Stewart (D. D.)

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BY

D. D. STEWART, M.D.,

OF PHILADELPHIA;

CLINICAL LECTURER ON MEDICINE IN JEFFERSON MEDICAL COLLEGE.

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Remarks on the Occurrence of a Normal, Constant Trace of Albumin in the Urine.[1]

BY D. D. STEWART, M.D.,
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CLINICAL LECTURER ON MEDICINE IN JEFFERSON MEDICAL COLLEGE.

IN a paper[2] read before the Society for Clinical Research, I drew casual attention to certain fallacies attending the use of delicate tests for the detection of serum-albumin in urine, laying stress on one especial source of error which I regarded as the most important and misleading. This related to the frequent presence normally of nucleo-albumin in amount sufficient to react to nearly all of these tests, such as Tanret's, Millard's, and Sebelein's reagents, and to

[1] Read before the College of Physicians of Philadelphia, May 2, 1894.

[2] "A Serious Fallacy attending the Employment of Certain Delicate Tests for the Detection of Serum-albumin in the Urine, especially the Trichloracetic-acid Test," THE MEDICAL NEWS, May 5, 1894.

all other similar test-solutions containing tannin, mercury, or a vegetable acid. This also applied to picric acid, especially the citrated solution, and to metaphosphoric acid, but particularly to trichlor-acetic acid, now much in vogue as a reagent for the detection of small amounts of serum-albumin in the urine.

I pointed out that a reaction could be so commonly obtained from several of these, with the urine of the healthy, that unless this were regarded, it were unquestionably better in clinical work to depend upon the less misleading, if also less delicate time-honored methods, especially that by ebullition, with, if necessary, the addition of acetic acid.

I had at one time regarded the slight reaction so frequently obtained with healthy urine, as indicative of the existence of a physiologic albuminuria, but within the past year or two the result of certain experiments of my own and the work of others in the same field has rather tended to convince me that in the vast number of cases the substance reacting to the very delicate tests is a mucoid body probably originating from the cellular elements of the extra-renal passages. My reasons for so believing I have entered into elsewhere,[1] so need not here detail them. Lastly, re experimenting with the delicate tests, in investigating certain cases of non-albuminuric nephritis, I have been especially interested to determine if the nature of the reaction often obtained by several of the very refined of these, and presumably not due to serum-albumin or to globulin,

[1] American Journal of the Medical Sciences, December, 1893. THE MEDICAL NEWS, April 14, 1894. Ibid., May 5, 1894.

could be exactly determined, and whether it be due to a mucoid body, settled.

Exact experiments with various percentages of artificially added nucleo-albumin to albumin-free urine seemed the most satisfactory mode of ascertaining with certainty the nature of its reactions, and their differences from those of serum-albumin, serum-globulin, the albumoses and peptone, bodies with which there is no doubt that nucleo-albumin has repeatedly been generally confused by many of the most accurate and perhaps all of the careless workers in urinalysis. As stated in the paper previously referred to, urinary nucleo albumin, which is now regarded as identical with the nucleo-albumin of the bile, is not true mucin, though still improperly so termed by many who are unaware of the important differences in these bodies which separate them both chemically and physically. Briefly, these bodies are distinguished in that concentrated solutions of mucin are mucilaginous and thready; with acetic acid mucin gives a precipitate insoluble in excess, while mucin, as a glycoproteid substance, after prolonged ebullition with dilute mineral acids yields, besides an albuminous body, a carbohydrate which reduces copper hydroxid.[1] Magnesium sulphate, also, is without precipitating effect upon solutions of mucin, totally unlike the case with solutions of nucleo-albumin. These differences in reaction have shown that it is doubtful if true

[1] See Hammarsten: Treatise on Physiological Chemistry. Sheridan Lea: The Chemical Basis of the Human Body. Neubauer and Vogel: Analyse des Harns. Gamgee: Physiological Chemistry of the Animal Body.

mucin is ever present in the urine, and that the reacting body is in reality nucleo-albumin. This fact is of the highest clinical importance, as apart from a local nucleo-albuminuria,[1] all the indications point to the fact that a nucleo-albuminuria, the nucleo-albumin of which is underived from any portion of the urinary passages, is of no uncommon occurrence. The clinical aspect of nucleo-albuminuria cannot now be discussed; suffice it here to state that it is a subject worthy of attention, and the study of which is in the line of the elucidation of not a little that is obscure concerning the question of albuminuria without nephritis.

In the course of recent experiments with urine from the healthy, specimens of day and food urine were often encountered which, with proper precautions to exclude precipitation of urates, gave a sharp cloud after appropriate treatment with acetic acid; but it was always found impossible to obtain by filtration, although dilution was first practised,[2] sufficient of a precipitate in this manner to be utilized for experiment, such really small amounts did the precipitate represent.

As urinary nucleo-albumin is unobtainable in trade the experiments were made with nucleo-

[1] That is, nucleo-albumin derived from the cells of some part of the urinary tract.

[2] Because of the soluble effects of certain of the urinary salts, it is more easy to remove the mucoid precipitate produced by acetic acid in dilute urine than in one of ordinary gravity. It is also on this principle that the interesting fact pointed out by Reisser is explained, that if to a specimen of urine which my be clouded by the addition of acetic acid, a second is added which is not so affected, no cloud may be produced by testing the mixture.

albumin (Merck's) derived from bile. This substance is regarded as identical, both chemically and in point of its various reactions, with nucleo-albumin occurring in urine. This body was found to be very slightly soluble in distilled water, and much more soluble in neutral or in alkaline saline solutions. With none of these in concentrated solution did it have a mucinous appearance, and the other tests, including ebullition with a dilute mineral acid, and subsequent testing of the precipitate for a reducing substance, showed that the body contained no true mucin. In the solid state it gave the xanthoproteic reaction, but reacted doubtfully to Adamkiewicz's reagent, and gave no response to Millon's, Liebermann's, or to the biuret test. This last was also examined for in strong solution, without result. This lack of reaction to certain of these color-reagents is interesting not only as a point in separating the nucleo-albuminous response from that due to other albuminous bodies, but also in indicating that the nucleo-albumin employed in the experiments made by Müller,[1] in which he obtained all the color-reactions, was contaminated with a second albuminous body.

Acting upon the fact that neutral salts, such as NaCl, AmCl, Na_2SO_4, and sodium acetate, serve to maintain the nucleo-albumin of the urine in

[1] See Neubauer and Vogel (loc. cit.). It is there stated that Müller obtained all the color-reactions with nucleo-albumin. The varying results there given can only be so explained. It is stated also that one observer noted that heat was without effect upon a solution of nucleo-albumin, and a second the contrary. The experiments I detail were made in a manner precluding error of this sort.

liquescence, no difficulty was encountered in obtaining a perfectly clear solution of this substance. The diluents used for the experiments were saline solutions of distilled water and normal acid urine; the former was prepared to contain approximately the amount of mineral salts, represented by NaCl, and a less amount of sodium acetate, that exist in urine of ordinary gravity. No allowance was made for urea, and this is regarded as being without auxiliary solvent effect. Specimens of very clear, filtered, morning urine from normal individuals were employed which, unfiltered, gave no reaction with trichloracetic acid until after five minutes' contact. Nucleo-albumin was added in proportions varying from approximately 0.4 per cent. to 0.02 per cent. In the preparation of the solutions this substance was first triturated with a small quantity of the diluent, subsequently thoroughly agitated with the total amount, and on each occasion thoroughly boiled for some minutes.[1] After a large number of filtrations a clear solution could be obtained. When an amount upward of 0.4 per cent. was added, some slight residue always remained on the filter; with smaller portions solution of the whole quantity was readily obtained, although filtration in removing turbidity naturally coincidently withdrew a certain amount in fine suspension.

[1] Lest ebullition have any decomposing effect upon the nucleo-albumin, altering its character, which, however, was not anticipated, in the form of menstruum employed, all the experiments detailed with the boiled solutions were repeated with solutions of similar strength prepared without heat. The results were identical. This likewise showed the absence of contamination by ordinary albumin, more than traces of which would, of course, be removable by boiling.

A very large number of experiments, approaching several hundred, were made with these solutions. A variety of tests in common use for the detection of albumin in urine were employed: ebullition with and without the addition of acetic acid; nitric acid; acetic acid and potassium ferrocyanid; metaphosphoric acid; plain and acidulated saturated solution of picric acid; Millard's reagent; trichloracetic acid, and a test-solution of mercuric chlorid with tartaric acid, a newly introduced reagent regarded of greater delicacy than Tanret's solution. Besides the employment of the color-reagents, notably the biuret test, the behavior of magnesium and ammonium sulphates in neutral solution was also ascertained.

The reactions with acetic and citric acids should be first mentioned. Citric acid was employed in both saturated and dilute solution. With these in all the various strengths of nucleo-albumin solution experimented with, a sharp contact-ring was obtained with a more or less diffused haze above. Acetic acid was used in the form of the liquid glacial acid, and in 2 per cent. and also in 25 per cent. strengths. The reaction with acetic acid was similar to those obtained with urines containing the mucoid body. The acid was applied both by the contact-method and by the addition of small amounts to 10 c.c. or more of the test-urine. A contact-ring was obtained with all strengths of acid, with a cloud above, though a less marked contact-reaction occurred with the undilute glacial acid, in which latter, indeed, nucleo-albumin can itself be dissolved. A cloud occurred instantly on the addition of small amounts of acid to the body

of a few centimeters of test-solution, its intensity and rate of appearance varying directly with the amount of nucleo-albumin present, and inversely with the proportion of salts present.

A reaction occurred with HNO_3, employed by the contact-method, with all strengths of nucleo-albumin solutions. It was still apparent as a slight contact-cloud in a dilution giving but a trifling haze with plain picric acid. With solutions approximately 0.1 per cent. or more, though carefully underlaid by the acid with a capillary pipet, the whole stratum of solution soon became opaque, and after standing a short time a deeper, opaque ring was found at the point of contact of the two liquids. With less strengths than 0.1 per cent., such as from 0.05 to 0.08, it was easy to obtain an instantaneous contact-reaction quite like the ordinary serum-albumin ring, although it lacked the appearance of denseness; the latter, if of any depth, usually has little or no opacity above, with these strengths of solution so tested.

A slight contact-cloud was still apparent with the weakest solution used, approximating 0.02 per cent., a dilution which gave but a faint contact-haze with unacidulated picric acid after standing a few moments. A few drops of fuming HNO_3 added to a few centimeters of urine also produced a slight opacity in urine containing this same dilution of nucleo-albumin.

A combination of acetic acid and potassium ferrocyanid, a test for serum-albumin in the urine first proposed by Pavy, and now much in use, gave the ordinary albumin-reaction, entirely indistinguishable

from that of serum-albumin.[1] This reaction was still apparent in the least strength tested. The reaction was decided at 0.05 per cent., and was still apparent at 0.025 per cent.

The various strengths of nucleo-albumin solutions also reacted similarly to metaphosphoric (glacial) acid. This reaction was in no way distinguishable from that of serum-albumin.

With considerable amounts of nucleo-albumin present, the whole solution above the piece of glacial acid became almost instantly opaque. With small amounts an almost instant contact-opacity occurred, typically such as is produced by serum-albumin, and gradually diffused from the portion of acid throughout the fluid. If but traces of nucleo-albumin were present, but still sufficient to react to acetic acid, though scarcely appreciable with unacidulated picric acid, the contact-response was delayed, not appearing for from two to three minutes, but soon becoming decided with longer contact.

A decided reaction was obtained with a saturated solution of picric acid, and with the citrated solution, indistinguishable in appearance from the contact-response with ordinary serum-albuminous urine. The ring was most sharply defined when the nucleo-albumin solution was of not too low a gravity (above 1010). No reaction, save a tardily

[1] Even so careful a chemist as Hammarsten (Text-book of Physiological Chemistry) does not speak of the similarity of reaction of nucleo-albumin and serum-albumin to either metaphosphoric acid or to the reagent of acetic acid and potassium ferrocyanid, in recommending these latter for the detection of albumin. No writer refers to confusion arising from the employment of metaphosphoric acid.

*

appearing contact-haze, occurred, however, to unacidulated picric acid with a nucleo-albuminous dilution approximating 0.02 per cent., although the citrated picric acid still gave a ring, and a response was still evident to metaphosphoric acid, and very markedly to trichloracetic acid, to Millard's, and to the mercuric test.

I had before shown that unacidulated picric acid solution reacted to the urinary mucoid body,[1] which is contrary to the view of Sir George Johnson. These experiments finally settle the question.

With Millard's reagent nucleo-albumin solution continued to give a sharp contact-reaction with a dilution at which plain picric acid had entirely ceased to react.

With trichloracetic acid up to the weakest dilution of nucleo-albumin employed, a sharp contact-reaction was obtained. With a dilution approximating from 0.025 to 0.04, with which unacidulated picric acid gave no more than a contact-haze, a markedly broad, opaque ring was apparent with trichloracetic acid.

With the tartrated mercuric reagent a similar reaction was obtained as that of the others, although it was found that this reagent did not compare in delicacy with trichloracetic acid.

It is now of importance to study in greater detail the reactions of nucleo-albumin with these albumin-tests, and to determine, if possible, how they may be differentiated from the reactions of serum-albumin,

[1] By the removal of the reacting body with acetic acid in the cold. See American Journal of the Medical Sciences, December, 1893; THE MEDICAL NEWS, April 14, 1894.

serum-globulin, the albumoses, and peptone, but especially from the first named. It will be recalled that as thorough ebullition was invariably practised with the acid artificial or natural urine in preparing the nucleo-albuminous solutions, more than traces of ordinary albumin could scarcely be present; and that as it was necessary to repeatedly filter to obtain a clear solution for testing, more than traces of the albumoses, or so-called urinary peptones, if present, would also be removed; as filtration of the solution was repeatedly done not only while hot but after it was thoroughly cooled.

The likeness of nucleo-albumin to urinary albumose or peptone is striking in several particulars. That the one has in the past often been mistaken for the other, I have no doubt, in view of the identity in many of the reactions of the two. This curious similarity in reaction of these bodies probably depends upon a close kinship. As Wright[1] and others have shown, nucleo-albumin is identical with cell-fibrinogen, which under the influence of ferments (proteolytic) is decomposed into a nuclein-moiety and albumose, or so-called peptone, the last of which is not infrequently present in the urine in cases of active leucolysis. It is conceivable and probable that traces of the albumoses are also present in the urine in conditions leading to proteolysis in the bladder of the urinary nucleo-albumin, through the action of pepsin and trypsin, which are normally present in the urine, and by the aid as well of formed

[1] See a paper on "Tissue- or Cell-fibrinogen in its Relations to the Pathology of the Blood." A. E. Wright, Lancet, March 5, 1892.

enzymes. But that nucleo-albumin is not identical with the albumoses or peptone is shown by its ready precipitation by acetic and other of the vegetable acids, and by the fact, as I have ascertained, that it will not give the biuret-reaction. This last seems a very positive mode of differentiating it from the other albuminous bodies.[1]

Its differentiation from ordinary peptone is quite easy, as I have found that by saturating hot urine containing nucleo albumin with ammonium sulphate, all traces of nucleo-albumin are removed, so that no response can be subsequently obtained with trichloracetic acid.

Its similarity in several points to serum-globulin is also quite striking. Its presence in the urine, I believe, has often been mistaken by those who are unaware that globulin very rarely, if ever, exists without the coincident presence of a greater amount of serum-albumin.[2] Like serum-globulin, if mucoid nucleo-albumin is present in some amount in the urine, as I have found is common in specimens of the day-urine (after food and exercise), it may not infrequently be precipitated from solution by considerable dilution of the urine with water, lessening the soluble effect of the urinary salts, or by dialysis to a very low specific gravity; or, like globulin, it

[1] It would become a clinical mode of differentiation of great value were the biuret-reaction for albumin one of any delicacy.

[2] This seems well established, and yet not infrequently in urine-reports of cases it is noted that serum-globulin but no serum-albumin is present. In these instances the reacting body is probably nucleo-albumin, which, I believe, is nowhere noted as likely to be a confusing factor.

may be readily thrown down by magnesium sulphate after neutralization with ammonia and separation of the earthy phosphates.

A strikingly interesting and important feature of the reactions of mucoid nucleo-albumin to the albumin-tests is the influence of heat. Although the presence of this body may be readily shown by the intelligent use of acetic acid or other of the vegetable acids, in the cold, yet without the assistance of heat it would be quite impossible by any reagent, were the reaction decided, save perhaps the biuret-test, to definitely assert that serum-albumin is not also present in some amount.[1] Heat with every test employed save two—the metaphosphoric acid, and acetic acid and potassium ferrocyanid—tends to diminish a nucleo-albumin reaction if the amount of this body present is in moderate or small percentage, such as is likely to be encountered in the urine. This result was invariable with a percentage upward of 0.15. With the presence of greater amounts, the soluble effect of heat was much less apparent; and with a percentage of from 0.3 to 0.4, heat was either without influence or slightly increased the reaction previously obtained. This effect, also, I have found varies in degree with different specimens of the diluent of approximately the same degree of acidity containing not only different strengths of nucleo-albumin, but the same amount. The cause of this variation seems largely due to the

[1] This is important, in view of the frequent coëxistence of serum-albuminuria with a so-called mucinuria (nucleo-albuminuria).

character and amount of salts present.[1] Albumin-free[2] urines, and artificial urines experimented with, showed this variation with different percentages of nucleo-albumin. The reagents, whether HNO_3, picric acid, trichloracetic acid, or others, seem to hold, after precipitation, a portion of the nucleo-albumin, so that a temperature approaching the boiling-point, even with very small percentages of nucleo-albumin present, will not entirely dissipate the precipitate.

Heat was most commonly applied in the manner detailed in the former paper,[3] by immersing the test-tube in hot water to a point beyond the upper line of fluid. In the trichloracetic-acid experiments there mentioned the bath was not always quite at 212° F., as so high a heat was not thought necessary. I was then unaware of the soluble effects of high heat upon traces of this mucoid body, accepting, as is commonly taught, that nucleo-albumin reacts to heat very much as does serum-albumin.[4] It evidently

[1] It is interesting to note that with approximately a 0.2 per cent. nucleo-albumin solution, containing NaCl, and a less amount of sodium acetate of a sp. gr. of 1017, the boiling-point has a slight precipitating effect. A further addition of a small amount of sodium acetate entirely prevented this precipitation in a second portion. Even without the presence of salts nucleo-albumin in small amount is apparently rendered more soluble by heat. A small amount shaken with distilled water, left, after a single filtration, a cloudy solution. Boiling at once cleared the latter.

[2] Save to trichloracetic acid.

[3] THE MEDICAL NEWS, May 5, 1894. This plan was pursued in order that mingling of reagent and urine is avoided when the contact-method is employed, as is so likely to occur by application of the flame directly to the tube.

[4] Treatises on urinalysis, save Neubauer and Vogel, state that urinary *mucin* is precipitable by heat. Reisser, quoted by Neu-

requires a temperature at or quite near 212° F., and prolonged—some minutes' contact—to insure a partial solution even with small percentages of nucleo-albumin. This is especially the case with trichlor-acetic acid. Nitric acid, and especially acidulated picric-acid solution, likewise retains a certain amount of the precipitant. With unacidulated picric acid the soluble effect of heat is more decided. The contact-coagulum obtained with it is dissipated to a greater degree by the aid of heat than with the others.

With potassium ferrocyanid and acetic acid, and especially with the metaphosphoric-acid test, however small the percentage of nucleo-albumin present, the soluble effect of heat is not evident. On the contrary, with the latter, heat tends to intensify the reaction obtained, so that it cannot in any manner be distinguished from that of serum-albumin. For this reason the clinical use of these reagents as precipitants of albumin must be regarded as absolutely untrustworthy, and should be unqualifiedly condemned.

It is important to study the effects of heat upon unprecipitated urine-solutions of nucleo-albumin. Heat, in any proportion of nucleo-albumin (in undiluted acid urine of ordinary gravity), upward of 0.15 per cent., is totally without precipitant effect. A few drops of dilute acetic or other acid which, added at ordinary temperature, cause a sharp

bauer and Vogel, found that ebullition does not change urine rich in nucleo-albumin (By rich, Reisser probably meant upward of 0 1 per cent., which is the greatest amount he at any time encountered.)

cloud, are without precipitating effect upon the boiling urine. As cooling occurs, clouding results. When nucleo-albumin is present in proportion of from 0.3 to 0.4 per cent., whether ebullition was previously employed in obtaining the solution or not, clouding always resulted without the addition of acid on boiling. This behavior of the various strengths of nucleo-albumin, with urine as a menstruum, was invariable in a large number of experiments. It shows that when nucleo-albumin is present in amount approaching saturation, heat exerts a totally different effect from what it does on solutions of moderate strength. As these latter (upward of 0.15 per cent.) are, perhaps, even greater than can occur in any condition leading to the presence of nucleo-albumin in the urine, apart from a pronounced vesical catarrh, which cannot mislead, heat becomes the most important mode of differentiating a nucleo-albuminuria from a serumuria.

From these results it would follow that the only reliable test for serum albumin, and that freest from liability to error, is the old-fashioned one by ebullition. But, apart from commonly recognized fallacies attending its employment, easily overcome, at least two important sources of error, strikingly developed in these experiments, are likely to arise with urine containing more than traces of nucleo-albumin. Treatises on urine-testing advise in the employment of the heat-test, the precaution that, should no reaction be apparent immediately on ebullition, the tube be inspected after standing and cooling, for cloud or precipitate, which, not being found to be due to acid urates, indicates albumin or

an albumose. Now, as heat is without precipitating effect upon a naturally acid, nucleo-albuminous urine, to which no further addition of acid has been made in process of testing, precipitation of the mucoid body, under the circumstances, would not occur; but if an acid be added in the application of the test, clouding will surely result as the urine cools, unless the acid have been added in too small an amount to overcome the neutralizing effects of the urinary salts. As related, the experiments with mucoid nucleo-albumin, artificially added in moderate amounts to urine, indicate that high heat tends to dissipate the cloud produced by acids, although, after forming, it will not entirely remove all its traces.

A second source of error is that relating to the presence of an excess of earthy phosphates in a decidedly nucleo albuminous urine. In the various experiments made with mucoid-fortified urines, specimens of the morning-urine were selected. On two occasions specimens of urine voided during the day were also employed as diluents, which, like the morning-specimens, were found not to react to trichloracetic acid within five minutes' contact in the cold. With these latter, and with one specimen of morning-urine, although all were acid, a cloud occurred in the fortified nucleo-albuminous specimens in the heated upper portion, which was not dissipated by the addition of a few drops of 25 per cent. acetic acid to 10 c.c. of the boiling urine. Control-specimens (containing no nucleo-albumin) were found to also cloud on heating, but were readily cleared by a small amount of acid. This indicates

that the presence of nucleo albumin in a urine rich in phosphates—a combination quite usual in the healthy in the day-urine, especially in that after food and exercise[1]—interferes with the solution of the precipitated phosphates by acetic acid, heat being coincidently applied to assist in the clearing of the mucoid reaction. This is an important point, ignorance of which has doubtless often given rise to error. It is of signal interest, as many urine-examinations, more especially those for life-insurance, are made of specimens voided under conditions giving rise to the presence of an excess of urinary mucin and of phosphates. But mucoid nucleo-albumin present in amount likely to originate error,[2] reacts to acetic or to citric acid, or to other of the vegetable acids, clouding more or less markedly at ordinary temperature, so that, by this means at least, the presence of excess is proved.[3] In testing for serum-

[1] Exercise and the process of digestion tend to increase the amount of mucoid substance in the urine. From the merest trace, undetectable in the morning-urine, it may rise in amount sufficient to cause a reaction to the contact nitric-acid test. I have noted this in the urines of several healthy male adults since examining into this point.

[2] In the phosphatic urine mentioned, an amount of nucleo-albumin was added approximating 0.1 per cent.

[3] I have elsewhere dwelt upon the precautions necessary to nsure a mucoid response with acetic acid, in urines with varying amounts of salts in which recognizable traces of mucoid nucleo-albumin are naturally present. The precipitating effect of acetic acid on urates must be borne in mind. The latter are differentiated by the fact that their precipitate is colored and has a characteristic microscopic appearance. They may be also recognized by the fact that a gentle heat, short of that necessary to diminish a mucinous reaction, clears the urate-precipitate. Dilution of the urine, before the addition of acetic acid, will also

albumin, should ebullition now not clear the acidulated urine, or at least not greatly diminish, the precipitate, the excess of earthy phosphates in a second portion of the urine should be removed in the usual manner, and heat later applied to the acid urine which has been first cleared by filtration.

It is of interest to here briefly detail the subsequent behavior of one of these albumin-free phosphatic urines to which about 0.2 per cent. of nucleo-albumin was first added, after the tendency to the marked precipitation of phosphates by heat was noted. The excess of the latter was precipitated by ammonia and removed by filtration. Later, the urine was rendered rather markedly acid and the nucleo-albumin thus thrown down removed. Repeated filtration gave a very clear solution, of a specific gravity of 1025. This, at ordinary temperature, still gave a cloud to acetic acid, but, however, tended to become quite clear on ebullition. Specimens gave a contact-cloud with nitric acid and a marked contact-ring with Millard's reagent; with trichloracetic acid the reaction was very decided; with metaphosphoric acid it appeared within a minute and was marked; with unacidulated picric-acid solution no immediate reaction occurred, but at the end of a minute a contact-haze was discernible, not increasing to an outspoken ring after standing for some time. With citrated picric-acid solution a fine, sharp ring was instantaneous. With all of these reagents, save metaphosphoric acid, the extent of reaction was diminished by the application of

tend to obviate a urate-precipitate and to increase a mucoid one.

decided heat, although with trichloracetic acid this reduction in amount of response was less manifest than with the others. The reaction of this specimen to the various tests was most instructive, as it contained a very small amount of the mucoid body, calculated by similar response of other specimens, to which measured amounts were added, to be 0.02 per cent. or less. This specimen was of especial interest for study because of correspondence in point of response to several specimens of day-urine from healthy subjects I had recently examined.[1] These gave a pronounced acetic and citric acid mucoid cloud, and responded quite markedly to the delicate tests for albumin, gave a narrow cloud at or near the meeting-line with HNO_3 by the contact-method, but did not react to heat alone.

So far as I am aware, the valuable aid heat may render toward promoting a response, in the application of tests used by the contact-method, for the detection of serum-albumin is nowhere mentioned. As apparently heat also becomes a factor of importance in the separation of the so-called mucin-reaction from that due to serum-albumin its employment is most essential when there exists the slightest doubt as to differentiation. I have frequently been able to bring out a serum-albumin reaction with HNO_3 used by the contact-method, by placing the test tube in hot water for some moments, which response otherwise was most tardy in appearing, if eventually noted at all. The use of heat in this way, rather than the application of the naked flame

[1] Tested in all points similarly to the reinforced mucoid urine save as regards the dissolving effects of strong heat.

to the tube, is essential, in order that actual ebullition in the tube be not permitted, otherwise disturbance of the relative relation of reagent and urine would interfere with the occurrence of a sharp contact-ring.[1]

Although I think it may be confidently asserted that a reaction so induced, as with one also apparent without heat, if decidedly sharpened by suspension of the tube in the water-bath, is due to serum-albumin (or serum-globulin), yet, on the other hand, as heat will not entirely remove a mucoid response after it has once appeared, there can be no great certainty as to the absence of recognizable traces of serum-albumin in the presence of the nucleoid body. If smaller amounts of albumin are looked for, the same must be said of the use of the delicate tests, although it would appear that if these are applied[2] with reagent and urine at the boiling-point—a method, of course, impracticable in ordinary routine urine-testing—no mucoid reaction will occur so long as these are maintained at a high heat. Of the many delicate reagents, unacidulated solution of picric acid has the least reacting power to traces of nucleo-albumin. Decided preference should, therefore, be shown it over all of the others, if the employment of a refined test seems essential. Citrated or otherwise acidulated picric acid should never be employed. Especially should it be avoided in the quantitative estimation of albumin by Esbach's method.

A serious fallacy attending the influence of heat

[1] By this method acid urates and a mucoid reaction are both prevented.

[2] Excepting metaphosphoric acid.

upon the trichloracetic-acid reaction in the experiments with nucleo-albumin should be here mentioned. It tends to further illustrate forcibly the impracticable, extraordinary delicacy of this reagent as a precipitant of albumin, and its inutility for clinical purposes. In the course of the investigation a control-specimen of acid urine previously unfiltered, of that portion used for experiment which gave no reaction at ordinary temperature with trichloracetic acid until some minutes' contact,[1] was thoroughly boiled and filtered while hot. This was done with the object of obtaining a control-solution in all particulars similar (save as regards the presence of nucleo-albumin) to the fortified nucleo-albumin specimen. To my surprise, it was found that this boiled and subsequently but once filtered control specimen reacted more markedly to trichloracetic acid than did the unboiled, unfiltered, original specimen. I then found that both cold and hot distilled water ran once or twice through specimens of all of the various makes of white filter-paper in my laboratory, gave either instantly or after the lapse of a few moments the contact white ring with trichloracetic acid.

I then procured specimens of the very best grades of fine filter-paper other than those which I had. It was thought that some of these would certainly not so react. Bearing as this matter does upon the subject of fallacies attending albumin-testing, the result is worth mention. The specimens, four in number, obtained from Messrs. Queen & Co., were marked

[1] And then but a very fine white ring, showing rather less than the ordinary normal traces of albumin.

Swedish; French; No. 390 and No. 589. They were of equal size, with a capacity of about 20 c.c. Two of each variety were used, one for hot distilled water, the other for distilled water at room-temperature; from 5 to 7 c.c. of each were passed through the filters in the usual manner several times. No pressure was exerted to force the water through. The distilled water in each instance was free from trace of cloud after filtration as before.[1] A slight contact white ring characteristic of traces of albumin occurred instantly with all of the specimens of distilled water filtered while hot, and tested in all save with the specimen of French paper while still warm. The albumin-reaction was quite decided with paper No. 390. With the cold, filtered, distilled water the reaction was instantaneous only in two, with but one filtration; in the others it appeared in from one-half to one minute. In all, the reaction became very much more decided on standing a short time; but although the use of the water-bath had no diminishing effect, heat did not seem to noticeably increase the reaction, as is the case with serum-albumin. A fine cloudy haze throughout the body of the tube was, however, developed by heat, indicating a precipitating effect of the trichloracetic acid, aided by heat, on the minute traces of albumin present. Experiments of various other sorts were tried with these solutions and trichloracetic acid, which, though interesting, cannot be detailed here. Millard's reagent, plain or citrated picric acid sol-

[1] For the sake of exactness, a control unfiltered specimen of distilled water was also tested with trichloracetic acid. As might be expected, it gave no reaction.

ution, and the reagents of the tartrated mercuric chlorid were found to give no indication of reaction, even after standing in contact for some time.

Millard, a number of years ago, called attention to the fact that the coarse French gray filter-papers which contains a great deal of cellulose or vegetable albumin, would yield sufficient to react to his reagent. This I long ago confirmed, finding that several of the delicate tests reacted to distilled water passed through the gray paper.[1] Trials made at that time with the white Swedish, French, and German makes, and the same tests, were resultless, so that it had not been suspected that a fallacy from this source, with a good quality of filter, existed.

To remove the traces of nucleo-albumin from suspension, it was necessary to repeatedly filter its menstruum ; from three to six papers were always employed, all of which probably contributed their quota of albumin to the filtrate, which would increase the amount of albumin recognizable by trichloracetic acid uninfluenced by heat.

Trichloracetic acid as a reagent for the detection of albumin is of such exceeding delicacy that, though evidently of little practical utility for clinical purposes, it apparently may be utilized to demonstrate, without need for concentration of urine, the existence of a constant normal albumin-trace. A physiologic albuminuria asserted to exist by Leube,[2]

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[1] Trichloracetic acid had not then been proposed as a test for albumin, and thus was not used.

[2] Virchow's Archiv, 1878, lxxii, 145.

Fürbringer,[1] Kleudgen, Senator,[2] Chateaubourg,[4] Capitan,[5] Posner,[6] and others, has been as strongly questioned by v. Noorden,[7] Millard,[8] Lecorché and Talamon,[9] and many others. The confines of this paper do not admit of even a cursory survey of existing data for or against this important question; the subject is one that requires much space for its consideration and will receive attention subsequently. Suffice it here to state that much of the work was done with concentrated urines, and the question of the frequent presence normally of extra-renal mucoid nucleo-albumin was often altogether ignored by those favoring the existence of a physiologic albuminuria. Leaving out of consideration the frequent high percentage of marked albuminurics among the apparently healthy, to be accounted for as a result of digestive derangements, circulatory weakness and the like, not omitting a latent nephritis, the fact that many of the experimenters in the field of physiologic albuminuria, who confidently hold to the existence of a normal minute albuminuria, fail to consider the presence of nucleo-albumin as a complicating factor, has thrown much doubt upon the results arrived at. Still regarding albuminous

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[1] Zeitschr. f. klin. Med., 1880, i, 346.

[2] Arch. f. Psych., 1881, xi, 5, 478.

[3] Die Albuminurie. Berlin, 1882.

[4] Thèse de Paris sur l'Albuminurie Physiologique. Paris, 1883.

[5] Thèse de Paris sur Albuminuries Transitoires. Paris, 1883.

[6] Berliner klin. Woch., 1885, xxii, 654: Virchow's Arch., 1886, civ, 497.

[7] Deutsches Arch. f. klin. Med., 1886, xxxviii.

[8] Bright's Disease. New York, 1892.

[9] Traite de l'Albuminurie et du Mal. de Bright. Paris, 1888.

bodies other than serum-albumin which may be present in the urine and are calculated to mislead, the experiments of many observers[1] have at least shown that at times no reaction may be obtained with such delicate reagents as Tanret's and Millard's; and Leube failed to get an albumin-response in some entirely healthy urines which had been subjected to much concentration. If the experiments which I have detailed with the reactions of mucoid nucleo-albumin to certain of the tests for albumin represent those obtainable when this body is present in traces in healthy urine, as I think there can be little doubt, then it can be proved that Posner is right in asserting that all urines contain demonstrable traces of albumin, and that a physiologic constant minute albuminuria exists, an albuminuria which apparently is not due entirely to the presence of a mucoid body, although it may perhaps still be accidental or contingent, and not arising from the blood-serum of the renal vessels.

In the paper on fallacies attending the employment of delicate tests for albumin it was stated that in a series of 105 urines from presumably healthy male adults, 102 gave at ordinary temperatures a contact-reaction characteristic of albumin within five minutes, and that the remaining three also readily reacted within this time on the employment of heat, showing a contact white ring of one or more lines in depth. The reaction in the 102, further tested by heat, was apparently increased.[2]

[1] Lecorché, Talamon, Millard, and others.

[2] As the influence of heat upon a slight mucoid reaction was not then understood, less attention was paid to long contact in

Since the series of 105 consecutive urine-examinations, in all of which the specimens reacted, as stated, to trichloracetic acid, I have examined a series of 35 morning-specimens from presumably healthy males in the prime of life, none of whom contributed any of the preceding specimens examined.[1] With the exception of but 2, applying the technique detailed in the other paper, without the aid of heat, a reaction was noted in all[2] within five minutes. In nearly all of these it was quite marked in this time. In the remaining 2 a contact-reaction was noted within ten minutes. In 13 the reaction was instantaneous, but in 2 only was it immediately very decided, and these were found to be examples of markedly albuminous urine reacting to coarse tests. In 6 only of the 33 could the reaction be termed slight at five minutes' contact.

The effect of heat upon all of the specimens

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hot water; nor was the latter always maintained at the boiling-point, it being more especially desired to differentiate as to urates rather than as to varieties of albumin. The nucleo-albumin experiments show that unless the heat be decided, and the amount of nucleo-albumin present small, the soluble effect of the former is not apparent. In fact, although lessening of reaction with heat was quite constant with traces of nucleo-albumin, it was not always so to the same degree. It was common, too, for a mucoid response to become again decided as the temperature of the water fell from boiling-point to a degree of heat still decided to the finger.

[1] Thirty-one of these 35 were collected for me by my friend Dr. G. M. Gould, to whom I am under warm obligations for them.

[2] In the specimens of the 105 urines less than 10 of the number had been filtered before testing. In none of the present 35 was filtration practised. The portion to be tested was always taken from the upper stratum, after subsidence of any mucous cloud in suspension.

which had reacted before its application was carefully studied, and was noted to vary somewhat with different specimens. In some it very markedly and promptly intensified the sharpness and depth of the contact-ring, which had not been very decided before the use of the bath. With 4 the effect of heat was negative, viewing the tube after some minutes' exposure to the bath. The contact-cloud remained as before, deepening, however, quite markedly, as was the case with all, after cooling and standing. It was certain that in all heat developed earlier a reaction which, without heat, was several minutes in becoming well-defined. In one of the 2 in which no response occurred in the cold until after five minutes' contact, it was noted that heat within a moment or so produced a contact-cloud of a depth of three or four lines, which cloud supplanted the slight, although more sharply defined, white ring evident without heat. The urine above in the tube also subjected to the bath became hazy. On inspecting the cooled tube subsequent to the use of heat, after it had been standing for some time, a sharply defined contact-ring was again noted of a much greater extent than at first, with a deep cloud above of some lines in depth.

One fact seems to me certain, developed by the examination of a series of nearly 150 specimens of urine from young male adults, the majority of whom are apparently in robust health, and none of whom had been regarded as renal suspects, that, excluding the 22 marked albuminurics, whatever the nature of the albuminous body reacting, a response may be obtained in any specimens of all urines of the healthy

by the intelligent employment of trichloracetic acid. This response may not occur instantly, but usually develops within a few moments, and may be brought out in any instance by the use of heat in the manner described.