with colouring matters also impairs the delicacy of the nitric acid test. After the soda the albumen

also coagulates readily with sulphuric acid; but there is now a reaction not only with colouring matters and urates, but also with lime salts, forming an insoluble sulphate immediately above the acid. There are thus four zones of reaction possible with an acid in an albuminous urine; first, an insoluble salt; second, colouring matters; third, albumen; and lastly, urates. These always occur in the same order from below upwards, and the lowest are those which interfere most with the albuminous reaction. They are all present with sulphuric acid, and would account for its inferiority as a test to hydrochloric acid, supposing the albumen itself to be equally coagulable by both.

Metaphosphoric acid has been proposed as a delicate test for albumen in urine, and I made some careful comparisons of it with nitric acid. I found it best, at the suggestion of a chemical friend, to use a saturated watery solution, which is a clear, heavy, liquid, having a sp. gr. of 2, and which therefore runs easily down to the bottom of the test tube like nitric acid. But I found that although it had great power to coagulate the albumen there were frequently constitutents in the urine, principally, I believe, salts of lime, which greatly interfered with its action, and not only so, but produced non-albuminous precipitates which might easily have led into error. I met with cases which gave very distinct reactions with nitric acid and none at all with metaphosphoric acid. I found the soda also useful, therefore, with this acid, and in dilutions with normal urine it frequently appeared to be more delicate, after the soda, than nitric acid. But after comparing the two in a variety of cases, I found the former not uniform, and this seemed due to a precipitation of a lime salt, even after the soda had been used.

Picric acid is said to be a delicate test for albumen, and in all instances in which I have compared it with the others, I have found it so. It also is best used in the form of a watery solution, which is light, and when allowed to run down the tube like the others, flows gently over the surface of the urine, when, if albumen be present, it shows itself by the formation of a narrow, greenish zone, where the two liquids meet. It forms in a minute, even when albumen is present in only the smallest quantities, and I have never seen anything in a non-albuminous urine which could be mistaken for it. It is not so applicable where there is turbidity from urates or phosphates; but in a comparatively clear urine I have always found it as delicate as the alkali and acid test, perhaps fully more so, while it is much less troublesome. But I should

mention that the most delicate test is not necessarily that which gives the largest precipitate when more than mere traces are present. When the albumen is in sufficient quantity to be recognised by nitric acid, the precipitate is often bulkier than that given by picric acid, although it can be detected by the latter after greater dilution than by the former.

The effect of the alkali on the albumen as used in the test, led to further investigation of the subject of alkali albumen. I found that if, instead of boiling the urine with the alkali, I allowed it to operate at ordinary temperatures, the same result was sometimes not produced after 12 or even 24 hours. But if the quantity of alkali was made double that used in the test, the albumen coagulated with acetic acid in about 4 hours. When tested by hydrochloric acid at successive intervals during this time, it was found to be gradually becoming more coagulable by it. In the course of these experiments it was observed that the proteid was never precipitated on neutralisation, which is the characteristic reaction of ordinary alkali albumen. When ovalbumen or serum albumen was treated in the same way they gave a precipitate on neutralisation which dissolved in either dilute acid or alkali. At a boiling temperature this effect was produced with very small quantities of alkali, quantities which seemed to have no effect of any kind on albuminous urine. There seemed to be a very decided difference between albumen in the urine and serum albumen with regard to the action of alkali, and an equally great, if not greater difference was also observed with acids, acid albumen being formed with the latter under circumstances which produced no such result with albuminous urine. Before treating this subject in detail I must request your attention to some experiments which were performed on the coagulation of albumen by heat and acids, which will serve to render the subject more intelligible.

We read of various anomalies in the behaviour of albumen with heat and acids, and many cautions are given against using too much or too little acid. With the view of determining the degree of acidity required in different instances, comparative experiments were made with albuminous urines, giving a precipitate of $\frac{1}{2}$ or $\frac{1}{3}$ of the tube; and with 1 in 20 dilutions of ovalbumen, blood serum, and serum from blistered surfaces, which also gave precipitates of about $\frac{1}{2}$ or $\frac{1}{3}$ of the tube. The dilute ovalbumen was prepared by breaking up white of egg thoroughly, removing the froth and mesh-work, and filtering from the globulin which was precipitated by the dilution. All the globulin, except, perhaps, mere traces, was

got rid of in this way. Concentrated ovalbumen coagulates on boiling, but a 1 in 10 or 1 in 20 dilution in water does not, but gives only a slight haze. It now requires, for perfect coagulation on boiling, the addition of a small quantity of either salt or acid. Both may be used together, but either will do singly. You are all aware of the statement made by Schmidt, that the small quantities of saline matter which are always associated with the albumens may be removed from them by dialysis, when they are no longer coagulated by heat. This has been much controverted,* and I may state that in a number of experiments, carried on chiefly by a chemical friend, who has frequently co-operated with me, we never met with an instance in which some coagulation did not take place on boiling, even after prolonged dialysis. But we generally found that the albumen coagulated much less perfectly than before, while the addition of a little salt made it coagulate as well as at first. Although, therefore, it may not be possible to remove all the salt by dialysis, it would seem as if it were essential to the coagulation, and that Schmidt's observation is a very important one. Indeed, it would appear that we can attribute the non-coagulability of the dilute ovalbumen to nothing but the dilution of the salt. Add to half an ounce of it (14·2 c.c.) 5 minims of saturated saline solution, and it again coagulates perfectly on boiling. Similarly, concentrated blood serum coagulates on boiling, but, as in the case of ovalbumen, when diluted to 1 in 20 of water it does not. It requires, like the former, the addition of 5 minims of saturated saline solution to the half ounce, or 2 or 3 drops of dilute acetic acid. According to Mathieu and Urbain the carbonic acid dissolved in the albumen combines with it under the influence of the heat, and is the cause of the coagulation. Solutions of albumen, deprived of carbonic acid by being placed in a vacuum, become incoagulable.† If this be so, is the amount of carbonic acid present in the serum so far reduced by dilution to 1 in 20 of water, as to render the albumen incoagulable, as happens with the salt in the case of ovalbumen? It would appear so, for I found that when the serum globulin is thrown down by passing a stream of carbonic acid through the liquid, which is thus saturated with the acid, the albumen again coagulates with heat. But it is important to bear in mind that the same dilute serum coagulates with the addition of a very few drops of saline

* Gamgee's *Physiological Chemistry*, vol. i, p. 63; and Foster's *Physiology*, &c.

† Quoted in M'Kendrick's *Physiology*, p. 695, from which the statement is taken.

solution, and I believe I shall advance reasons for supposing that dilute acid is never the sole agent, but that the salt present, however small its quantity, is always a factor. Experiments were conducted with this dilute serum before separation of the globulin by carbonic acid. The blister serum (from blistered surfaces) was diluted to the same extent, and any globulin precipitated by dilution was removed by filtration. These dilutions gave a precipitate of $\frac{1}{3}$ to $\frac{1}{2}$ of the tube.

I now proceeded with equal quantities ($\frac{1}{2}$ oz. = 14·2 c.c. of each) of these 4 albuminous solutions; namely—(1.) neutral albuminous urine; (2.) dilute ovalbumen; (3.) dilute blood serum; and (4.) dilute blister serum (the latter three slightly alkaline). Boiling these and adding a drop of 28 per cent acetic acid at a boiling temperature, I found they all coagulated except the last, which remained perfectly fluid. Even after dilution with only 8 or 10 volumes of water this blister serum would not coagulate on boiling, with or without a drop of acetic acid, and the same result was observed with various specimens. With a few drops of saline solution, with or without acid, it coagulated perfectly. I was surprised at this result, but my friend, the Rev. Mr. Gibson, performed an experiment which threw new light on the matter. Repeating the boiling of this serum, and observing that on adding the acid a precipitate apparently formed and re-dissolved, he was induced to try a drop of dilute acetic acid, when he found that the albumen coagulated. He also found that if the same drop of dilute acid was added before boiling no effect was produced. Now it is well known that solutions of globulin must be very cautiously treated with dilute acid on boiling to produce coagulation; but there was no globulin in this solution, which gave no precipitate with a stream of carbonic acid or by saturation with salt. The fact that it coagulated with more acid if salt were also added, pointed to the conclusion that the acidity must bear a certain proportion to the salt present. To use a large quantity of salt and acid is a well known method of precipitating the proteids; but nevertheless the true relation of salt to acid in the process in all cases, and more especially the fact that the acidity must be reduced if the salt be partly removed by dialysis, does not seem to be generally recognised. I think this will be apparent from the following experiments, performed with the view of elucidating this point.

Mr. Gibson found that the blister serum coagulated equally well with acetic, nitric, hydrochloric, or sulphuric acid, if sufficiently diluted, a drop of 1 per cent sulphuric acid being

enough. If a drop more was used the albumen remained in solution and passed at once, at the high temperature, into acid albumen. Repeating the experiment with ovalbumen and blood serum, I found that although they had coagulated with a drop of acetic acid, the result was much better when only one or two drops of dilute acid ($3\frac{1}{2}$ per cent) was used. It was observed in this case also that if the drop of strong acid was added *before* boiling no coagulation took place. With albuminous urine it was found that coagulation could be produced with much larger quantities of acid, and that there was a very considerable range between the minimum and maximum of acid which might be used. It also appeared that when an excess of acid was employed a change in the character of the precipitate was manifested, long before a quantity of acid was reached which kept the albumen in solution. The range of acidity frequently extended from 2 or 3 drops of dilute, to 12 or 15 drops of strong acetic acid, the maximum therefore being 30 or more times greater than in the case of the other two. Now, it is evident that the urine contained a very much larger quantity of salts than the other solutions, over and above the urea, which would probably have much the same influence as a salt. It was found, after removal of most of the salts and urea by dialysis, and the reduction of the specific gravity to 1002 or 1003, that the acidity required to be reduced to $\frac{1}{50}$ or $\frac{1}{100}$ of the former maximum, a drop of 1 per cent acetic acid being all that was necessary, while a larger quantity now produced fluidity. This almost infinitesimal quantity of acid was, however, essential, coagulation not taking place without it. At the same time it must be confessed that coagulation was sometimes imperfect after the most careful regulation of the acidity. If salts were again added to the urine more acid could also be used. It was remarkable that after dialysis of albumen the acidity required was not reduced to so small a quantity as in the case of urine. Thus, ovalbumen ($\frac{1}{2}$ oz. of 1 in 20 dilution) was found to coagulate with a drop of 1 in 20 hydrochloric acid, a drop of 1 in 10 dilution keeping it in solution and producing acid albumen. After 48 hours' dialysis the necessary amount of acid to produce coagulation was reduced to a half of what it was formerly, or a drop of 1 in 40 hydrochloric acid, 1 in 20 now causing fluidity. Albuminous urine will coagulate with a drop of strong hydrochloric acid as a rule, or at all events after the acid is diluted with one or two volumes of water; after dialysis this may be reduced to 1 in 50, or less. It would appear from this that there is a larger amount of salt in intimate

combination with the ovalbumen which it cannot be so readily robbed of by dialysis.

I never found, when the acid was used in such excess in the urine as to keep the albumen in solution, that it passed into acid albumen, as the others had done. What, moreover, was the explanation of the fact that a quantity of acid which had no effect in causing coagulation if added *before* boiling might do so if added at the boiling point? This was found to depend on the acid being in excess; if in proper quantity, it does not matter when it is added. But when in excess it produces no result if added before boiling, as it is everywhere diffused before the temperature which produces coagulation is reached, and so keeps the albumen everywhere in solution. But when added to the boiling liquid (without shaking), it acts unequally owing to its unequal diffusion, and it is remarkable how slowly it diffuses, especially when much salt is present. Where the drop falls, therefore, and where the acid is in excess, the albumen is kept in solution; but further away, in parts to which the acid comes in smaller quantities, the precipitate forms, and having once formed, the excess of acid cannot altogether re-dissolve it after complete diffusion has taken place.

The fact, already alluded to, that the character of the precipitate changed as the acid was increased, before the quantity became so great as altogether to keep the albumen in solution, requires further attention. It was found that the changes produced by varying quantities of salt and acid were very considerable. These will be best explained by a reference to the accompanying diagrams, in which I have attempted to illustrate the several effects on albumen thus far recorded. The salt and acid may be compared to two forces acting on a body at right angles to each other. Let A (diagram I), be the albumen, A C the line of action of the acid, and A S the line of action of the salt. Suppose A to be a body, as a ball, free to move in any direction, and the acid and salt to be forces pulling it in the directions A C and A S respectively. It is obvious that the movement of the body will be the resultant of the two rectangular forces, and that this may be any line between A S and A C. This, I think, represents accurately what takes place every time we boil an albuminous urine. There is reason to believe the effects may be as various as the different resultants lying between A S and A C. If the resultant be in one direction we have a certain effect on the albumen; if in another direction there is a different effect. These various effects, however, pass by such insensible gradations into each other

that it is only at certain intervals they become so pronounced that we can fully appreciate the difference, and it will be sufficient to single out two or three for especial notice. And first of all let me request your attention to the resultant A–P which bisects the angle S A C. This is the resultant of the two forces when they are equally balanced, and it is that which produces perfect precipitation of the albumen. With this resultant we have the perfect flaky or curdy precipitate, and a clear supernatant liquid, and this is the result which should always be attained when we use heat and acid as a test. But if the acid be in excess, and the resultant inclines towards A C, as the line A–P G, the precipitate undergoes a change, the flakes becoming viscid or gelatinous, until finally, with a sufficient amount of acid, the flakes are lost sight of altogether, and the precipitate becomes a homogeneous, gelatinous mass. This precipitate does not settle down, nor become visible for some time—it may be several hours after boiling. During the boiling, and for some time afterwards, the albumen appears to be in perfect solution, but it is found that it does not pass through the filter, and that it throws down this gelatinous precipitate after a length of time. This precipitate may generally be obtained with 20 to 30 minims of acetic acid. It will be observed that this reaction resembles that shown by the form of albumen to which Bence Jones has given the name of metalbumen. If the acid be in still greater excess, we have the resultant A–A F, or what we may call the acid fluidity, the albumen now giving no precipitate whatever.

If we now add salt beyond the proportion which gives the flaky precipitate and produce such a resultant as A–P S, the precipitate becomes sandy or finely granular. This is better seen in albuminous urine than with ovalbumen or serum albumen. In the latter two a very little salt alone coagulates the albumen, and the precipitate takes the form of lumps or masses, the entire liquid becoming extremely viscid. But if an albuminous urine be neutral, it will seldom coagulate on boiling, even with large quantities of salt, and I have met with instances in which, after saturation with salt, it refused to coagulate on boiling till the acidity was made greater than before. Sometimes, however, I have found the neutral urine to precipitate, wholly or partially, after saturation with salt. It has sometimes appeared to me that the after dinner urine was more prone to do so than the morning urine, but I would not speak positively on this point, as the greater quantity of albumen in the urine in the former case may have had a good deal to do with the result. But if any change in the pro-

portion of acid to salt causes a change in the direction of the resultant, and so in the character of the precipitate, how was it that perfect precipitation might be obtained with a considerable range of acidity, the quantity of salt remaining the same? To explain this it was found necessary to take into account the effect of the temperature. Observe that there are two things to be considered in connection with the resultant; (1) its intensity, and (2) its direction. The former depends on the sum of the two forces; the latter on their relative amounts. Thus (diagram II), if the salt be 4 and acid 4, the resultant will be in the same direction as when the former is 6 and the latter 6, but the resultant will now be one of greater intensity. In both cases there will be perfect precipitation of the albumen, but with the weaker resultant a higher temperature will be required. As long as the two forces are in the proportions to give the resultant A P, their effect is intensified by the temperature to such an extent as to produce coagulation, however small the sum of the two. But as the sum of the two forces increases, the temperature falls. Thus, if the temperature be 70° C. when salt and acid are each 4, it will be lower, say 60° or 50°, when the salt and acid are 6 or 8 each (diagram III.) But if the salt be 4 and acid 6, it is evident that the resultant will be in a different direction, as A–P G, at least at the same temperature as before—namely, 70° (diagram II.) If, therefore, the same resultant is still obtained, it will be at a different temperature, and it was observed that as the acid was gradually increased the temperature of coagulation fell as long as the character of the precipitate remained unchanged. Thus it happened that 4 of salt and 6 of acid might give the same resultant, A–P, at 60°, as 4 of each did at 70°. This is represented in diagram II, where A–P G is the resultant of 4 of salt and 6 of acid at 70°, and A–P the resultant of the same at 60°, the curved dotted line indicating the veering round of the resultant with the change of temperature. But if the excess of acid be too great, so that the salt present cannot counteract its tendency to keep the albumen in solution, we observe that the temperature of precipitation does not fall further, but the precipitate gradually assumes a different character. This does not become evident, however, till there is a considerable increase of acid. It must be observed that the acid used in these experiments was acetic acid.

The following experiments will serve at once to illustrate these principles and show the proofs on which they are based.

1. If salt and acid be both increased, the temperature of coagulation falls in proportion. This is well known, and

experiments showed that the temperature might thus be reduced from 90° C. to 30° or 20°.

2. If the salt be made a fixed quantity, precipitation may be obtained with various qualities of acid, but the temperature falls as the acid is increased. Half an ounce of a neutral albuminous urine, with one drop of acetic acid, became turbid at 70°, coagulating fully at 80°; with four drops of the same it became turbid at 60°, full coagulation, 70°. With increase of acid, therefore, the temperature had fallen 10°, the character of the precipitate remaining the same. When the acid was still more increased no further fall of temperature resulted, but the precipitate gradually assumed the gelatinous form. It even appeared, when the quantity of acid became considerable, that the temperature of precipitation rose somewhat, but it was observed that a faint turbidity still appeared at a low temperature, and that its character was different. Instead of appearing as flakes shooting through the tube, a uniform opacity spread through it, extending over a range of temperature of 40° before the turbidity became denser. When the amount of acid became sufficiently great (30 drops of acetic acid from a burette = 12 minims), the urine remained altogether transparent, depositing a perfect gelatinous precipitate on cooling. An interesting fact was observed with this quantity of acid. On boiling *slowly* the liquid became turbid at 70° to 80°, but did not become dense even at 100°, so that the bulb of the thermometer was never altogether hidden, and the precipitate took the form of viscid gelatinous flakes. Boiling another specimen *rapidly* with the same amount of acid, it remained perfectly transparent throughout and gave no precipitate till it cooled. The reason of this is evident. The acid, being in excess, had a greater tendency to keep the albumen in solution at the high temperature, and by passing too suddenly the temperature at which the resultant of precipitation was produced, fluidity was the consequence. Hence, it sometimes happens, with excess of acid, that the precipitate which forms at a certain temperature partially redissolves when we reach a higher. The same principle also explains why a precipitate may appear only on cooling, since the liquid again passes slowly through the same range of temperatures, though in the reverse direction. The precipitate would certainly have appeared had the temperature not been raised too rapidly in the first instance.

Instead of only reducing the temperature 10° by increase of acid, as in the above experiment, it might have been reduced much further by adding more salt at the commence-

ment. This widens the range of temperature of precipitation, and by raising successive quantities to various temperatures, as 90°, 80°, 70°, 60°, &c., and adding acid at each of these temperatures, it may easily be proved that the higher the temperature the less the amount of acid required; or, which is the same thing, that the temperature falls as the acid is increased. Increase of salt, the acid remaining the same, also as a rule lowers the temperature, but in albuminous urine at all events this is not invariable.

The facts thus far recorded favour the idea that in the entire absence of salt, dilute acid would probably not coagulate albumen at any temperature. Along with salt it brings about coagulation; but alone, or in a certain excess over salt, it keeps the albumen in solution, but reduces it to a state in which it is more coagulable by salt at low temperatures than before. In the same way I have found that after the prolonged action of a certain quantity of salt the albumen coagulates more readily with acid. It has been shown that it does not require a very large amount of salt to effect coagulation on boiling without acid, but it is evident that the latter combines to produce the same result with a small quantity of salt not of itself sufficient to do so. This precipitate, therefore, may be called the *salt and acid* precipitate. A precipitate apparently due to salt alone, as when we boil dilute ovalbumen with 5 minims of saturated saline solution, or any larger quantity, may be called in contra-distinction the *salt* precipitate. This is represented in diagram IV, where it will be observed that in the case of albuminous urine this is very small, as if neutral it sometimes coagulates very imperfectly even after saturation with salt.

The solubility of the salt and acid precipitate in acids depends essentially on the same principles which determine its formation. Thus, if the acid be already somewhat in excess of salt, the precipitate will necessarily be readily soluble in additional acid, especially at a high temperature. On the other hand, if salt be in excess, additional acid will sometimes have the effect of increasing the precipitate till the resultant is brought into the line A–P. When the salt and acid are properly balanced, the precipitate will be insoluble in acid in proportion as it was originally formed by large quantities of both agents. The insolubility in acid, in short, must always be in proportion to the amount of salt present. Whenever by inadvertence we use an excess of acid in testing, this can be at once counteracted by adding salt—a fact recognised in the tests given in some text books. It must be remembered that the precipitate is always most soluble at the moment of its forma-

tion. Some of the supposed anomalies in the action of heat on albumen will be found, it is believed, to be explained by these principles.

We constantly read of proteids separable by mere boiling, but it would appear that elevation of temperature alone is never to be considered the principal agent, but that its effect always depends on the presence and relationships of other forces. In the same way we read of the precipitation of albumen by the mineral acids without any reference to temperature, but as I shall have occasion to mention presently, these acids act much more energetically at high temperatures. It is obvious, therefore, that all the factors concerned in the result must be taken into consideration.

I next proceeded to determine whether albumen occurring in urine would not, after dialysis, pass into acid albumen, with large quantities of hydrochloric acid and boiling. In doing so it was found that when the acid rose to a certain amount the albumen was again precipitated on boiling. That is to say, a small quantity of hydrochloric acid, not exceeding a single drop to the half ounce of urine, gives the salt and acid precipitate, any excess over this producing the acid fluidity, but a third and still larger quantity again precipitates the proteid. This precipitate was of a dirty violet colour in dialysed urine, and dissolved in excess of hydrochloric or sulphuric acid. When the amount of acid became sufficiently large (about equal volumes of acid and urine), this precipitate did not appear, the albumen again remaining in solution. The precipitate, with successive quantities of acid, gradually became larger till a maximum point was arrived at, beyond which it became gradually smaller till the second fluidity appeared. This is figured in diagram V, where will be seen along the acid line (1) the salt and acid precipitate; (2) the first acid fluidity; (3) the acid precipitate passing gradually into the fluidity at the two extremes; and (4) the second acid fluidity. We are told in text books that the strong mineral acids precipitate albumen, and also dissolve it, but the proportion of acid which produces any result, together with circumstances of time and temperature, seems not to be sufficiently attended to. I made a comparison of nitric, hydrochloric, and sulphuric acids, with respect to the acid precipitate in dialysed and undialysed albuminous urine, and then a comparison of the latter with ovalbumen and serum albumen. I must confine myself to the briefest summary of the results. With $\frac{1}{2}$ oz. of neutral albuminous urine (undialysed) it was found necessary to have one small drop of nitric, hydrochloric, or sulphuric acid, to produce

the salt and acid precipitate. A single drop more produced fluidity. Hence, when these acids are used as a test they should be first diluted with 5 or 10 volumes of water, and of this dilution a few drops should be added cautiously after boiling. This salt and acid precipitate is always white, with whatever acid produced. When I reached from 5 to 10 drops of nitric acid, or 30 to 60 of hydrochloric or sulphuric acid, traces of the acid precipitate began to appear. This was red with all three acids on boiling, the colour depending almost entirely on the effect of the acids on the colouring matters of the urine, for the colour was pale in dialysed urines. When the maximum point of the reaction was passed, the quantities of acid being such that the precipitate began to diminish, the colour became yellow with nitric and sulphuric, and this was also the colour of the second fluidity (xanthoproteic reaction.) I may here remark that this is much less marked than with serum albumen, but more especially ovalbumen. The precipitate came at a low temperature with nitric acid, and was then white, but required a temperature of 60° to 80° with hydrochloric or sulphuric.

A comparison of ovalbumen and serum albumen, with albuminous urine (diagram VI), showed that the precipitates came with smaller quantities of acid in the case of ovalbumen than either of the other two. In all three it came first with nitric acid. In the case of ovalbumen and serum albumen, the precipitates with both hydrochloric and sulphuric acids came at a low temperature, although on boiling it was observed that traces of them appeared with smaller quantities of the acids. At the lower temperatures, however, the smaller quantities seemed to have the same effect if sufficient time were allowed. The precipitate in the case of ovalbumen began to appear at a high temperature, with 6 drops or so of hydrochloric acid, serum albumen requiring twice as much, and albuminous urine still more. But the chief difference between ovalbumen and serum albumen lay in the large amount of acid required before the second fluidity was reached with the former. It required 4 volumes of acid to 1 of ovalbumen, instead of equal volumes of each, as with the other two.

From a general view of these diagrams, it will be seen—

1. That all the spaces of precipitation are greater, and those of fluidity smaller, at high temperatures, although this is partly compensated by time at low temperatures.

2. All the spaces of precipitation are greater, and those of fluidity less with ovalbumen than serum albumen, and the same holds with regard to the first fluidity between the latter

and albuminous urine. The difference between the three may be best seen, however, by reference to temperature. Given the same amounts of each in solution, the same quantity of acid, and the same time, the precipitate requires 25° higher temperature with serum albumen than with ovalbumen, and about 25° still higher with albuminous urine.

Did time permit, it might be shown that these diagrams (V and VI) give a comprehensive view of the behaviour of albumen with mineral acids. Take an instance. It is well known that the precipitate formed by nitric acid dissolves in a large quantity of water, and you see that by diluting with water you reach the first fluidity. Similarly, the precipitate will also dissolve with additional acid, for this will bring you to the second fluidity. Again, if you add in the first instance sufficient acid to produce the second fluidity, a precipitate will form on diluting with water, re-dissolving when the amount becomes sufficiently large; that is to say, by so doing you travel, so to speak, from the second to the first fluidity. And so you may travel in the opposite direction, with the effects reversed.

It was now found that quantities of acid which produced the first fluidity were those necessary (or at all events best suited) to form acid albumen. This space, it must be observed, is wholly obliterated if sufficient salt be added, when nothing but the salt and acid precipitate forms, and acid albumen is impossible. In the case of ovalbumen and serum albumen, it was found that quantities of acid which gave no trace of precipitate on boiling produced acid albumen in one minute at 37° C. or any higher temperature. With ovalbumen the quantity of hydrochloric acid was found to be from a drop of a 1 in 10 dilution (1 in 20 producing precipitate) upwards to 4 or 5 drops of the strong acid, and with serum albumen from a drop of a 1 in 5 dilution to about 10 drops or so of the strong acid. The smaller quantities did not produce, perhaps, so perfect a result as the larger in the time specified. With albuminous urine, whether dialysed or not, it was found that no such effect was produced. It seemed to pass with difficulty, if at all, into acid albumen.

I would here observe that when acid albumen is formed, the position of the acid precipitate is not altered; it appears as before, when the acid is increased to the proper quantity. Moreover, the precipitation of acid albumen from its acid solutions by excess of sodium chloride, which is given as one of its reactions, is simply the production of the salt and acid precipitate, as before—the resultant A–P of the two forces.

Had the salt been added in the first instance, this precipitate would have formed in the same time, and at the same temperature as sufficed for the transformation into acid albumen. The effect of the acid in rendering the albumen coagulable by salt at a high temperature continues after cooling to a much lower temperature. The instantaneous production of acid albumen at a sufficiently high temperature is worthy of note. We read in books that the change into acid albumen by dilute acid is gradual, as proved by the fact that a portion of the liquid taken soon after the addition of the acid gives little precipitate on neutralisation, and still gives a precipitate on boiling. In this case, it is evident that the quantity of acid present is such as to give a trace of either the acid precipitate on the one hand, or the salt and acid precipitate on the other. I have not yet sufficiently investigated the formation of acid albumen under these circumstances, but, from the observations I have made, I am disposed to think that, if the quantity of acid is such as to produce any acid precipitate, the proteid cannot be wholly transformed into acid albumen, since the precipitate will form slowly, even at a low temperature. And if all the albumen present can be slowly changed into acid albumen at a low temperature, with a quantity of acid which, at first, gives some precipitate on boiling, this would seem to suggest that the action of the acid is an undoing, so to speak, of the influence of the salt on the albumen, and hence requires additional salt to counteract it, and again bring about precipitation. But it should be noticed that the non-coagulation of acid albumen on boiling is by no means a distinctive feature. Given a quantity of acid which produces the first acid fluidity, and the proteid does not coagulate at any temperature, although it does not form acid albumen, precipitable on neutralisation, till it acts for some time, or until the liquid is raised to a certain temperature. That is to say, it does not coagulate on boiling, although it has not yet become acid albumen.

To return to alkali albumen. A saturated solution of caustic soda was now made, three parts of which were found nearly equivalent to one of hydrochloric acid, and another solution equal to the 1 in 10 dilute acid. With quantities ranging from 1 drop of the weak solution upwards, it was found that both ovalbumen and serum albumen passed into alkali albumen, precipitable on neutralisation, in one minute, at 37° C. or any higher temperature. At 20° this did not take

place in the time specified. When the amount of alkali was increased to 10 drops of the strong solution in the case of ovalbumen, or 20 drops in the case of serum albumen, the neutralisation precipitate dissolved with difficulty in acid. This appeared to be due to the salt formed in neutralising the alkali, rendering the precipitate insoluble in acid. With less than this amount, however, the precipitate readily re-dissolved in acid, and the acid fluidity and the acid precipitate occupied the same relative positions as before. There is always some increase of coagulability by acids, so that the temperature of coagulation, with the same amount of acid as before, is less with alkali albumen than native albumen. With albuminous urine no neutralisation precipitate was ever obtained after the action of alkali; the effect was totally different. There was simply a considerable increase of coagulability by acids. This is represented in diagram VII, where the acid precipitate to the right is that obtained with hydrochloric acid at 50°. After the action of the alkali, the temperature falls, so that at a certain stage, represented by 1 on the alkali line, it will be 30°, by and bye at 2 it will fall to 20°, and at 3, or the extreme end of the line, it will fall nearly to zero, and less acid also will be required to produce the coagulation; the precipitate has moved, as it were, in the direction of the neutral point, but has failed to reach it. To produce this last result, however, a considerable amount of alkali is required; so much, in fact, that the salt formed in neutralising it contributes to the bringing about of coagulation with smaller quantities of acid than before. It is evident that, if the combination of alkali with the albumen of albuminous urine is to be regarded as an alkali-albuminate, it is of a different kind from that produced with ovalbumen or serum albumen.

While ovalbumen is more coagulable by acids than serum albumen it is also, as far as I could ascertain, more coagulable by salt. It is, in short, more readily coagulated in every way. Acid albumen formed from ovalbumen required one drachm (3·55 c.c.) of saturated saline solution at 10° C. to precipitate it, while the same quantity prepared from serum albumen was found to require 3 drachms or more at the same temperature.

It is interesting to observe that, in some respects, the difference between the albumen we find in urine and native serum albumen is one of degree only, like that which obtains between the latter and ovalbumen. Just as serum albumen

is less coagulable than ovalbumen, so the proteid of albuminous urine is still less coagulable than the former. But the effects of dilute acids and alkalies seem to point to a more radical difference. Now the albumen which occurs in urine is said to be either serum albumen or globulin, but most frequently the former. If so, it is evident, if my observations are well founded, that it has been altered in some way or other, and the question arises—are its reactions not so widely different from other proteid bodies as to entitle us to regard it as a proteid *sui generis* to which we might appropriately give the name of *ren*-albumen? For what can be the origin of this modification of albumen? It is obvious that it must be referred either to the blood or to the kidneys. If such a body exists in the blood, it has certainly never been isolated, for it differs still more from globulin than from serum albumen. Indeed, it could easily be shown that ovalbumen is more closely allied to globulin than is serum albumen, and the latter more than ren-albumen. These proteids form a scale, so to speak, each member of which from ren-albumen to ovalbumen approaches more nearly the characters of globulin. If it is only through the kidneys, therefore, that we become acquainted with ren-albumen (if I may so call it), is its origin not rather to be regarded as due to some action on the part of the renal cells? Such a view would, in my opinion, render the causation of albuminuria much more easily understood than otherwise, and would quite coincide with views which have already suggested themselves to eminent authorities. Let me quote the following from Niemeyer.* "The presence of albuminuria, which usually persists throughout the whole course of the disease" (speaking of chronic Bright's disease), "and only disappears now and then for short periods, unfortunately cannot be satisfactorily accounted for. One might be led into mistaking the albumen and the exudation cylinders for the products of inflammation, excreted from the free surface of the tubules, were it not that in other and non-inflammatory diseases of the kidney the urine contains both tube casts and large amounts of albumen. I believe (he continues, and he prints this sentence in the German in larger type) the presence of albumen in the urine to depend upon the destruction or degeneration of the epithelium. That normal urine should not contain albumen is, confessedly, extremely perplexing to physiologists. They are almost

* Translation of 8th German Edition by Dr. Humphrys and Dr. Hackley, Vol. II, page 27 ; or 6th German Edition, Vol. II, page 22.

forced to suppose that the albumen does transude into the kidney, together with the water and salts; and they are reduced to the hypothesis that its absence from normal urine is in some way connected with the epithelial lining of the uriniferous tubules, the transuded albumen either becoming assimilated for the nutrition of the epithelium, or else its diffusion into the tubules, receiving some other modification, as yet unknown to us, from the epithelium. The observation that albuminuria exists in all diseases of the kidney, in which its epithelium is either degenerated or destroyed, fully confirms this physiological hypothesis."

A similar opinion, I believe, is held by Dr. George Johnson and others. If this be so, we can readily understand why the albumen we find in the urine should be different from serum albumen. For it is in the highest degree probable that this action of the renal cells, although not sufficient to change all the transuded albumen into other products as in health, will still be exercised in Bright's disease to a certain extent, and so may give rise to this modified albumen. At the same time, if the distinction whch I have attempted to establish between ren-albumen and other proteids bear the test of further investigation at the hands of others, it would seem to corroborate the physiological hypothesis of Niemeyer and others. Neither does such a view exclude the influence of diseases of the blood or morbid constitutional states in producing albuminuria. We can quite understand, too, that albumen might appear in the urine, in consequence of increased transudation, as when not only albumen but blood and blood corpuscles transude through the capillaries, in consequence of excessive venous obstruction. Such an occurrence does not disprove this view of the action of the renal cells, for it is evident that, although the renal cells may be unimpaired, their functions may be so far interfered with by such vascular disturbance that they cannot transform the albumen completely into other products, as in health.

Some other features of difference between ren-albumen and serum albumen, as well as some points in which albuminous urines differ from each other, I have also had under observation, but I cannot enter into these on the present occasion.