On bedside urine testing : qualitative albumen and sugar / by Geo. Oliver.

ON

BEDSIDE URINE TESTING:

QUALITATIVE

ALBUMEN AND SUGAR.

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GEO. OLIVER, M.D., LOND.,

Member of the Royal College of Physicians of London, &c., &c.

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H. K. LEWIS, 136, GOWER STREET, W.C.
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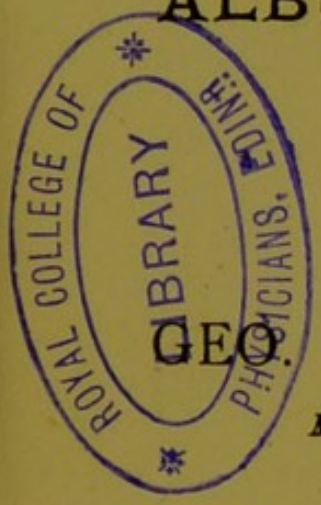

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PREFATORY NOTE.

THE following pages embody the pith of my observations on the qualitative testing of albumen and sugar; they form the first part of the work, which I began some time ago, with a view to facilitate urinary examination at the bedside.

While printing them for circulation among my professional friends, it occurred to me, that, perhaps, there may be others, interested in the matters here advanced, who may wish to refer to them in a collected form during the summer and autumn, which must pass over before I can take up what remains to be done. I have therefore, asked Mr. Lewis to publish this instalment.

Harrogate,
June 1883.

CONTENTS.

CHAPTER I.

THE
OLD AND THE NEW METHODS
OF
BEDSIDE URINARY TESTING

1. The Inconveniences which arise from carrying about Solutions of the reagents, especially when caustic or corrosive.

IT is not necessary to describe the miseries of Nitric acid, and of Fehling's solution: for I have merely to point to dilapidated and spoilt urinary cases, and to the fact that many medical men have been driven by the objectionable properties of these caustic fluids to give up urine testing entirely during their rounds. Hence, I fear there has often arisen some neglect of an important branch of practical medicine: for it is surely the duty of a medical man to examine the urine of every case—whatever the ailment—and not merely to trust to a suspicion of there being something wrong here and there, and then only to order a sample of the urine to be sent to his house.

But solutions, though physically unobjectionable, are undesirable in the daily round, when

compact dry preparations can be obtained, which will remain unimpaired by free exposure, and which can be freely handled, and as harmlessly—to the fingers and the tests—as ordinary writing paper. Fluids must have bottles, which take up room, and the best corking cannot be trusted, especially when the contents are required at every turn in the daily work.

II. Urinary Examination by Test Papers.

About six months ago the idea occurred to me to run the test solutions into chemically inert filtering paper, linen, or other fabric, and after drying, to use the product cut up into test papers.[1]

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1 Very shortly after the appearance of my first paper on Bedside Urinary Tests (*Lancet*, Jan 27th, Feb. 3rd, 1883), my friend, Dr. Geo. Harley, informed me, that in 1862, Professor de Luna, of the Central University, Madrid, showed him some urinary test papers. I have since, through Dr. Harley's kindness, examined Professor de Luna's Bolsa Chemica, and the printed instructions contained therein. I found six test papers, viz: (1) litmus (blue), (2) litmus (red), (3) turmeric, (4) molybdate of ammonia, (5) acetate of lead with nitrate of silver, (6) ozone papers after Schönbein. There were no test papers for albumen and sugar: the detection of which is clinically so important. None of the test papers were of any practical value, so that I think it fair to conclude, that is the reason why what Professor de Luna inaugurated remained sterile. He must, however be credited with the idea of presenting urinary test-reagents on paper. I need scarcely say that I was totally ignorant of these facts, until my attention was directed to them by Dr. Harley. The concluding words of Professor de Luna's memorandum are interesting. "Perhaps further studies, improving this pouch and rendering it more useful in medical practice, will enable us to ascertain with analogous results, sugar, albumen, &c. * * * My intention has been to put into real practice a thought which has occupied my mind for many years. As to the rest time will take upon itself its realization." (Quizá nuevos estudios escaminados á perfectionar está bolsa, haciendola cadu, viz: más útil en la practica-medica, permitan reconocu con reactivos áuálogos, el azuca, albumina, &c. * * * Yo solo me he propuesto dar forma real á un pensamiento que viene preocupàudome hau algunos anos: lo demás el tempo se encargará de realizarlo).

It appeared to me that we should thus secure the deposition of the reagents in a finely divided and concentrated state; a condition, it was hoped, which would be favourable to such a rapid resolution of them in the urine as to produce a quick and sensitive action on the constituents sought for. I soon discovered that the pieces of chemically charged paper were, when dropped into the urine, very delicate and cleanly tests; and being in the most portable and compact of all forms for clinical work, and, moreover, affording better results than I had previously obtained from the old corrosive test solutions it was not long before I cleared my spoilt urinary case of the latter; and I did so with feelings of satisfaction and comfort.

Apart from ministering to the personal convenience of the observer, these test papers possess clinical advantages, such as:

(*a*) The concentrated condition of the reagents: increasing their sensitiveness.

(*b*) Each kind of test paper being charged with the same quantity of the reagent, a uniformity is introduced into the qualitative testing, which cannot be attained when powders and liquids are used, without weights and measures.

This fact is well illustrated by the indigo-carmine test for sugar: a test which possesses higher powers— reminding one, of those of a microscope — proportionate to the amount of carbonate of soda mixed with the carmine. The indigo-carmine test paper alone sharply separates diabetic from non-diabetic urine: but on using it with one of the papers definitely charged with carbonate of soda, the delicacy of the test for the detection of small quantities of sugar is so much

increased, that it becomes specially available for the study of the traces of sugar about the borderland of diabetes, or that fill in the gap that exists between the normal and diabetic proportions; and the sensitiveness of the test is still further increased by the addition of a second carbonate of soda paper. I should also remark, that the test papers have not merely devoloped the capacity of this reagent as a clinical instrument, but have rescued it from obscurity and neglect: for the mixture of the constituents in water undergo a change, which, rendering the test valueless, doubtless led to its disuse. (See p. 38).

(*c*) Inasmuch as the papers can be made of known quantitative value, they provide a ready means of applying at the bedside the volumetric method of analysis. The clinical observer, though not requiring the decimal refinements of the laboratory, often desires, nevertheless, to infuse some of the accuracy of chemical observation into his work: these test papers will enable him to do so at the bedside, where the burette and other similar appliances are out of place, and can be dispensed with.

CHAPTER II.

THE QUALITATIVE ESTIMATION OF ALBUMEN.

1. Observations on the working of the old Tests (Nitric Acid and Heat) by the side of the others on the same urines.

No one can form a reliable opinion of the value of any one test compared with that of others, unless he devotes no small amount of pains to a careful observation of the working of the various tests side by side. This rule I have followed since I began this enquiry and my observations have now been very numerous.

In deciding the sensitiveness of the various tests, I preferred to draw conclusions from clinical evidence, rather than from experiments on dilute solutions of ov-albumen or ser-albumen. And, for this purpose I took a series of urines but faintly impregnated with albumen, presumably derived from the presence of a small quantity of pus, or of blood, or of both, as determined by the microscope. The table of results annotated at the time of every testing is before me. All the urines were acid except one, which was faintly alkaline. The reagents employed were the following :—

1. Strong nitric acid.

2. Boiling the urine and afterwards adding dilute acetic acid.

3. Saturated solution of Potassium ferrocyanide, and the urine freely acidulated by citric acid, as suggested by Dr. Pavy.[1]

4. Saturated solution of picric acid as advised by Dr. George Johnson.

5. Acidulated brine after Dr. Wm. Roberts.[2]

6. Standard solution of potassio-mercuric iodide, after Tanret, with, however, this modification —strongly acidifying the urine with citric acid instead of acetic.

The test fluid and the urine were in all the experiments brought into contact, as in Heller's method of using strong nitric acid, and the line of juncture was carefully examined for at least five minutes.

Out of the twenty urines nitric acid failed to indicate the presence of albumen in sixteen instances, boiling in fourteen, acidulated brine in fourteen, and potassium ferrocyanide in twelve; while picric acid and potassio-mercuric iodide gave a distinct and generally a sharply defined ring of precipitated albumen in every case.

By the side of these reagents I likewise experimentally tried citric acid dissolved in the picric solution—two drams to one ounce—and it invariably afforded a more rapidly formed and better defined zone than resulted from Picric acid alone; and, moreover, when the urine and the acidified picric solution were afterwards shaken together, the opacity from precipitated albumen

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1 See *The Lancet*, vol. ii., 1882, p. 823.
2 *The Lancet*, vol ii., 1882, p. 613.

was greater than when the simple picric solution was used. Hence, I conclude that, in detecting small quantities of albumen in urine, the power of picric acid is quickened and intensified by the presence of citric acid.

The reaction was indicated by varying degrees of rapidity by the different tests; I must name the potassio-mercuric iodide, picric-cum-citric, and picric acid as the readiest; and of the three I would, if asked for a preference, decide in favour of the first. I found as a rule nitric acid, acidulated brine, and potassium ferrocyanide much slower in bringing to light mere traces of albumen.

The same method of testing (Heller's) was followed throughout these observations for the purpose of securing uniformity in gauging the results. But I must say with regard to potassium ferrocyanide, that I am not quite satisfied as to whether the capacity of this test as an albumen precipitant was in this way fairly put to the trial; for, on several occasions I noticed the production of a very slight opacity all through the urine instead of a well-defined zone. I am, therefore, with this qualification in my mind, inclined to think somewhat better of it than the above recorded number of failures might lead one to suppose.

The outcome of these observations, as well as of many more recent ones, suggests to me the grouping of the tests in the following rising order of power to detect small quantities of albumen:— 1. Nitric acid and boiling. 2. Potassium ferrocyanide and acidulated brine. 3. Picric acid, sodium tungstate, potassio-mercuric iodide. I have, as a rule, found the members of each

group to be nearly equivalent, and confirmatory of each other; and, further, the albumen which nitric acid and boiling discovered was always detected with greater facility by all the other reagents, and those tests which comprise the third group frequently revealed traces which the others failed to bring to light; lastly potassium ferrocyanide and acidulated brine certainly took precedence over nitric acid and boiling.

As confirmatory of the foregoing observations I may mention that I supplied an analytical chemist with some albuminous urine, and he subjected it in the following way to a comparative examination by nitric acid and the tests I am introducing in the paper form. After diluting the urine until the albumen was just detectable by the acid, he proceeded to further dilution, when, the reaction failing to appear, the more delicate paper tests still distinctly indicated the presence of the albumen.

Undoubtedly the most delicate tests for the detection of albumen are the potassio-mercuric iodide, picric acid, and sodium tungstate. Experiment shows that these three albumen precipitants are of equal keenness: for, after precipitating the albumen in a urine by either the iodo-mercuric or the tungstate, the clear supernatant liquid obtained after complete subsidence of the albumen, gives no zone on running the saturated solution of picric acid upon it. The albumen precipitating power of picric acid is not therefore, greater than that of the other tests.

As the result of many observations and experiments, I conclude that one part of albumen may be discovered in

Parts of Urine.		Tests.
20,000	by the	Iodo-mercuric.
		Picric.
		Tungstate.
10,000 to 12,000	" "	Ferrocyanic.
		Brine.
6,000 to 7,000	" "	Heat.
		Nitric.

It follows, therefore, from the standpoint of delicacy in detecting albumen, that the clinical observer will, in using the more sensitive tests, instead of heat and nitric acid, score a clear gain : for they possess two and three fold the albumen detecting power of the latter. I do not class heat with nitric acid in this inference to suggest its exclusion in testing for albumen, because there is sufficient evidence to show that it ought not to be dispensed with. (See p. 30.)

II. The Clinical Aspects of the most Sensitive Albumen Precipitants.

Passing from the chemical to the clinical aspect of these delicate tests, this question suggests itself : Apart from their convenience, will they be more helpful than nitric acid in medical practice ? Experience is not sufficiently ripe to enable one as yet to garner the fruit : there is but discernable the early promise, which must be left to the realizing influence of time. Clinical observation will, however, ultimately define the place of these tests in medical practice. I am led by it to regard them as specially fitted to aid enquiry in such directions as the following :—

(1) *Albuminuria of Gout.*—The liability of the gouty to irritation of the urinary apparatus, from

the convoluted tubules of the kidneys to the end of the urethra, is well known; and the occasional or intermittent detection of albumen in gouty urine by heat or nitric acid is not an uncommon observation. But the very much more frequent, though perhaps not invariable, presence of a trace of albumen, as shown by these delicate tests, in such cases, is to me a revelation. It is true it is only now and then proved by the microscope to be of renal origin, and in the majority of cases, it is only due to a small portion of pus, which mingles with the urine as it passes over the irritated urinary mucous membrane. In itself it is, therefore, of but little moment: but though not generally giving rise to symptoms, it points to the advisability of putting a new condition—such as solvent waters — into the existence of the gouty, who should be regarded as a variety of the species *homo*, and then possibly the development of the more important renal disease to which it may lead, may be postponed or prevented. Then again, may not this microscopic form of albuminuria—the tell-tale of gouty blood—be utilized for diagnostic purposes?

(2) *Albuminuria from disturbances of the renal circulation*, as in heart disease, &c. I have sometimes found albumen, not otherwise to be accounted for in such cases, when heat and nitric acid failed to bring it to light.

(3) *The albuminuria of adolescents*, which, according to heat and nitric acid, is so apt to be intermittent. More watchfulness in the treatment, and more satisfactory results may follow the guidance of the more sensitive tests.

(4) *The complete removal of all traces of albumen from the urine during convalescence from acute renal*

dropsy, must be a point of great practical importance, especially with the view of preventing chronic renal disease, which may otherwise supervene years afterwards.

(5) *Every case of Bright's disease does not become full blown all at once;* it has a beginning in a slight departure from normal non-albuminous urine. Can any one say how often does concretionary irritation of the kidneys, which may induce traces of albumen not recognisable by nitric acid or boiling, merge into chronic renal disease? It is only the systematic examination of the urine in all cases—the apparently healthy as well as the unhealthy—that enables one to pick out here and there the kidneys that are gradually becoming crippled: though there may be no other sign of the fact than the mere trace of albumen brought to light by these sensitive tests. It is true nitric acid or boiling may, perchance, detect the albumen when it rises in quantity, so as to come within the range of these tests—as it does occasionally; but such an occurrence may not be caught, while the smaller quantities of albumen, which the more delicate reagents can demonstrate, are much more frequently present.

III. The Albumen Test Papers.

Observation having proved the following to be the best and most trustworthy albumen precipitants, viz., potassio-mercuric iodide, sodium tungstate, picric acid, and potassium ferrocyanide: it was found they were all soluble in water, and could then be evenly distributed through filtering paper, which, when dried, possessed the albumen precipitating power of the reagents unimpaired.

The selection of the precipitants was, in the first place, determined by an enquiry which I undertook in order to decide for myself which were the tests of most promise, and the production of them as test papers was quite a secondary matter.

1. *Potassio-mercuric iodide* was brought forward as an albumen precipitant by M. Chas. Tanret, of Paris.[1] According to my observations it is the most sensitive and reliable test known. The precipitate it produces is dense and bulky, and being white, it is, moreover, much more distinctive than the tawny yellow picric opacity, which resembles the colour of the urine; it likewise coagulates and subsides more readily than the latter. The standard solution, from which the papers are prepared, is the following:—

Grammes.

Mercuric chloride 2·70
Potassium iodide 6·64
} aq destill, 100 c. c.

2. *Sodium Tungstate.* According to the Journal of the Chemical Society, for March, 1874, it is stated that this salt had been employed by F. L. Sonnenschein as a sensitive blood test—producing with ammonia a deep green colour, even when the blood was so dilute as not to be recognizable by the spectroscope—and as an albumen precipitant in the presence of acetic, or phosphoric acid. I suppose this important observation has not attracted the notice of clinical observers, for I am not aware of any reference to it in the medical journals in its obvious applications to urinary analysis. On mixing together equal parts of the saturated solutions of the tungstate (one in four)

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1 See *Journal de Connaissances Médicales*, Mai 15, 1872; also, "*Recherche et dosage de l'albumine dans l'urine*" *Bulletin de Thérapentique*, 15 août 1877 p. 308.

and of citric acid (ten in six), and of water, I obtained an albumen precipitant of great delicacy, rapid in operation, and one moreover, so far as I have ascertained, devoid of all objectionable qualities. When merely dropped into the urine, or used after the manner of Heller, it has always quickly revealed the same minimal proportions of albumen as could only be brought to light by the other keenest tests. This combination, when evaporated to dryness on filtering paper, gives results very satisfactory, and the test paper thus produced, is in my opinion, one of the readiest and most sensitive of this series of albumen precipitants.

3. *Potassium Ferrocyanide,* when deposited to saturation on filtering paper is a very reliable work-a-day test; in my hands it has proved itself almost as sensitive as the other test papers. According to my experiments on peptones, this is the only albumen precipitant of the series, which will not throw down peptones as well as albumen. In this respect it may therefore be classed with heat and nitric acid, which cannot detect peptones. (See p. 29.)

Citric acid.—All the foregoing reagents are inoperative as albumen precipitants unless the urine is highly acidified; their application should, therefore, be preceded or accompanied by a sufficient charge of acid. For this purpose citric acid is easily made available, when deposited to saturation on filtering paper, and in this form it has afforded me uniformly satisfactory results with all the albumen test papers.

Compound papers.—Instead of using citric paper separately prior to the reagent paper, it has been combined by a thin layer of rubber with the latter,

as a single test paper, in the case of potassio-mercuric iodide. The picric and the tungstate are likewise presented as compound papers; but in these instances chemical reasons do not necessitate the separation of the citric acid from the reagent: the two are, therefore, united in the same paper.

4. *Picric acid* can be deposited to saturation on filtering paper, which becomes a most compact and cleanly vehicle, and which, moreover, quickly delivers its charge to water or urine. Repeated observation has shown me, that when united with citric acid, as in the test papers, picric acid is divested of most of the objections that have been urged against it. A few drops of albuminous urine instantly turn the bright picric solution, extemporaneously prepared from the test paper, into a muddy one, while the addition of more urine does not dissolve the precipitate with anything like the same readiness as when picric acid alone is used. Then, again, I have found that picric acid is apt to fail in albuminous urine when alkaline—a condition which is met by the picric-cum-citric test. This test paper is, however, in my opinion, the least satisfactory member of the series.

IV. The Mode of Testing.

No urine, while it remains turbid, should be submitted to the tests, or indeed to any test for the detection of albumen; it is best to remove the turbidity by filtration; this simple process, can, of course, be performed anywhere by a piece of blotting paper, or filtering paper carried for the purpose. If the opacity be due to urates it should be

removed by warmth, such as that of a wax match or taper—(See p. 31)—or of a little hot water added to the urine.

The nipple pipette—(see p. 54)—is a most useful article at the bedside ; it enables the observer to take up a perfectly clean specimen of urine, perhaps otherwise unprocurable, and to examine separately the deposit, or any particular portion of it.

From thirty to fifty minims of the urine are transferred to one of the short test tubes—(see p. 54.) It is now rendered strongly acid by dropping into it a citric paper, which may be allowed to remain, or may be withdrawn after the interval of a few seconds.[1] It is not now needful to ascertain if the urine be sufficiently acidified, unless it was distinctly ammoniacal, when at least two citric papers should be used,[2] therefore without delay the test paper selected is allowed to fall into it. A simpler plan, and one which answers almost as well, is to drop both the acid and the reagent papers (or a compound paper) into the urine, so that they may fall together to the bottom of the test tube, or the papers may be placed first in the test tube, and then the urine may be run in. Shaking of the tube is not at all

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1 A correspondent wrote me he had observed a turbidity in one instance after the addition of the acid paper. This event must be rare, for out of many hundreds of testings I have only once witnessed it in a slight degree, and it was then clearly due to urates thrown out of solution—it quickly vanished with the warmth of a wax match. However, I retain the description of the separate use of the acid paper, on the mere chance of a recurrence of such an opacity. I have for long followed the simpler plan of using the acid and the reagent paper together, or a compound paper.

2 It is best to heat ammoniacal urine before the testing, and to take a smaller quantity of it—say half that advised—when one citric paper will suffice.

necessary or even advisable, it may now be held in a vertical position, or be set aside for a minute. If albumen be present in a small quantity—*e.g.* below a sixth or eighth of a per cent.—a whitish cloud will very quickly gather about the paper, and will collect at the bottom of the test tube, or in the lower half of the column of urine: if there be only a trace, the opacity will of course be slight, and will be more readily detected by intercepting the light by the hand, &c., while, in striking contrast, the upper part of the urine will remain clear. If, however, the albumen exists in larger proportions, it will not usually produce a haze, but will coagulate about the paper and will fall from it in clots. The observer will now note the rapid precipitation of it into the lower portion of the urine, where it will gather as a cloud, the density of which will vary according to the albuminous impregnation, while the urine above will retain its transparency. Or, he may now shake the tube, when the urine will become less or more opaque, according to the amount of albumen present. If, on the other hand, the urine preserves its brightness, or if any slight turbidity it possessed prior to the introduction of the test paper is not increased, it may be safely inferred it is free from albumen. The whole proceeding, of course, takes up very much less time than that occupied in reading this description of it. The reaction is practically instantaneous when the urine has been freely acidified prior to the introduction of the test paper. It is, however, not quite so quickly obtained, though the delay only amounts to a few seconds, when, without previous acidification, the compound test papers are used.

Other simple and effective ways of using the papers will suggest themselves to the observer, such as the following.—

(*a*) Those who prefer to develop a zone of precipitation along the plane of contact of a test solution and the urine, can do so by aid of these papers. Two test tubes (see p. 54), or a test tube and a wineglass are required. Into one the reagent paper rolled up is placed with about fifteen minims of water, and, without shaking, is set aside, while a similar quantity of urine is put into the other test tube with a citric paper. After withdrawing the latter, the reagent, now in solution, is taken up by the pipette and is allowed to trickle down the side of the tube, in which it will either glide over the urine or collect below it. After developing the ring, the two fluids may be shaken together, when the albumen will be more largely precipitated as a milky cloud.

(*b*) I am indebted to Dr. S. C. Smith, of Halifax, for the suggestion of a very good and useful method. The papers are bent into a circle, so as to fit within the test tube, and are then pushed some way down: the tube is then filled with the urine. If albumen be present, the whole of the urine below the papers becomes opaque, while that above them remains transparent or unaltered. This is a very pretty experiment, and provides the means of comparing the urine under the influence of the tests with that unaffected by them.

If it be suspected that there may be more albumen in solution in any sample of urine than is within the range of one precipitant paper, it is only necessary to add another, taking care to use a citric paper also, if one of the compound test papers is not selected.

V. The Keeping Power of the Papers.

None of the papers have been bottled or kept from the air and light during the past six months. Still, I cannot discover the least deterioration of their power to precipitate albumen, or any change of colour, or other physical quality. The stability of the papers in the exposure and friction of daily work has now been definitely proved.

VI. Fallacies which may be met with.

No one test—as at present known—for the detection of albumen in the urine can, on all occasions be implicitly relied upon: whatever reagent be selected, the results it provides require every now and then to be qualified, or if need be corrected, by other tests or procedures. For instance, heat alone is clinically valueless: it must be supplemented by an acid, *e.g.*, acetic, otherwise the phosphatic opacity which it frequently produces, cannot be distinguished from the albuminous one; then again, if the acid modication of albumen be present, heat will fail to bring it to light. Nitric acid is, with the unwary, also apt to mislead, by its liability to precipitate a zone of urates or of urea: hence this test is not always to be trusted unless corroborated by heat. Picric acid has been shown by Dr. Geo. Johnson to precipitate artificially prepared peptones, and now and then urates also.[1] Potassio-mercuric iodide and sodium tungstate are likewise open to the same fallacies; and though potassium ferrocyanide does not throw peptones out of solution, it may, like the other

1 See *British Medical Journal*, vol. i. 1883, p. 614 and 859.

reagents on rare occasions, cause an opacity consisting of amorphous urates.

In selecting an albumen test for ordinary use, the choice, therefore, cannot hinge on the immunity of any particular reagent from fallacies—for there is none that can claim such perfection—but on the comparative absence of them.

On using the albumen test papers, certain precautions have been suggested by experience, which it is advisable to keep in mind, so as to reduce to a minimum the possibility of drawing an erroneous inference from the working of them.

(*a*) When I introduced these test papers, I thought it possible that urines might be met with, so strongly impregnated with albumen, that the instant the test paper was dropped into them, a dense film of coagulum might take place all over it, which would prevent further precipitation by the reagent thus locked up. I have not myself met with an instance of this kind, though the fluid of a hydrocele has provided me with a good example of the fact to which I refer. In this case none of the papers gave a precipitate, but on withdrawing them a greasy looking track was left along the inside of the test tube, and they felt soapy; but when the fluid was diluted to about one half, the albumen was thrown down abundantly by all the test papers. On adding picric acid, either in fine powder or in crystals, to the undiluted fluid, it would not dissolve, but merely caked together, and fell to the bottom, without precipitating any of the large amount of albumen present. Then on diluting the fluid with an equal bulk of water, still, after shaking, the albumen remained in solution. But, on taking a fresh portion of the diluted solution, the

picric powder or crystals threw down the albumen. A similar explanation of the failure of the reaction to that given above applies, therefore, also to this reagent in a solid form—even though finely pulverized—when added to a highly albuminous fluid: namely, the reagent becomes encased in a film of coagulated albumen.[1]

A urine so highly charged with albumen as to cause this behaviour with the tests, is not likely to be met with, without there being present such obvious and unmistakeable evidence of renal disease as, on the failure of the usual reaction, to cause the observer to dilute the urine, or to prepare a solution of the reagent from the test paper in the second test tube. See p. (54).

(*b*) The precipitation of urates is but a comparatively rare event: still it may happen; but the observer merely requires to be warned of the possibility of it, in order to discriminate between it and an opacity due to albumen. On two occasions I have noted the deposition of urates in large quantity after adding the mercuric iodide and the ferrocyanic, but not the tungstate test papers; both urines contained a small portion of albumen; and in both instances nitric acid produced a thick dense zone of urates. From repeated observation on these urines, I noticed some points of difference between the precipitation of the urates and that of albumen. When the latter—even when present in small quantity—is thrown out of solution, it very quickly collects at the lower end of the paper, and first of all forms a white cloud in the hollow of the test tube; then the opacity there collected steadily increases, until it may occupy the lower half of the column of urine. On the other hand,

1 See the *British Medical Journal*, vol. i. 1883, p. 859.

urates are apt to appear at almost any part; and several observations showed, they had a decided disposition to form a detached cloud with fleecy edges at the upper or middle portion of the urine under examination. The slight warming of the tube—as when the latter was held in the heated air above a wax match—quickly dissolved the cloud of urates, and left a haze due to precipitated albumen.

Experiments have shown me that no other constituent of the urine is likely to be thus precipitated. A strong solution of urea remains transparent after dropping into it the test papers: and an opacity due to phosphates—as when neutral or alkaline urine is boiled—is rapidly cleared up on adding a citric paper; these salts cannot, therefore, fall out of solution after acidifying the urine.

(*c*) I feel some hesitation in mentioning the last precaution I wish to adduce, because it is obviously so insignificant. But I do so, lest there should be such an obtuse observer in the profession, who cannot distinguish between a fine cloud of albumen, and the particles of fluff which may be shaken from the papers; should, however, any doubt arise a pocket lens will soon resolve it. When the testing is properly performed—shaking being avoided — there is no fluff to interfere with the detection of the merest trace of albumen.

VII. The forms of Albumen and Peptones.

(*a*) *The forms of Albumen.*

Albumen as ordinarily met with in the urine is serum albumen; and, like that which exists in

the blood, it is coagulable by heat. In some urines, however, it is associated with an alkali, and in others with an acid: then it is no longer thrown down by heat alone. But serum albumen, and the alkaline and acid forms of it, are coagulable by nitric acid, as well as by all the test papers; the latter, therefore, cover the same ground as the acid.

I have no experience of Bence Jones' albumen, which, judging from the statements of its properties, appears to stand midway between serum-albumen and peptones.

Is there any practical advantage to be derived from discriminating between these modifications of albumen? Inasmuch as the alkaline and acid forms of it are in all probability merely serum albumen accidentally associated with an alkali and an acid respectively, is it necessary, clinically, to differentiate between them? Is it not better and safer,[1] to use first of all one of the albumen precipitants that throw down all forms of albumen alike; and then, if it be thought necessary to enquire into the form in which albumen exists in the urine, to appeal to heat? (See table.) As bearing on this point, it may be well to keep in mind that Béchamp never found the albumins of the blood in the liquids of discharges: a fact which, therefore, indicates not merely transudation, but transformation.[2] But, for the detction of the latter, something more than heat is required.

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1 I say 'safer' because if heat be chosen as the precipitant of the albnmen, acid albumen, even in large quantity, will remain undetected: and to meet this possibility, it will still be necessary to use nitric acid, on the other tests.

2 *Compt. rend* v. 88, p. 608

THE BEHAVIOUR OF ALBUMEN TESTS ON THE FORMS OF ALBUMEN, AND ON PEPTONES (ARTIFICIALLY PREPARED).

	TESTS.					
	1. Heat.	2. Nitric.	3. Mercuric.	4. Picric.	5. Tungstate.	6. Ferrocyanic
1. SERUM ALBUMEN	+	+	+	+	+	+
2. ACID ALBUMEN	0	+	+	+	+	+
3. ALKALINE ALBUMEN ...	0	+	+	+	+	+
4. PEPTONES (Digested serum albumen).....	0	0	+	+	+	0

Reagent 3, 4, or 5 being used.

The precipitate when heated.

1. Coagulates, and separates from the urine, which remains clear in the cold = ALBUMEN.
2. Clears up entirely, and re-appears in the cold as a diffused opacity, which is soluble by heat, and returns without precipitation in the cold = PEPTONES.
3. Clears with coagulation: coagula settle, and the sepernatant liquid becomes turbid as it cools:—the turbidity dissolving with heat and re-appearing in the cold = ALBUMEN with PEPTONES or URATES.

Picric peptonic opacity soluble in Nitric Acid.....
Mercuric " " Hydrochloric Acid .....
} which do not dissolve Urates.

A slight opacity when heated.

1. Soluble, but re-appears on cooling = PEPTONES or URATES.
2. Persistent—not coagulating or depositing = ALBUMEN

(*b*) *Peptones.*

In 1852[1] Mialhe asserted that digested albumen (the peptone of Lehmann) may appear in the urine: but the matter still remains much in *statu quo*. Inasmuch as it has been found that picric acid, potassio-mercuric iodide, and sodium tungstate precipitate artificially prepared peptones, peptonuria has been assumed to raise an objection to the conclusions to be drawn from the working of these tests as albumen precipitants, when not otherwise corroborated. Experimentation is, however, one thing, and practical experience of disease is another: and the two are not always confirmatory, even though one may anticipate complete conformity. After a careful scrutiny of the working of the albumen test papers, I have hitherto failed to glean any evidence, to cause me to distrust, on the score of peptones, the results obtained by them. The table on the previous page gives the pith of my observations on peptonous urine—derived from digesting albuminous urine—and on the mixture of peptonous with albuminous urine: and on the forms of albumen.

VIII. The Value of Heat in testing for Albumen.

Inasmuch as the tests introduced as substitutes for nitric acid at the bedside perform their work satisfactorily without the aid of heat, the latter is apt to be discarded entirely. Though observation has shown me, it is but now and then really necessary as a verifier of results, I have, nevertheless, found the systematic application of warmth after the precipitation valuable: as

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1 *L'Union Médicale*, 1852.

(*a*) When small quantities of albumen are detected; it rather intensifies than diminishes the true albuminous haze, though it may not coagulate it:[1] while on the other hand, any urates which may have been likewise precipitated will vanish. In working for traces of albumen—such as generally exist in gouty urine—it is easy to prove the superiority of first using the albumen precipitant and then heating, over the comparatively imperfect search effected by boiling only. An albuminous urine is mixed with a non-albuminous one, until boiling fails to precipitate any albumen: to a portion of this mixture the test papers are added, and the haze is warmed, when the opacity—slight though it may be—is obvious when placed by the side of the boiled urine.

(*b*) When albumen is precipitated beyond mere traces; it causes the opacity to collect quickly into coagula, which mass together and float up bodily like clotted cream to the surface—leaving the urine transparent, or only slightly opaque from a trace of albumen that will not coagulate by heat. A better rough notion of quantity can thus be obtained on the spot, than from the density of the precipitate.

The Mode of Heating. At the bed-side cleanly heating or boiling may be effected without the spirit lamp, which is cumbrous and apt to get out of order. It is merely needful to use a long wax match, or taper, or candle, and to hold the tube just above the tip of the flame, when smoking will be entirely avoided.

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1. I have proved by experiment, that when a trace of purified albumen, or a small portion of albuminous urine, is added to normal urine, and precipitated by the test papers, the opacity is slightly intensified by heating, and does not always coagulate, as albumen does when present in larger quantity.

IX. The Selection of Test Papers.

The first matter to be decided is : should preference be given to the single or the compound papers ? The use of the acid and of the reagent apart has undoubted advantages over that of the combination of them in one paper, such as the following:—

(*a*) Any precipitation of urates and of oleo-resins[1] induced by the acid can be readily detected.

(*b*) The reaction is quicker.

(*c*) Those who prefer heat as the reagent, will find the citric paper convenient for the solution of precipitated phosphates. As previously stated, it is best to use the test papers first of all in the search for albumen, because they precipitate all modifications of this body; then the observer, if he wish to do so, may differentiate serum albumen by heat, when the solvent power of the acid paper may be required. Alkaline albumen may likewise be worked for by heat aided by the previous use of the citric paper; and acid albumen may also be precipitated by heat after neutralizing — care being taken not to alkalinize — the acid by means of carbonate of soda paper. (See p. 51.) The separate use of these papers, therefore, enables the observer to utilize heat, not merely as a detecter of serum albumen, but as a discriminator between the three modifications of

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1 When an oleo-resin (such as balsam of copaiba) is present in the urine, citric acid produces a milkiness, which disappears on boiling, but quickly returns intensified, even though the urine is still quite warm. Potassio-mercuric iodide, potassium ferrocyanide, and sodium tungstate, in the absence of the acid, do not precipitate the oleo-resin; but picric acid in solution throws it down as a dense opacity, which, like an albuminous one redissoives, until an excess of the acid has been added; it is, however, soluble on boiling, but very quickly reappears.

albumen. I, therefore, prefer to use the reagent apart from the acid.

Then, it may be asked—as I have frequently been—which of the four test papers should be selected as the best ? It is very difficult to form an opinion when all do their work with much the same efficiency. In, however, giving my preference, I am but expressing the gist of the opinions of others which have reached me. The Picric paper should be eliminated as the weakest member of the series. The rest are clinically of nearly equal value, but of the three, I would, upon the whole, decide in favour of the Potassio-mercuric iodide and the Ferrocyanic papers.

CHAPTER III.

THE
QUALITATIVE ESTIMATION OF SUGAR.

I. The Detection of Sugar by Means of Test Papers.

All the reagents which are employed as tests for sugar require to be associated with an alkali: some, such as the cupric, must have a caustic alkali; while others, as indigo-carmine, sub-nitrate of bismuth, and picric acid, will work satisfactorily with an alkaline carbonate. The former are for obvious reasons unavailable as test papers; while only the latter can be so employed.

I have chosen the indigo-carmine as being, in my opinion, the least open to fallacies, and the best and most sensitive test. Picrate of soda with an excess of carbonate of soda has, however, provided me with a very good test paper: its constituents are so proportioned, that when boiled in thirty minims of water, five drops of non-saccharine urine afford no appreciable reaction, while with one drop of diabetic urine the colour deepens very considerably. But, inasmuch

as the indigo-carmine covers exactly the same ground as the picrate test in revealing the presence of all the saccharoid bodies which may appear in the urine, I fail to perceive any advantages for bedside work which would arise from the introduction of the latter as a test paper.

II. Indigo as a Test for Glucose.

When casting about for a good and at the same time convenient test for sugar in the urine, I was particularly struck with a fact relating to indigo; and that was the presence of this intensely blue substance in a colourless state, when associated with glucose or some similar sugar; for instance,

(*a*) When in the sap of living plants (Indigofera and others) it is combined with Indiglucin, ($C_6H_{10}O_6$) which has a chemical formula not far removed from that of Glucose($C_6H_{12}O_6$;)[1]

(*b*) When the dyer mixes Indigo with grape sugar and dilute caustic alkali to produce a colourless solution in which to immerse his fabrics, which acquire a blue colour on exposing them to the air, and

(*c*) When Indigo appears in normal and pathological quantities in the urine, it does so—according to Schunck—in the form of colourless Indican: a substance which, moreover, as just stated, exists in the woad and other indigo-yielding plants. When this compound is broken up, it is supposed by Schunck, that on the one hand, Indigo-blue (Indigotin), or its isomer, Indigo-red (Indirubin),

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1 Indiglucin in some of its properties resembles glucose; for instance, when heated, it gives off an odour of caramel, it also reduces cuprous oxide from an alkaline cupric solution, and the metals from the salts of silver, gold, &c. It does not, however, ferment with yeast—it only turns acid.

and on the other, Indiglucin, are set free, according to the formula—

$$C_{26}H_{31}NO_{27} + 2H_2O = C_8H_5NO + 3C_6H_{10}O_6$$

(Indican). (Indigo-blue or red). (Indiglucin).

Almost every clinical observer must have met with ammoniacal urines tinted blue, or violet, or reddish violet; such urines are probably good examples of this reaction; the blue tint arises from free indigo-blue, and the violet one from a mixture of the red with the blue isomeric forms of indigo —but the source of this colouring matter must in all cases be referred to the colourless Indican, a normal constituent of the urine, which is apt to be split up into Indigo-blue or red, and Indiglucin. Therefore, it would appear according to Schunck's investigations, that Indigo associated with a sugar, passes from the system in the urine unrecognised, because it is thus deprived of its colours (blue and red), and it only becomes evident to us when disassociated—the Indican being split up.[1]

It then appeared to me a reasonable question to ask, Can glucose in the urine be made in some way to discharge the deep blueness of indigo, and thus to tell the tale of its presence? Experiment gave a positive answer: for when indigo was suspended—it did not dissolve—in a weak solution of soda, or in a stronger one of carbonate of soda, a test solution was obtained, which, when heated with a few drops of diabetic urine, underwent a series of remarkable changes of colour—from blue to green, then to violet, to red, and finally to yellow. I longed to run the liquid containing carbonate of soda and indigo into filtering paper,

1 I am aware of the theory of Indol—a derivative of the proteids—broached by Jaffé in 1873 to account for the presence of Indican in the urine, and supported since by Baumann, Tiemann, and others

and use it as a test paper; because, with carbonate of soda as the alkali, the test papers would have been more durable than with solution of soda. But unfortunately, after deposition on the paper, the indigo would not leave it.

III. The Alkaline Indigo-Carmine Test.

My attention was directed to the use of indigo-carmine as a test for glucose by a paragraph in a work by M. Méhu, which stated that when the carmine of indigo is heated with carbonate of soda, and a solution of glucose or saccharine urine, the blue colour is converted gradually into green, then into red, and finally into yellow.[3]

Carmine of indigo is the sulph-indigotate of sodium—a salt intensely blue and soluble (solubility 1 in 120 parts water). Sulphuric acid when heated with indigo produces the soluble sulph-indigotic acid, which, after combining with a base (such as sodium, calcium, magnesium, &c.), provides us with indigo as a reagent in a perfectly dissolved state. When carbonate of soda is mixed with a solution of the carmine the latter is precipitated in a fine state of division; when freshly made and shaken this mixture may pass for a solution much like that of Fehling in colour and general appearance. A perfect solution of a greenish blue tint is, however, obtained on heating the liquid.

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3 *L'Urine*, par Dr. C. Méhu. Paris, 1880. Since the above was written, Dr. Ralfe has shown me a passage to the same effect, in Neubauer and Vogel p. 73 (Sydenham Society) which I had not previously seen. These authors refer to Mulder, who appears to have been the introducer of this reagent for clinical purposes. I find no reference to indigo as a test in that invaluable, if not indispensable index of medical progress—Dr. Neale's Digest—or in the works of English authors, within my reach.

IV. The Test Papers Possess Advantages over the Solution of Indigo-Carmine.

The mixture of the indigo-carmine and carbonate of soda in water, undergoes a gradual change, which renders the test useless; the rich indigo blue slowly gives place to a faded pale green. The test in an aqueous form, is, therefore, not available, unless the constituents are kept apart as two solutions. The inconvenience of doing this is obvious, but it is surpassed by that which arises from the necessity to use on every occasion exactly the same proportions of the carmine and the alkaline carbonate, otherwise—as I have frequently observed—the results of the testings are not comparable. The liquid preparation of the test is valueless as a clinical instrument, and its grave defects must have speedily led to its disuse.

The test papers, however, not only meet all these disadvantages, but amplify the powers of the test.

(*a*) Every paper is charged with the same definite quantity of the reagents; it thus provides a uniformity for the qualitative testing, which also, becomes a standard of known value for the quantitative estimation. An additional range of sensitiveness is, moreover, provided by the paper containing a uniform charge of carbonate of soda, when used with the ordinary test papers.

(*b*) The paper furnishes a perfectly transparent[1] alkaline solution of the sulph-indigotate,

1 A clear solution cannot be obtained from the papers heated in the London or other hard drinking water. The characteristic reaction of the test is, however, precisely the same.

and all the reagent with which it is charged becomes completely reduced by the sugar, so that in the quantitative estimation the colourlessness of the paper as well as that of the solution will be found to be the guide as to the termination of the completed reaction. When the liquid preparation is boiled it assumes a green colour: Mehu, Neubauer, and Vogel are, therefore, incorrect in giving that hue as the first stage of the reaction of indigo with grape sugar. On the other hand, when heat is applied to the test papers in water, a fine true blue solution is obtained, much resembling Fehling; and no amount of boiling will induce a green tint—even though saccharine urine be added. Unlike the solution, the test paper, therefore, provides a clean start for the testing: so that the urine to be examined may, with some saving of time, be added before heat is applied, and the first change of colour, which after ebullition appears, can be safely taken as the earliest step in the reaction.

(*c*) The stability of the test papers is beyond question. The constituents, being dry, remain unchanged: and, when dissolved out of the paper, they furnish a freshly prepared solution at each observation.

V. The Reaction.

The characteristic reaction which indicates the presence of glucose in the urine, arises shortly after the first simmer of the solution prepared from the papers, a drop, or at most two of diabetic urine having been added before the heating. Then a beautiful violet tint suddenly spreads throughout the bright-blue solution; very quickly the violet deepens and passes into purple; this in

its turn melts into reddish-purple, which gives place to various tints of red, and these as quickly merge into orange-red and orange, and finally the solution becomes of a straw colour, which remains without further change, though heated ever so long. At this point the paper assumes the same light-yellow colour as the liquid. The complete range of this striking colour reaction embraces all the prismatic colours except green, and the order of the appearance of the successive hues is always the same. The reaction is one of great beauty; for the primary colours are not merely pure and sharply defined, but all the transitional and intermixed tints pass quickly before the eye in such rich profusion as one rarely sees in nature herself. Now, on shaking the tube the colours return in the inverse order to that in which they appeared. This remarkable thing is not due to cooling, but to admitting the oxygen of the air into the liquid; for the various hues at any stage of the reaction may be caught and retained for days, merely by corking the tubes full of the solution, and the return of the colours, when the test tube is at rest, always appears first at the surface, and slowly spreads downwards—so slowly that after putting the solution aside for some hours, at least the lower half will still retain its acquired colour.[1]

1 Inasmuch as the return of the colours is clearly due to oxidation, it will probably be safe to presume that the reaction of glucose on indigo blue is a process of deoxidation. I do not think the facts admit of a more specific inference. The first stage of the reaction is pretty clearly the conversion of the blue into the red isomeric form of indigo—hence the shades of violet, purple and red; and just that small amount of glucose can be added so as to secure only these steps of the partial reaction. The second stage is the gradual merging of red into pale yellow—a colour which indigo-white produces when dissolved in aqueous alkalis.

Experiment has shown that the tint reached in any particular observation depends on the quantity of glucose added to the test liquid—e.g., the reaction may stop at violet, purple, red, &c., and when it thus halts, it can be easily made to proceed to the final stage by adding more of the diabetic urine—the liquid the while being kept warm. This suggests a principle on which to found a quantitative analysis. The method I propose to myself is a very simple one; it is based on the complete removal of all the colours below the pale yellow, except when the sugar exists in such small quantities as are not gaugable by any known procedures, then the delicate scale provided by the different colours may be made available.

VI. The Mode of Testing.

One of the papers should be dropped into the half-inch test tube, and water poured in to the 50*m* mark; a column one inch in height and half an inch in diameter will thus be produced, and the solution obtained will always acquire the same concentration. Then not more than one drop[1] of the suspected urine is let fall into the tube from the pipette, heat is applied (See p. 31.), and on shaking the carmine will dissolve. As with the qualitative testing of albumen, so in that of sugar a tea-spoon can be used instead of a test tube, and the urine can be dropped from the end of a penholder or similar article.

After freely boiling and the appearance of the first change of colour, it is advisable to move the tube away from the flame, and merely to keep its contents hot in the higher part of the column

1 The pipette held vertically will give drops of nearly equal size, *i.e.*, half a minim.

of heated air above it. Then all the colours will follow in the order I have given, without disturbance from ebullition, until straw-yellow is reached, providing the amount of glucose present is sufficient to develop all the colours: if not, another drop of urine should be added.

VII. The Results of the Testing by the Side of Fehling's Solution.

In applying the test papers to different urines, I took Fehling's solution as my guide, because it is the best glucose test.

The results of the working of the two side by side were briefly as follow:—

(*a*) On always submitting one drop of urine to the indigo test, and the presence of sugar being shown, confirmation was invariably provided by Fehling used in the ordinary way.

(*b*) On the other hand, whenever one drop of urine gave no reaction with the test, Fehling's solution did not give a precipitate.

(*c*) On, however, taking more than one drop of urine a different kind of experience was opened up. Then with various urines a deep violet or purple tint would strike up on the addition of the second, third, fourth, fifth, sixth, or more drops, and Fehling employed in the usual way gave negative results. But I am inclined to think in the cases in which from two to four drops developed the partial reaction, that Fehling, when applied as follows, showed a change which suggested the presence of a very minute quantity of sugar. The urine—either in its normal state or decolourized by animal charcoal—and the solution were mixed in equal proportions, and, it being found the true blue remained

intact—not having been turned to green by an excess of the yellow urine—heat was applied, when, though no precipitate was visible, the blue quickly turned to a decided olive-green tint, contrasting strongly with the pure blue of the solution held by it in another test tube. In several such instances the transparent green became muddy, either in the act of boiling, or in a few minutes, but, even then, the clear supernatant liquid, after the subsidence of the precipitate, was of the same green hue, while others remained free from turbidity. By adding mere traces of glucose, or small quantities of diabetic urine to normal urine, I have repeatedly tried to reproduce these results with Fehling, but, whenever a reaction was induced, by however small a quantity of glucose, I could never secure a permanent transparency of the green colour, for, either while the boiling was proceeding, or during cooling, it invariably became milky. I can, therefore endorse the statements of Dr. Wm. Roberts respecting the detection of small quantities of glucose in the urine. "The copper solution having been heated to ebullition, and something less than an equal bulk of the suspected urine having been added, the mixture is again raised to the boiling point. * * * If the urine contains less than half a grain per cent. of sugar, the precipitation does not take place immediately, but occurs as the liquid cools, in five, ten, or twenty minutes, and the manner of the change is peculiar. First, the mixture loses its transparency, and passes from a clear olive-green to a light greenish opacity, looking just as if some drops of milk had fallen into the tube. This green milky appearance is quite character-

istic of sugar. By this proceeding one-tenth of a grain per fluid ounce, or less than one-fortieth of a grain per cent., can with certainty be detected."[1] Inasmuch as, according to these observations, glucose only accounts for the green opacity, an explanation of the transparent green is yet to be found.[2] The intensity of the green reaction (whether milky or clear in the cold) was always proportionate to the colour change afforded by the carmine test papers: and the urines that gave no reaction with the latter below the fourth

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[1] "*A Practical Treatise on Urinary and Renal Diseases, &c.*," by Wm. Roberts, M.D.

[2] I am now inclined to view this obscure matter by the light derived from the following facts. While proceeding to extract from the heart of the ox, Kreatin, inosite, and other constituents of muscular tissue which may appear in the urine, I found the clear concentrated solution, after precipitation of the albumen and phosphates, afforded the characteristic play of colours with indigo-carmine, and a green reaction with Fehling: in the latter case, after the separation of a green tinted opacity, the solution remained for some hours olive-green and transparent. When the creatine crystallized out and was recrystallized, it gave no reaction with either test. The mother liquor, containing the inosite, still reacted as before, and the crystals of inosite dissolved in water, turned undiluted Fehling into a clear olive-green solution, and reduced indigo-carmine. Prof. Arthur Gamgee remarks that inosite "does not reduce Fehling's solution, but changes its colour to green." (*A text-book of the Physiological Chemistry of the animal body.* p. 338.) Cloetta, many years ago, found that after the subsidence of a green precipitate, the supernatant liquid became blue, and the filtrate was again turned green by heat (Watts' *Dictionary of Chemistry.*) This fact I have noted with several urines, which, though remaining transparent for some time after the boiling, let fall a green precipitate over night, and then presented a clear greenish blue appearance: on, however, heating the transparent supernatant liquid, it became again distinctly green, but not quite so much so as after the first boiling with Fehling. This matter will, however, require a good deal of observation, before it can be made available as positive knowledge for clinical purposes; but, from what I have already seen, I am persuaded that inosite—in small quantity—is a frequent constituent of the urine. Whatever clinical bearing it possesses, has yet to be worked out. I have certainly detected the reaction most frequently, and to a more marked degree in cases of functional disorder of the liver.

or fifth drop, either did not disturb the azure blue of Fehling, or merely turned it to a greenish blue shade.

VIII. The behaviour of the Indigo-Carmine, the Cupric (Fehling's) and the Picric Acid Tests when boiled in the presence of various substances.

A test so little known and understood as the Indigo-carmine should be critically examined, before it can be safely admitted as a clinical reagent by the side of Fehling's solution and Picric Acid. In order to obtain a preliminary gauge of its position as a glucose test for urinary work, I, therefore, boiled each of the following substances —which are either constituents of the urine, or medicines which may appear in that fluid—with it and the other tests.

*The substances marked with an asterisk * reduce the Alkaline Picric Solution.*

I. CONSTITUENTS OF NORMAL URINE.

No reaction with Indigo-carmine or Fehling.

Urea.	Hippuric Acid.
*Kreatin.[1]	Sulphates.
*Kreatinin.[1]	Lactates.
Urates.	Oxalates.
Chlorides.[2]	Ammonia.[2]
Phosphates.	Butyric Acid.
Unoxidized Sulphur.	

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1 Kreatinin strikes in a few seconds a red colour with the cold picric solution, (Liq. Potass., a dram; sol. acid Picric saturat. *m*xx. aq. ad. seven drams; sol. Kreatinin one dram): the reaction soon attains a maximum intensity - though it is quickened and advanced by heat. Normal urines react in the same way with the picrate test without heat. A solution of Kreatin or of Glucose produces no reaction in the cold. Thudichum estimates the average daily excretion of Krea-

Indigo-carmine unchanged, but Fehling reduced.

URIC ACID. OXALIC ACID.

LACTIC ACID.

Indigo-carmine and Fehling reduced.

*UNOXIDIZED PHOSPHORUS.

II. CONSTITUENTS OF ABNORMAL URINES.

No reaction with Indigo-carmine or Fehling.

LEUCINE.

TYROSINE.

ALBUMEN : purified Ov-albumen (after Wurtz).

Purified Ser-albumen.

Albuminous urine free from sugar.

After boiling the indigo-carmine test with albumen, a drop of diabetic urine reacted as freely as in the absence of albumen; and glucose was detected by it in mixtures of any proportions of albuminous and diabetic urines. But when the quantity of glucose was small, while that of albumen was large, Fehling could not discover the former.

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tin and Kreatinin (chiefly the latter,) as 11·5 grains (*A treatise on the Pathology of the urine*, Lond. 1858, p. 416.) or about ½gr. per oz. This proportion in water gives a reaction with the cold alkaline picric solution very nearly that afforded by the normal urine: but it does not develop so quickly as the latter. Kreatin is not present in normal urine, but Kreatinin is a constant constituent (*A Text Book of Physiology* by M. Foster, M.A., M.D., &c. Lond. 1883).

2 Ammonium Chloride (even in very small quantity), ammonium urate or other ammoniacal salts were shown by Dr. Beale to prevent the precipitation of cuprous oxide, when sugar was present in small quantity. These salts do not reduce the Indigo-carmine, and the glucose detecting power of this test is not impaired by the addition of even more than 3 p. c. of ammonium chloride or other salt of ammonia, or of free ammonia to diabetic urine.

PEPTONES.

BILE: non-saccharine bile charged urine (Jaundice), and ox-bile added to water in which the test was boiled.

BLOOD, PUS, or MUCUS in non-saccharine urine.

Indigo-carmine and Fehling reduced.

*AMMONIUM SULPHIDE: When the solution is dilute—as in ordinary state albuminous urine—Indigo-carmine is unaffected.

Indigo-carmine reduced, and Fehling turned olive-green.

*INOSITE.

III. CARBO-HYDRATES.

No reaction with Indigo-carmine or Fehling.

CANE SUGAR.
PURE GLYCERINE.
MANNITE.
BOILED STARCH.
*GUM (Acacia).
GLYCYRRHIZIN.
SALICIN.

Indigo-carmine and Fehling reduced.

*MILK SUGAR.
*DEXTRIN.

IV. MEDICINAL AGENTS, &c.

No reaction with either Indigo-carmine or Fehling.

QUININE.
MORPHIA.
CODEIA.
ATROPINE.
CAFFEINE.
SANTONIN.
STRYCHNINE.
BALSAM OF COPAIBA.
BENZOATE OF LITHIA.
HYPOPHOSPHITES.
IODIDES.
LIQ. PEPTICUS (Benger).
ETHER.
GELATIN.

Reaction with Indigo-carmine and Fehling.

GALLIC ACID merely turns the carmine green, produces no play of colours, and there is no return of colour on shaking (therefore no reduction); but reduces Fehling.

GELSEMINE reacts green with carmine on admixture, bnt the colour is not further altered by boiling or shaking (no reduction); while it also turns Fehling green, and reduces it on boiling.

Indigo carmine unaffected, while Fehling reduced.

CHLOROFORM.	CARBOLIC ACID.
TANNIC ACID.	SALICYLATE OF SODA. *(Green, not reduced).*
RESIN (B.P.)	[1] CHLORAL. *(Green, not reduced).*
JALAPIN.	GUTTA-PERCHA SOLUTION.

Indigo-carmine merely decolourized or bleached, and Fehling unaffected.

TURPENTINE.

The PIGMENTS of urine have not been isolated, and experimented on apart. The following facts, however, disprove of their taking any share in the characteristic reaction of the Indigo-carmine test.

(1) The depth of colour of the urines has borne no definite relation to the reaction. In diabetic cases, in which the urine is often very pale, any suspicion of the colouring matter is practically excluded. But in non-diabetic urines—such as those which give an earlier reaction than normal

1 The urine of persons taking chloral-hydrate reduces Fehling's solution, the Bismuth test, and the salts of silver. This redncton is said to be due to uro-chloralic acid. See *L'Urine*, par Le Dr. C. Méhu, Paris, 1880, p. 120.

urines and a green colour with Fehling—it has often happened, that quite pale specimens have afforded a quicker and more developed colour change than the darker ones.

(2) Dark urines invariably gave the same reaction after being decolourized by animal charcoal as before.

Of the sixty-four substances experimented with,

Fehling	was reduced	by	15
Picric acid	„	„	8
Indigo-carmine	„	„	5

The five materials (unoxidized Phosphorus, Ammonium Sulphide, Milk-sugar, Dextrin, and Inosite) that produced the characteristic play of colours with Indigo-carmine, also reacted with Picric acid, and all of them—except Inosite—reduced Fehling's solution.

So far my experiments have demonstrated, that the Indigo-carmine test is not reduced by any of the constituents of normal or abnormal urines, except the saccharoid. I think it is probable, when the sugars of the urine have been worked out and isolated, it will be found to react with all of them, but in this respect it is not inferior to Picric acid, which has not been shown to differentiate between glucose and allied saccharoid bodies.

IX. The Clinical Advantages of the Indigo-Carmine Test Papers.

The high position of Fehling's solution as a test for the glucoses in urine cannot be questioned; but for two disadvantages which belong to it—the liability to change on exposure to light and air, and the caustic properties which condemn it for bed-side or out of door work—no one would desire

a substitute. It has undoubtedly yet much sound work to do, and I have no wish to suggest the dismissal of it from our service. Still, like every other urinary test, it is not equally good all round. Where it is weak and apt to fail, the indigo-carmine test, as here presented, appears to me to supply useful supplemental aid—apart from other clinical advantages.

(1) Sugar in small quantity along with much albumen, may be overlooked by Fehling. As a rule, the search for sugar by this test in albuminous, bloody, or purulent urine, should be preceded by precipitation of the albumen and filtration. These procedures are, however, unnecessary when the indigo-carmine test papers are used; for it has been proved by repeated observation, that they will detect sugar—in any proportion, and as readily as in ordinary diabetic urine—in the presence of albumen, peptones, blood, pus, &c.

(2) It is well known that uric acid will reduce Fehling's solution: but it has no reaction with the carmine test.

(3) I have observed that the test papers do not produce a reaction with one or two drops of decomposing albuminous urine: and ammonium sulphide in weak solution (but sufficient to reduce Fehling) used in the same way is equally negative, but, when more concentrated, it will reduce the carmine. When ammonia is freely added to diabetic urine, the reaction is not retarded or prevented; and ammonia of itself cannot produce it. It is, however, advisable, before testing for sugar to thoroughly boil ammoniacal urines.

(4) The stability of the handy and cleanly carmine test is an unquestionable gain.

A good qualitative test should readily display

the coarser variations of quantity, which are often more helpful to the practitioner than fine gradations. This working property the carmine test possesses. Each one will doubtless apply it in his own way: but it can be easily shown by adding one drop at a time of diluted diabetic urine, until the reaction is completed; or after the final stage has been reached by the undiluted urine, the fresh addition of another paper, or a portion of a paper may be made, when, perhaps, the straw-yellow will fail to appear. Then again, rapidity of the reaction is proportionate to the amount of sugar, and roughly suggests a large or a small charge of it.

Besides definitely deciding the presence or absence of sugar in the ordinary diabetic proportions, the carmine papers appear to me to possess exceptional powers for detecting small quantities. This fact is pointed out also by Neubauer and Vogel, who write: "This reaction is a very brilliant test, and is capable of determining the presence of a very small quantity of sugar. If only traces of sugar be present, of course only a very weak blue indigo solution must be employed." The sensitiveness of the solution prepared from the test papers can be readily proved by dropping the same small portions of diluted saccharine urine into it and other test solutions, and comparing the results after boiling. The saccharoid constituents of normal urine, as a rule, give a violet reaction after the addition of the fourth, fifth or sixth drop, though the appearance of it is frequently delayed beyond this limit. Urines containing small quantities of sugar beyond this range will afford reactions in proportion to the amount: for instance, either the first, second, or

third drop—as the case may be—will induce a distinct violet reaction, or carry this on to the purple, the red or the yellow stages.

But experiment has shown, that the glucose detecting power of the test paper is greatly amplified by using in addition a paper charged definitely with carbonate of soda: so that, for instance, the traces of sugar, otherwise detectable on adding the second or third drop, may become evident with the first, and then further additions of the urine will advance the reaction.

The test papers may, therefore, be made available not merely for the detection of ordinary diabetes, but for the study of all the variations of saccharoid matter which appear in non-diabetic urine, or in urines which fill in the gap between health and diabetes.[1]

This is undoubtedly a delicate instrument of clinical enquiry: and I, therefore, trust it may be found useful—how far, in the absence of sufficient experience, I will not presume to say—in the study of other forms of disease than diabetes; such, for instance, as those which are apt to induce the appearance of sugars in small quantities in the urine, as in functional disorders of the liver and of the vaso-motor nervous system.

As at least suggestive of an explanation of the appearance of small quantities of sugars—which

1 The value of the Indigo-carmine as a detecter of glucose for clinical purposes will not be impaired, even though the frequent presence in the urine of the non-fermentable isomer of grape-sugar, inosite ($C_6H_{12}O_6 + 2H_2O$), or any similar body, should even be demonstrated: for, according to all observation, such saccharoids will merely appear in very small quantities, with which the carmine will not react, perhaps, until after the addition of two or three drops of urine; while the test will decide with the first drop for or against such proportions of glucose as are of clinical significance, as suggesting diabetes, or perhaps a proclivity thereto.

may be readily found and estimated by the indigo-carmine test papers—in functional disorders of the liver, we have the fact, that bile will convert amyloid matter—starch and its isomer, glycogen—into sugar, even at the ordinary temperature. Von Wittich proved this.[1] I have repeatedly confirmed it, and traced the development of the sugar hour by hour by means of the Indigo-carmine test papers, as well as by Fehling's solution. Thirty minims of absolutely fresh bile from the gall-bladder of the sheep were added to four drams of boiled starch mucilage (cold). This solution boiled with Fehling in equal proportions gave no reaction: nor did one drop of it react with the test paper, but four or five drops produced with the solution of the latter a deep violet tint.[2] The testings with Fehling gave the following results: in an hour the red oxide boiled out as a fine deposit on the inside of the test tube, and as the conversion into glucose went on, the amount of the precipitate increased, until, in from twelve to twenty hours, it produced a chocolate red mixture. The Indigo-carmine afforded confirmatory reactions: after an hour the first drop of the solution produced a purple hue, and ten drops completed the colour changes; and the complete reaction was secured by

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1 "*Diseases of the Liver*," by Dr. Geo. Harley, F.R.S., Lond., 1883, p. 62.

2 "Among the constituents of the bile I must mention sugar, for both in the normal bile of man and of the lower animals, the ox and the dog, I have always detected that substance. On one occasion I even found torulæ in the bile within twenty-four hours after its removal from the gall-bladder of a healthy dog, and the torulæ were of course the product of the fermentation of the sugar contained in the bile."—Dr. Geo. Harley, *op. cit.*, p. 72.

5 drops at the end of the 2nd hour.
3 „ „ „ 12th „
2 „ „ „ 17th „
1 „ „ „ 20th „

Does the ferment existing in the freshly secreted bile convert the glycogen within the parenchyma of the liver into sugar? In hepatic disorders may not the sugar forming function of the liver be frequently so disturbed as to lead to a rise—however small—of the normal amount of sugars in the blood, and then in the urine? Or, may not the liver sometimes fail to fix as glycogen some of the sugars—such as glucose, dextrin, maltose—derived from the operation of the amylolytic ferments on carbohydrates in the alimentary canal, and allow them to pass into the general circulation?

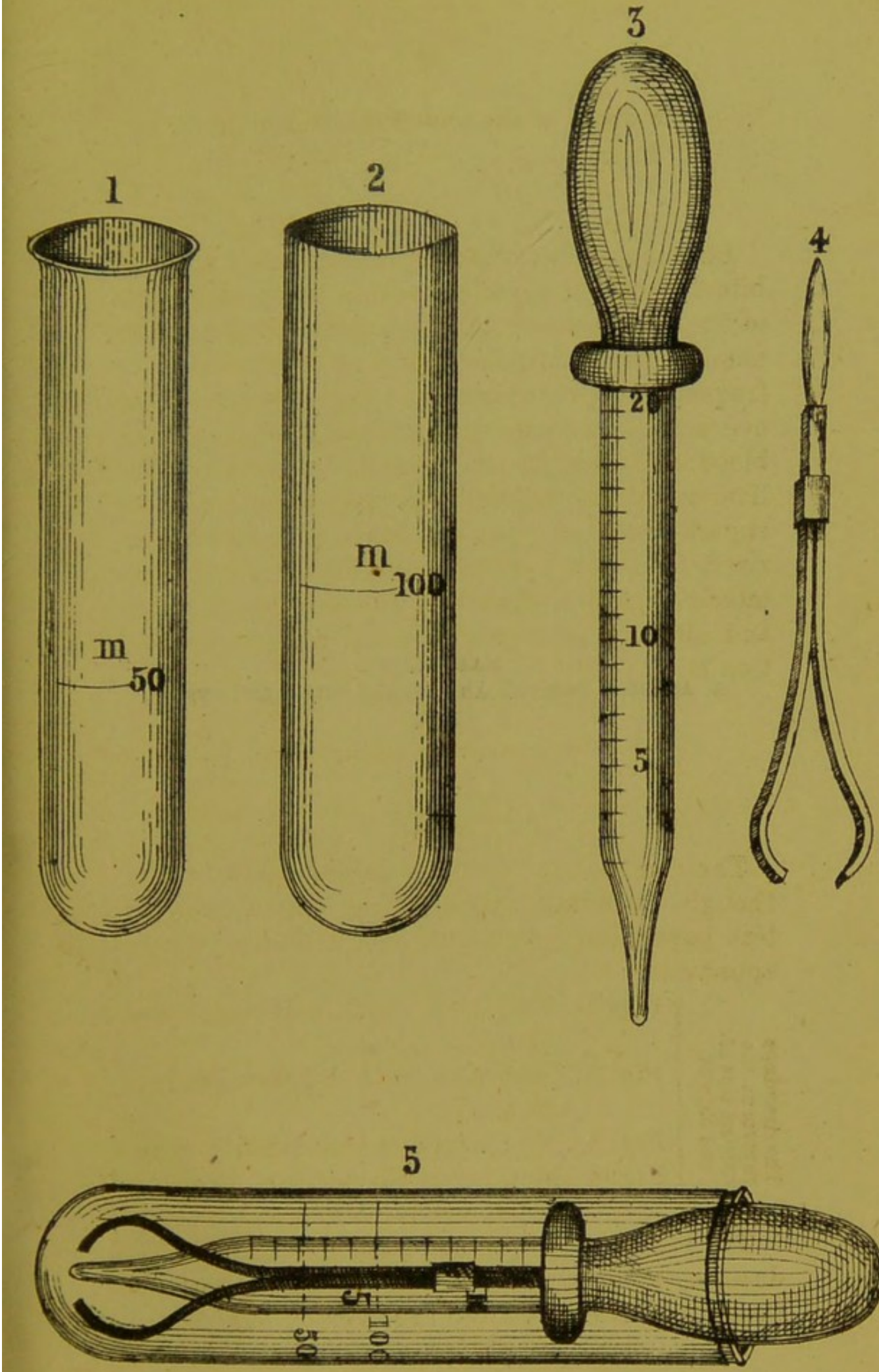
APPARATUS.

The method of urinary observation — even though including quantitative estimations—by test papers requires merely the following simple apparatus :—

The drawings represent the natural size of the objects.

Fig. 1 Test tube with a 50*m* graduation.
Fig. 2 Test tube with a |100*m* graduation.
Fig. 3 Nipple pipette graduated in mins
Fig. 4 Metal clip for boiling, drawing out test papers, &c.

The rubber nipple of the pipette can be pushed down so as to include part of the tube : and all the articles can be packed as shown by Fig. 5.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