

cancer, have the power of proliferation. Of course, this fact cannot prove any such power, since analogy can prove nothing, but can merely suggest; still, if it could prove it it would not be necessary to corroborate the power of proliferation of generative cells, since the first infant one comes across is ample and convincing proof of that! At the same time there is a very suggestive parallel between the two proliferations. *Neither generative tissue nor other normal tissue cells can proliferate without a specific stimulus.* In the case of generative tissue the ovum only commences to proliferate when it has been invaded by the spermatozoon, which acts practically as an extrinsic specific protozoan parasite. The proliferation is enormous and rapid. The resulting tumour is benign, though some might consider it to be a malignant tumour in a normal situation! It is easily got rid of, and "recurrence" does not take place unless there is reinfection! In the case of somatic cells proliferation only commences when they are attacked by the cancer parasite. Mitosis becomes heterotype, proliferation goes on apace, and the tumour is malignant and impossible to eradicate. Nothing is known of the method of the influence of the cancer parasite on the somatic cell, any more than the influence of the spermatozoon on the ovum is understood, but it will be admitted that the one process is equally as marvellous as the other.

With regard to the second of these facts, among the vertebrata in which cancer has been discovered to exist are certain fishes, found both in rivers and in the ocean round our coasts. This fact has induced the director before mentioned to state<sup>20</sup> that "the wide zoological distribution of malignant new growths indicates that the cause of cancer is to be sought in a disturbance of those phenomena of reproduction and cell-life which are common to the forms in which it occurs." Thus he makes another statement of opinion which clearly indicates how little is the help towards solution that may be expected from intrinsic hypotheses and their advocates. To the "man with the open mind," however, it might probably occur, as it certainly does to me, that this wide distribution of cancer throughout the vertebrata, with the resulting great diversity in environment, food, and conditions of life generally, was suggestive of the omnipresence of a specific malevolent agent of great vitality; that, with regard to the fluvial and marine fishes found bearing cancerous growths, this fact might well suggest that the specific cause of cancer is water-borne and, like the bacillus typhosus, it is not injuriously affected by sea-water; and, finally, that since the neoplasms found in fish are identical in every way with those occurring in man, infection may well have arisen through the agency of parasite-bearing sewage polluting both the rivers and the ocean into which they flow.

Regarding the third discovery of the transmissibility of cancer from one individual to another of the same species, although this has had to be admitted, it is qualified by the assertion that such transmission is merely a transplantation of a graft of a malignant tumour and not the genuine inoculation of a specific disease. It would be more graceful to concede that since transmission between individuals of the same species is proved to be possible it may be eventually found that transmission between animals of different species, and even the development of one variety of malignant growth from another, is possible also.

An admission which may be looked for sooner or later is that a carcinoma of epithelial origin has been observed to give rise to a sarcoma in the adjacent connective tissue. Then will be confirmed the dictum that cancer is a specific infective disease and that one specific micro-organism is capable of originating every variety of malignant neoplasm, the variety depending upon, and only limited by, the anatomical diversity of the structure and the situation of the tissue invaded.

#### DEDUCTIVE EVIDENCE.

As it is necessary to apply the strictest canons of logic to the arguments brought forward in support of any hypothesis I beg to submit the following, which to my mind fulfils this postulate. Every specific disease is infectious to the individual. By this term, "infectious to the individual," I mean the gradual evolution of disease, more or less rapid, locally and constitutionally, over the body from the point of origin of the disease. Specific diseases infectious to the individual are very numerous and are caused by the agency of some extrinsic parasite, either a microphyte or a micro-zoon. These diseases are communicated to others, directly from individual to individual, or indirectly by inanimate objects, or through an intermediate host.

Now cancer is, emphatically, a specific disease, and it is intensely infectious to the individual; therefore, the only logical conclusion that can be drawn is that cancer is both transmissible to others and that it is caused by an extrinsic agent. Except for the solitary fact that the elements of a malignant neoplasm—i.e., its cells—are themselves transported, in addition to the infective agent (which is the peculiar and characteristic idiosyncrasy of cancer), this disease very closely resembles in its origin and evolution a chronic infectious disease.

My argument may be condensed into two syllogisms—viz: 1. All diseases which are infectious to the individual are transmissible to others. Cancer is infectious to the individual. Therefore cancer is transmissible to others. 2. All diseases which are infectious to the individual have an external origin. Cancer is infectious to the individual. Therefore cancer has an external origin.

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## ON ANOMALOUS REACTIONS OBTAINED IN TESTING URINE FOR SUGAR WITH FEHLING'S SOLUTION.

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THE application of Fehling's test for the detection of sugar in urine is both simple and reliable in the presence of a fair amount of sugar. Urines in which a distinct reaction is obtained when boiled for a short time with an equal amount of Fehling's solution may, in almost all cases,<sup>1</sup> be held to contain excess of sugar, and such urines present no further difficulties. Practical experience, however, as well as an examination of the plentiful references to the reaction in clinical and other text-books, &c., show that the test as applied to urine in general is complicated by many difficulties and ambiguities. Different authorities seem to hold very divergent views with regard to the exact significance of certain results obtained with Fehling's test when the reaction is somewhat modified either with regard to the general appearance of the precipitate or the time taken to produce it. Thus on testing urine it is sometimes found that no result is evident after a good deal of heating with Fehling's solution, but that some time after standing an opalescent mixture is found to have taken the place of the blue solution, or perhaps there is a fairly definite yellowish-green precipitate. In other cases it is found that the mixture of urine and Fehling's solution gives on boiling for some time a dirty opalescent greenish milky liquid without any sign of a definite precipitate; at other times a more yellowish-green solution is in evidence; and all stages from a faint dirty greenish opalescence to a definite yellowish mixture exhibiting undoubted evidence of a precipitate may from time to time be seen in clinical work.

The significance of these ambiguous reactions has not been thoroughly dealt with in the literature of the subject and at present the general tendency is to explain them in a convenient, but not very helpful, way by reference to some disturbance by "interfering" substances. How these latter substances are supposed to interfere with the test is generally not very plainly indicated, but the general impression conveyed seems to be that they act in virtue of their direct reducing action and thus simulate sugar, though giving at

<sup>1</sup> Possible complications with glycuronic acid, &c., are not discussed here.

the same time a result somewhat different from that obtained from sugar, inasmuch as the reaction is often modified, the result being a greenish and greenish yellow mixture, as mentioned above.

#### *Causation of the Anomalous Reactions.*

Some years ago the writer undertook an investigation into this subject in order if possible to elicit the causation of these ambiguous results and the clinical significance to be attached to them. The results of this investigation were published *in extenso* in the *Biochemical Journal*,<sup>2</sup> but as the subject is exceedingly important from the standpoint of practical medicine I propose to deal here with the part of greatest interest to the clinician.

If we take a normal urine giving no reaction with Fehling's solution and add to it a trace of sugar it will generally be found, on subsequent testing in the usual way, that no evidence of the presence of the added sugar can be detected; that this is not due to any lack of delicacy on the part of Fehling's solution is evident from the fact that this reagent will readily detect such a small quantity of sugar as 1 part in 125,000 parts water (0.0008 per cent.). Now since several times this amount of sugar often gives no result when added to normal urine and tested as usual we at once arrive at the conclusion that normal urine contains something which prevents small quantities of sugar giving a reaction with Fehling's solution. According to Dr. F. W. Pavy this inhibition of precipitation of cuprous oxide is due to the ammonia evolved by the action of the alkali of Fehling's solution on the nitrogenous constituents of the urine holding the suboxide in solution. In this way, of course, precipitation might be prevented provided the ammonia were present in sufficient abundance, but it will be shown later that the quantity of ammonia actually present is much too small to be of any account in this respect.

An examination of all the ordinary constituents of urine resulted in disclosing the fact that the substance which causes this inhibition of precipitation is kreatinin. Kreatinin has the power of holding in solution the reduced suboxide and so the mixture gives no evidence, so far as precipitation is concerned, of the presence of sugar. Now, since normal urine always contains a certain amount of sugar every urine would give a reaction with Fehling's test were it not for the kreatinin. The normal sugar of urine, of course, reduces its equivalent amount of Fehling's solution in the ordinary way but since the reduced suboxide is held in solution by kreatinin we get no evidence of the presence of sugar and thus with Fehling's solution average normal urine gives no apparent reaction. It will be seen that this action of kreatinin is more beneficial than otherwise, for it prevents mistakes being made with urine containing very little sugar—sugar of normal amount and only of physiological importance. The first effect therefore of kreatinin is to prevent a reaction with small amounts of sugar.

#### *Nature of the Precipitate.*

Kreatinin, however, possesses another very marked property—that of materially modifying the nature of the precipitate when there is a slight excess of sugar present. It will be shown later that this characteristic of kreatinin is a most marked one and exceedingly important in affording an intelligible explanation of the various different-coloured solutions and precipitates described above. In a paper published in the above-mentioned journal for April, 1907, I entered fully into the phenomenon and showed that the different-coloured precipitates obtained in urine testing were associated with, and dependent on, a difference in the degree of granularity of the particles of the precipitates. Thus, in a urine giving, after boiling for some time, a dirty greenish opalescent solution, the modified colour is due to the fact that the precipitate of cuprous oxide is present in an exceedingly finely divided state; in the case of a greenish-yellow precipitate the particles are still very fine but rather coarser than in the last; with a yellow precipitate they are still somewhat larger, and this increase in the size of the particles goes on until in a distinct red precipitate the size of the individual granules is much more marked.

It is customary to state that the red precipitate seen in testing distinctly diabetic urine is cuprous oxide, whereas the yellow precipitate in evidence when urines containing comparatively small amounts of sugar (say from 1 to 2 per cent.) are tested is cuprous hydrate. The difference in colour, however, is not dependent on the chemical nature of the precipitate present but on the state of subdivision of the particles,

and it is likely that all urines really give a precipitate of cuprous oxide and that the statement to the effect that the yellow precipitate is cuprous hydrate is incorrect. The lower hydrate of copper,  $\text{Cu}_2(\text{OH})_2$ , is such an unstable chemical body as immediately to suggest a doubt whether it is possible for it to appear in urine testing in the form of a permanent precipitate. With regard to this difference of colour of the precipitates being associated with the state of subdivision of the particles of the cuprous oxide, it is of interest to observe that some months after the publication of my paper dealing with the above a paper by Dr. Pavy appeared in THE LANCET<sup>3</sup> in which my general investigations were repeated. It is satisfactory to note that the above authority has quite confirmed my observations concerning the relations of colour and state of subdivision.

A consideration of the above facts enables us to understand the causation of modified results in urine testing. When the urine contains just a slight excess of sugar it reduces its equivalent amount of Fehling's solution in the ordinary way, but the kreatinin present modifies the nature of the precipitate so that it separates out in exceedingly fine particles; these fine particles floating in the liquid give a dirty, milky, greenish appearance to the liquid. It is thus obvious that these ambiguous precipitates are just modifications of the usual form, and are often (in fact generally) indicative of a slight excess of sugar above the amount present in normal urine. Of course, it is obvious that the presence of a sufficient amount of some other reducing body—e.g., glycuronic acid—would act in the same way, but observation shows that sugar is really the substance which most often gives these modified reactions. Again, it is sometimes necessary, in the case of those ambiguous precipitates, to boil for some time before a result is obtained; this is due to the fact that the kreatinin holds in solution the cuprous oxide formed during the initial stages of boiling, and it is only after the kreatinin can hold no more in solution that the modified precipitate separates out. Since a hot kreatinin solution is a more effective solvent than a cold one, there is a consequent tendency for the precipitate to settle out after standing for some time.

#### *Reaction with Normal Urine.*

Even normal urine, when boiled long enough with Fehling's solution, will give a distinct precipitate, and a consideration of what happens here will help us to make clear the processes involved in the case of urines with slight excess of sugar. With regard to this the following quotation from my paper may be of interest:—

The chief reducing substances present in all normal urines are uric acid, carbohydrate material (of which the larger part seems to be dextrose), and kreatinin. Uric acid and sugar, however, differ greatly from kreatinin in regard to the time required to cause reduction. Both substances, in the percentage in which they occur in normal urine, are capable of effecting reduction almost immediately on the boiling point being reached; in the process of reduction they undergo destruction.

Kreatinin, on the other hand, reduces very slowly indeed. When a normal urine is boiled with Fehling's solution, the uric acid and sugar present almost immediately reduce their equivalent amounts of the solution; no effect is perceived owing to the fact that the reduced suboxide is held in solution by the kreatinin; after boiling for a very short time the full reducing effect of both uric acid and sugar is completed. On continued boiling the kreatinin gradually causes further reduction, at the same time becoming gradually diminished in amount; part of it is probably converted into kreatin while part is destroyed.

Ultimately a point is reached, at which the suboxide reduced by the sugar and uric acid, added to that reduced by the kreatinin itself, is no longer capable of being held in solution by the amount of kreatinin and its derivatives actually present in the urine at that moment; at this point precipitation occurs.

Thus it will be seen that the reaction obtained from a normal urine is very similar in character to that obtained from a urine containing more than the normal amount of sugar, the chief difference being that the more sugar present the quicker the reaction occurs.

In the presence of great excess of sugar the effect of kreatinin is of course quite obscured and of no practical importance. It will be seen that the influence of kreatinin on Fehling's solution is very marked and important, though somewhat different from that ascribed to it in the text-books where its action is generally considered in relation to its direct reducing power. This direct action occurs but slowly, and is therefore very insignificant when Fehling's test is used in the ordinary way; its direct inhibitory action, however, explains many points which are otherwise obscure, and for which no definite explanation has been forthcoming.

#### *Dr. Pavy's Criticisms.*

In THE LANCET of August 3rd, 1907, p. 290, Dr. Pavy in a paper advocating his own theory of the phenomenon takes exception to certain statements published by the writer in the *Biochemical Journal* for February, 1906, under the heading "Observations on Fehling's Test for Dextrose in Urine." After satisfying myself as the result of prolonged

<sup>2</sup> February, 1906, and April, 1907.

<sup>3</sup> THE LANCET, July 27th, 1907, p. 223.

experimental work that the quantity of ammonia evolved from the ordinary nitrogenous constituents—i.e., urea, uric acid, kreatinin, &c.—was quite incapable of giving rise to the reaction, I performed some experiments with a view to determine the possible effect of the ammonia always present in "loose" combination. By this, of course, was meant any ammonia that might be present in the form of salts as distinguished from ammonia generated from the nitrogenous constituents. All observers agree that a certain quantity of ammonia is always present in this form; in order to get rid of this ammonia I boiled the urine for a short time with the alkaline part of Fehling's solution. It this way the action of any ammonia present in loose chemical combination was disposed of. With regard to this point the statement made in my paper was as follows: "Were the reaction due to ammonia present in loose combination boiling the urine with the alkaline part of Fehling's solution for a comparatively short time should be sufficient to remove it." Dr. Pavy, however, seems to argue that my meaning was that the total ammonia of the urine—both the ammonia present as such and the ammonia generated from the nitrogenous constituents—would be evolved after boiling for a short time with the alkaline part of Fehling's solution. After quoting my statement as given above he proceeds as follows: "Dr. Maclean surmises that boiling the urine with the alkaline part of Fehling's solution for a comparatively short time should be sufficient to remove the ammonia. If he had ascertained by observation the effect produced he could not have expressed himself as he has done. As a matter of fact it is difficult to get away from the evolution of ammonia," &c. He then goes on to say that even if boiled to solidification ammonia is still freely evolved on the addition of water. That ammonia comes off after boiling urine and caustic alkali for a very long time I have often verified. This fact only adds further proof to the assertion that the amount given off in any given short period must be very small indeed. This ammonia, however, is generated from the nitrogenous constituents of the urine, and such ammonia could hardly by any play of imagination be referred to as ammonia in "loose combination." In quoting my statement (given above) Dr. Pavy italicises the words "should be sufficient"; if at the same time he had italicised the words "loose combination" all confusion would have been avoided.

It is, of course, commonly known that sugar is easily destroyed by the action of boiling caustic alkali and this fact is often taken advantage of in physiological chemistry when we wish to destroy the sugar in any substance previous to certain processes—quantitative estimations, &c.—which would be interfered with by the presence of sugar. For instance, in Neumann's well-known method for estimating phosphorus, when the substance to be operated on contains sugar—e.g., milk—it is first boiled with caustic alkali. This destructive action of caustic alkali towards sugar would, of course, destroy the sugar normally present in the urine when the latter is boiled with an alkali. Therefore after boiling urine with caustic alkali for some time in order to remove the loosely combined ammonia as described above it is necessary to add a little sugar to the boiled urine in order to test its inhibitory power when treated with Fehling's solution, since the sugar normally present has been destroyed. When this was done it was found that the urine still possessed marked inhibitory power, proving, of course, that the loosely combined ammonia was not itself the cause of the inhibitory action. With regard to this point, my paper reads: "Urine however to which small amounts of dextrose have been added previously do not give any more indication of the presence of sugar after boiling than before." "Previously" of course refers to the boiling of the urine with Fehling's solution. In other words, urines which have been boiled for some time with caustic alkali, and to which a small amount of sugar is added after the boiling is completed and previously to boiling with Fehling's solution (to represent the sugar of normal urine destroyed by the boiling alkali) do not give any more indication of the presence of sugar than if they had not been boiled at all. It may be that the above sentence as given in the *Biochemical Journal*, is ambiguous as far as the mere wording is concerned, and that it is possible to assign to it the interpretation adopted by Dr. Pavy. With reference to this point, Dr. Pavy says that I seemed "to have overlooked the destructive action exerted by a fixed alkali on dextrose. Instead of their being more indication of the presence of sugar after boiling with the alkaline part of Fehling's solution as is suggested should be the case through the expulsion of ammonia the actual

effect that occurs is a disappearance of the sugar. It is easy for anyone to boil some dextrose-containing urine with the alkaline part of Fehling's solution, then add the copper portion and see the effect produced. Even with as much as 2 per cent. and over of dextrose present and boiling only for one minute sufficient destruction occurs to prevent any sign of reaction being obtainable, &c." It was, of course, to make up for the sugar destroyed that I added sugar to the boiled urine and while it is admitted that the sentence as it stands might possibly be interpreted as Dr. Pavy suggests such an interpretation would hardly have been expected from such an authority as Dr. Pavy.

In the same journal (April, 1907) another article was published in which I incidentally emphasised the destructive action of caustic alkali on sugar not as something not well known already but in regard to the *very rapid* action of the boiling alkali on small amounts of sugar. In the course of my research work I happened to ascertain that in many cases this fact was not generally appreciated and so thought it worth while to draw attention to the point, stating at the same time that "while it is a well-known fact that boiling with hot alkali destroys sugar it would seem that the extreme facility with which this is accomplished is hardly appreciated." With regard to the above Dr. Pavy says: "In this [article] Dr. Maclean shows that he has discovered the facility with which dextrose is destroyed by the agency of a fixed alkali and that he has learnt the caution that is required to be exercised in experimenting with sugar in the presence of an alkali to escape arriving at a fallacious conclusion." It is obvious from what has been said that the foregoing statement is inapplicable.

#### *Inhibitory Influence of Ammonia.*

In the same journal I gave the following table with regard to the inhibitory effect of ammonia on a weak dextrose solution:—

	Ammonium hydrate, pure (0.88%), 1 in 10.	Ammonia calculated as nitrogen in milligrammes.	Results.
—	1 drop.	1.5	Immediate precipitate.
	2 drops.	3.0	"
1 cubic centimetre of a 0.1 per cent. dextrose solution	4 "	6.0	"
	6 "	9.0	Precipitate after a few seconds boiling.
	8 "	12.0	"
	10 "	15.0	Precipitate after 6-10 seconds boiling.
1 cubic centimetre of Fehling's solution boiled with—	12 "	18.0	Precipitate after 8-12 seconds boiling.
	16 "	24.0	Precipitate after a little time.

In these experiments the urine was boiled with the alkaline part of Fehling's solution in a test-tube, just as ordinary urine is tested, in order to obtain a result as nearly as possible in harmony with the effects of the ammonia generated when urine is tested in the usual fashion. Dr. Pavy performed similar experiments but under materially modified conditions—the mixtures being heated in a boiling salt solution and not boiled directly over the flame—and makes the statement that "viewed in this way the table given by Dr. MacLean does not supply a correct representation. It is not correct to represent no effect as being producible by one to four drops of dilute solution of ammonia ..... after four drops the interval amounted to seven seconds ..... after six drops of the ammonia solution the interval was observed to stand at 12 seconds; after eight drops, 15; after 12 drops, 20; after 16 drops, 38," &c.

Now boiling the mixture rapidly over the flame is a very different matter from heating in a boiling salt solution and so no comparison can be drawn between the two sets of experiments, more especially since the intervals during which precipitation is prevented are, comparatively speaking, so insignificant when compared with the time taken for the production of a precipitate when normal urine is boiled with Fehling's solution. Such a large amount of ammonia solution as 16 drops is capable of preventing precipitation only for 38 seconds, and since urine (which would never contain this amount of ammonia per cubic centimetre) does not as a rule give a reaction until the boiling is continued for several minutes, I think that my table when viewed in comparison

with what obtains in urine gives a fairly true idea of the relative action of ammonia as an inhibitor of precipitation. It is certainly not customary when testing urine for sugar in the ordinary way to apply heat by means of a boiling salt solution, and as my experiments were purposely so conducted as to coincide as nearly as possible with the methods adopted generally in urine testing it may fairly be claimed that these results are representative of the effects produced in urine. A urine which with Fehling's solution would give a precipitate after, say, four minutes' vigorous boiling would require a longer period of heating before a similar precipitate would be given if the urine were immersed in a boiling salt solution; this can be very simply proved by simple comparative experiments, using portions of the same sample.

I think that the above explanations make it clear that in Dr. Pavy's article in THE LANCET of August 3rd my observations are unfortunately represented in a manner quite different from the true meaning assigned to them by me.

Dr. Pavy refuses to accept the view that kreatinin is the chief substance in urine which gives rise to this inhibitory effect, and argues that the evolution of ammonia generated by the action of caustic alkali of Fehling's solution on the nitrogenous constituents of the urine is sufficient to hold the reduced suboxide in solution when the latter is present in small amount. While it is common knowledge that ammonia has the power of holding cuprous oxide in solution, my assertion was that ammonia "is ordinarily evolved in too small an amount to markedly interfere with the reaction"—i.e., the precipitation of the cuprous oxide. In order to settle the point conclusively, however, I annex some experiments performed with ordinary urine in which a certain quantity of the urine was boiled with an equal volume of caustic alkali of the same strength as is present in ordinary Fehling's solution; such a mixture, of course, contains the same percentage of alkali as urine when mixed with an equal volume of Fehling's solution. The ammonia was collected in the ordinary way by means of  $\frac{N}{10}$  sulphuric acid,

and the latter titrated against  $\frac{N}{10}$  sodium hydrate solution.

The total amount of ammonia given off from the urine could in this way be easily and accurately determined, and affords a direct and simple means of judging of the part played by ammonia as an inhibitor of precipitation of cuprous oxide. Different amounts of urine were used, and boiling continued for different periods, but the following tables deal with mixtures boiled for five minutes, as this space of time represents roughly the time required to produce a precipitate in many normal urines when boiled with Fehling's solution.

TABLE I.—25 Cubic Centimetres of Urine Boiled with 25 Cubic Centimetres of Caustic Alkali for Five Minutes.

No.	Specific gravity.	Total amount of ammonia evolved in milligrammes.	Average amount per cubic centimetre of urine in milligrammes.
1	1020	25.5	1.02
2	1026	39.1	1.56
3	1018	24.6	0.98
4	1025	33.8	1.35
5	1026	40.1	1.6
6	1018	25.1	1.004

The average of the six experiments gives an ammonia evolution equivalent to 1.252 milligrammes  $\text{NH}_3$  to each cubic centimetre of urine, this amount being evolved in five minutes' boiling.

TABLE II.—10 Cubic Centimetres of Urine Boiled with 10 Cubic Centimetres Caustic Alkali for Five Minutes.

No.	Specific gravity.	Total amount of ammonia evolved in milligrammes.	Average amount per cubic centimetre in milligrammes.
1	1020	12.3	1.23
2	1026	16.8	1.68
3	1015	14.1	1.41
4	1023	20.1	2.01
5	1020	26.2	2.62
6	1015	18.5	1.85

Average of above experiments = 1.80 milligrammes  $\text{NH}_3$  per cubic centimetre evolved in five minutes.

TABLE III.—15 Cubic Centimetres of Urine Boiled with 15 Cubic Centimetres of Alkali for Five Minutes.

No.	Specific gravity.	Total amount of ammonia evolved in milligrammes.	Average amount per cubic centimetre in milligrammes.
1	1015	18.7	1.24
2	1012	21.2	1.41
3	1022	32.6	2.17
4	1024	22.1	1.47
5	1018	19.7	1.31
6	1015	20.5	1.36

Average of above experiments = 1.49 milligrammes  $\text{NH}_3$  per cubic centimetre in five minutes; and the average of three sets of experiments from 18 different urines = 1.514 milligrammes  $\text{NH}_3$  per cubic centimetre evolved in five minutes.

The above specimens were obtained from different sources and give a fair representation of the amount of ammonia evolved in a given time when urine is boiled with the alkaline part of Fehling's solution. Urines, therefore, may be considered as capable of evolving from 1.5 to 2 milligrammes of ammonia per cubic centimetre when boiled for five minutes with an equal volume of sodium hydrate containing the same percentage of caustic alkali as is commonly present in ordinary Fehling's solution; in other words, when urine is tested for sugar in the ordinary way with Fehling's solution 2 cubic centimetres of the mixture if boiled for five minutes would generate from 1.5 to 2 milligrammes of ammonia in that time. Now since the ammonia formed in the boiling liquid must be quickly driven off the amount of this substance present in the mixture at any given moment must be exceedingly minute. If we assume that the ammonia is formed at an average fixed rate per second, then the average amount generated per second in the boiling liquid will be from 0.005 to 0.007 milligramme  $\text{NH}_3$  per cubic centimetre of urine. Now Dr. Pavy states that with one cubic centimetre of urine mixed with one cubic centimetre of Fehling's solution the addition of one drop of pure (diluted 1 in 10) ammonia (0.880) solution just appreciably hinders precipitation; observation proves that if there is any interval it is really exceedingly minute. If, therefore, such a comparatively large amount of ammonia as one drop which represents about 1.5 milligrammes of ammonia has but the very slightest effect it is obvious that the insignificant amount of ammonia evolved in urine testing with Fehling's solution has practically no effect in acting as an inhibitor of precipitation.

Again, if for purposes of comparison we assume that all the urea present in one cubic centimetre of urine is changed into ammonia say in five minutes' boiling, a simple calculation gives the total amount of ammonia formed. Average urine contains about 22 milligrammes of urea per cubic centimetre. The total ammonia evolution of 22 milligrammes of urea is 12.4 milligrammes  $\text{NH}_3$ .

$$\text{CON}_2\text{H}_4 = 2(\text{NH}_3) = \frac{34}{60} \times \frac{22}{1} = 12.4 \text{ mgs. } \text{NH}_3.$$

(mol. wt. 60) (mol. wt. 34)

If we add another 2.6 milligrammes<sup>4</sup> for the ammonia generated from kreatinin and other nitrogenous bodies that may be acted upon, as well as for any ammonia present as such in "loose" combination, the total ammonia evolution per cubic centimetre of urine would amount to 15.0 milligrammes. Now the inhibitory effect of 15 milligrammes  $\text{NH}_3$  is exceedingly small. This amount of ammonia corresponds to the amount present in ten drops of a 1 in 10 pure ammonium hydrate solution (0.880).

According to Dr. Pavy's results ten drops of ammonium hydrate of above strength added to a mixture consisting of one cubic centimetre of Fehling's solution and one cubic centimetre of a 0.1 per cent. solution of dextrose would prevent the ordinary reaction for about 17 seconds.<sup>5</sup> Since Dr. Pavy's experiment was performed under conditions entirely different from those generally adopted in urine testing (heat being applied by means of a boiling salt solution) the above period of 17 seconds may be taken as

<sup>4</sup> This is of course a very liberal allowance and does not represent the actual relationship between urea nitrogen and other nitrogen present; it is merely given for purposes of comparison.

<sup>5</sup> Dr. Pavy's actual figures are: 8 drops = 15 seconds delay; 12 drops = 20 seconds delay. From this it is obvious that 10 drops would be equal to about 17 seconds.



the maximum time. Here, then, according to Dr. Pavy, the total amount of nitrogen that can be evolved from one cubic centimetre of urine is capable of inhibiting the usual reaction when 0.1 per cent. of sugar is mixed with an equal volume of Fehling's solution only for about 17 or 18 seconds. Now as Dr. Pavy assumes that ordinary urine contains from 0.3 to 0.5 per cent. sugar it is obvious that a reaction with urine would not be inhibited for so long a period as with a 0.1 per cent. dextrose solution (seeing it contains more reducing substance). In fact, with a 0.5 per cent. solution we might reasonably expect little or no inhibitory action. Experiment shows, however, that one cubic centimetre of ordinary urine may be boiled with an equal amount of Fehling's solution for anything from three to five minutes before a reaction is obtained.

In the above experiments it is assumed for purposes of comparison that all the nitrogenous substances of the urine generate all their ammonia when boiled for five minutes with the caustic alkali. This, of course, is far from true, for it is proved by observation—as shown above—that but a comparatively small fraction of the total ammonia is evolved in this time. The following quotation from Dr. Pavy's paper is of interest in this respect: "The boiling [of the mixture boiled with the alkaline part of Fehling's solution] may be carried on in any open capsule until solidification has begun to take place and then on adding water and transferring to a test tube renewed boiling will be found to be attended with the free evolution of ammonia made manifest by moistened litmus paper introduced into the mouth of the tube." Seeing, therefore, that but a portion of the total ammonia is generated after boiling for five minutes it follows that the total amount present in the urine at any given time must be exceedingly insignificant both in quantity and inhibitory effect; in fact, it is certain that the ammonia can count for little or nothing in preventing precipitation of cuprous oxide in urine.

It might possibly be argued that the addition of ammonia as such to a mixture of sugar and Fehling's solution is not tantamount to the gradual production of ammonia in urine, and that after a few seconds' heating the ammonia is driven off. This objection is not a valid one, for the usual tests disclose the fact that ammonia when added to a solution of sugar boiled with Fehling's solution comes off in considerable abundance after the cuprous oxide has been precipitated; in fact, it is obviously much more abundant after the addition of a drop or two of the above dilute solution, than it is in the case of urine where, though ammonia is continuously evolved during the process of boiling, the indications of its presence given by prepared test papers held over the boiling liquid suggest that it is present in comparatively small amount at any given time. Again, as above mentioned, an amount of ammonia amounting to about 1.5 milligrammes when added to one cubic centimetre of 0.1 per cent. dextrose with one cubic centimetre of Fehling's solution and boiled, has but a very insignificant, if any, action as an inhibitor of precipitation, and here the precipitate occurs before the ammonia could possibly be boiled off; this is a case in which a fair amount of ammonia is present and yet there is little or no inhibitory action.

#### *Influence of Kreatinin in Association with Urea.*

Dr. Pavy again quotes from my paper the following observation:—

Urea, in the percentage in which it occurs in urine, yields much more ammonia than the kreatinin of an equal amount of urine, and yet it possesses no apparent retarding effects; for a very dilute solution of dextrose (less than 0.01 per cent.) introduced into a 2.3 per cent. urea solution and mixed with equal parts of Fehling's solution gives quite a distinct reaction.

He then proceeds to say that when kreatinin is associated with the ammonia-generating product—urea—an effect is producible beyond that which is capable of being occasioned by kreatinin alone. Now, it is a fact beyond dispute that urea in the maximum percentage in which it ever occurs in urine produces no inhibitory reaction in a weak (say 0.01 per cent.) solution of sugar when the test is performed in the usual way. Dr. Pavy, however, finds that one cubic centimetre of a 0.1 per cent. sugar solution containing four times the urea present in normal urine gives a precipitate slightly in arrear of a similar mixture without urea and so argues that urea has of itself an inhibiting effect. This statement conveys a wrong impression with regard to the real facts, for Dr. Pavy admits that urea in the percentage in which it occurs in urine produces no inhibitory effect when boiled with sugar and Fehling's solution, and it was urine—

or mixtures equivalent in strength of urea to urine—that I had under consideration when I published the above. The statement that four times the amount of urea present in urine has a slight effect in inhibiting the reaction is practically irrelevant, seeing that urine never varies in its urea content to such an extent as this. The urea of urine, therefore, though generating much more ammonia than kreatinin, has no effect in preventing precipitation.

Again, it is argued by Dr. Pavy that urea greatly enhances the inhibiting effect of kreatinin in virtue of the ammonia given off. The statement is made that with 0.05 milligramme of kreatinin + 1 cubic centimetre of Fehling's solution + 1 cubic centimetre of 0.1 per cent. sugar solution, the latter being mixed with two drops of 40 per cent. solution (i.e., twice the amount of urea for an equivalent amount of urine), inhibition was delayed for 35 seconds. Now, if this inhibition was caused by ammonia it is obvious that a very small amount of ammonia must be capable of increasing the inhibitory effect of kreatinin. Shortly after the above, however, Dr. Pavy makes the statement that with one or two drops of a 1 in 10 solution of strong ammonia there is no effect produced in inhibiting the reaction of the kreatinin when one cubic centimetre of a 0.1 per cent. solution of dextrose, one cubic centimetre of Fehling's solution, and one milligramme of kreatinin are heated. Now two drops of the above ammonia solution are equivalent to about three milligrammes of ammonia, and since experiment proves that urea does not give up more than about one-fifth or one-sixth or so of its nitrogen when boiled for five minutes it is obvious that the amount of ammonia generated in 35 seconds must be very small; the total possible evolution (assuming that all the N. of the urea is changed) is only 24.8 milligrammes of ammonia, and taking one-sixth of that amount we get 4.2 milligrammes of ammonia for five minutes' boiling. We are therefore justified in assuming that not more than three milligrammes of ammonia are generated in 35 seconds' boiling, and this amount of ammonia, according to Dr. Pavy's second statement, produces no effect in augmenting the inhibitory power of kreatinin. Even the untenable assumption that all the urea is changed in 35 seconds may be examined with profit; here 24.8 milligrammes of ammonia would be generated—an amount corresponding to about 16 drops of above ammonia solution, and we have it on Dr. Pavy's authority that 15 drops produce no effect. If, on the other hand, we assume that a good deal more than three milligrammes of ammonia is evolved, then, according to the same authority, this amount of ammonia should decrease instead of increase the inhibiting effect of kreatinin, for it is stated that over three milligrammes up to about 15 milligrammes of ammonia added (in solution of corresponding strength) to one cubic centimetre of a 0.1 per cent. sugar solution containing one milligramme kreatinin and heated with an equal amount of Fehling's solution actually decreases the kreatinin inhibitory power. It is only after 15 milligrammes are added that an increased effect is noticed, and of course it is impossible to imagine that anything beyond 15 milligrammes  $\text{NH}_3$  could be evolved from the urea in 35 seconds.

Thus the statement by Dr. Pavy that urea increases the inhibitory effects of kreatinin in virtue of the ammonia generated is, according to his own observations, incapable of being accepted. Much more could be said with regard to the above, but it is the experience of the writer that urea in the percentage in which it occurs in urine neither inhibits the production of a precipitate in a weak sugar solution nor adds to any material extent to the inhibitory power of the kreatinin. Intervals of a few seconds are of no importance, and emphasising the importance of such short intervals tends only to produce an erroneous impression with regard to what actually happens in normal urine, where the time of boiling with Fehling's solution necessary for the production of a precipitate usually extends to minutes instead of seconds.

#### *Effectiveness of Kreatinin.*

Dr. Pavy bases his objections to my statement that kreatinin is the substance responsible for the reaction on the argument that the amount of kreatinin present in urine is too small to be answerable to any material extent as an inhibitor of precipitation. Now, according to very exact determinations of the amount of kreatinin in urine made by Folin,<sup>6</sup> it is estimated that the amount for average urine is from

<sup>6</sup> Zeitschrift für Physiologische Chemie, Band xli., S. 223.

1.5 to 2 milligrammes per cubic centimetre. The following table taken from his paper is of interest:—

Urine.	Amount of kreatinin in milligrammes per 10 cubic centimetres of urine.	Amount of kreatinin in milligrammes per cubic centimetre.	Urine.	Amount of kreatinin in milligrammes per 10 cubic centimetres of urine.	Amount of kreatinin in milligrammes per cubic centimetre.
1	6.15	0.61	6	17.4	1.74
2	12.5	1.25	7	21.3	2.13
3	19.1	1.91	8	16.6	1.66
4	11.6	1.16	9	13.1	1.31
5	20.25	2.025	10	19.8	1.98

Now one cubic centimetre of a 0.1 per cent. dextrose solution when mixed with one cubic centimetre of Fehling's solution is on boiling prevented from giving the usual precipitate of cuprous oxide for a considerable time by the addition of such a relatively small amount of kreatinin as one milligramme. Now, since normal urine generally contains, according to the above authority, from 1.5 to 2 milligrammes of kreatinin per cubic centimetre, it is obvious that urine, if it contains about 0.1 per cent. dextrose, would be prevented from giving a reaction for a considerable time by the amount of kreatinin normally present. Now average urine may be said to contain generally about 0.1 per cent. sugar, or at any rate to have an average reducing action due to carbohydrates equivalent to that exerted by a 0.1 per cent. solution of pure dextrose. This can be shown by various reagents which are not acted upon by the so-called "interfering" substances of urine. Such reagents are safranin and sodium-nitro-phenyl-propionate. With safranin as an indicator the following examples of the results obtained by the writer may be cited:—

Urine.	Specific gravity.	Percentage of sugar calculated as dextrose.	Urine.	Specific gravity.	Percentage of sugar calculated as dextrose.
1	1020	0.11	6	1025	0.13
2	1014	0.08	7	1023	0.095
3	1023	0.10	8	1024	0.14
4	1021	0.125	9	1016	0.09
5	1022	0.08	10	1026	0.115

Here, then, it is seen that the average amount of sugar corresponds roughly to about 0.09 to 0.11 per cent. The examination of several hundred normal urines gave on an average a reducing power equivalent to 0.08 to 0.1 per cent. glucose—due to urinary carbohydrate.

The statements made by some observers that average urine contains anything up to 0.3 per cent. or so of sugar are not borne out by observation, for if this were the case such an amount of sugar could be easily detected by Fischer's phenyl-hydrazin test. Normal urine does not, as a rule, give anything corresponding to a typical reaction with the test; this fact and various other tests show that sugar is seldom present in an average urine to this extent. That a urine may contain occasionally 0.2 or 0.3 per cent. of sugar and still be obtained from a healthy or "normal" subject is quite a different matter, but it is certain that average normal urine does not contain in general more than about 0.1 per cent. of sugar. Thus it is seen that the amount of kreatinin present is quite sufficient to act in a very effective manner as a retarder of cuprous-oxide precipitation when minute amounts of sugar are in question. Dr. Pavy, however, reasons as follows: "Kreatinin dealing with the quantity existing in healthy urine has but a limited power in delaying suboxide precipitation, and the power is only made manifest when the amount of suboxide being dealt with is exceedingly small. In the case of one cubic centimetre of a 1 per 1000 solution of sugar, one cubic centimetre of adjusted Fehling's solution, and one milligramme of kreatinin, compared with the counterpart without the kreatinin, marked delay is seen to occur. With a large quantity of suboxide, however, brought into the question, as, for instance, when a 5 per 1000 solution of sugar is used with the ordinary Fehling's solution, it may be said that practically no material indication of delay is perceptible.

In urines giving the anomalous reaction it may be considered that somewhere about 3, 4, or possibly 5 per 1000 of sugar may be reckoned to be present, from which it follows that the kreatinin constituent cannot be regarded as answerable to any material extent for the delaying effect that may be noticed to occur."

Now the admission that one milligramme of kreatinin per cubic centimetre causes considerable delay is tantamount to saying that this amount would cause considerable delay in urine. As above stated, the amount in urine is generally considerably above this (from 1.5 to 2 milligrammes per cubic centimetre), and since the sugar generally corresponds to about 0.1 per cent. it is obvious that the kreatinin present is answerable to a very material extent for the delaying effect that occurs. The statement that urines giving the "anomalous reaction" (if this really means, as judged from the context, delayed precipitation or, in other words, average normal urines) contain about 0.3, 0.4, or possibly 0.5 per cent. of sugar is, in the opinion of the writer, as the result of exhaustive experimental work, absolutely unfounded. It has been my constant experience that urines giving a sugar equivalent of such a comparatively small amount of sugar as about 0.25 per cent. (or even less) invariably gave the usual modified reaction with Fehling's solution after boiling for a very short time, and never corresponded in their behaviour in this respect with average urine. Dr. Pavy then proceeds to argue that an increase in the alkali of Fehling's solution will prolong the interval prior to precipitation. With large amounts of alkali it is quite possible that some prolongation may be in evidence, but at most the difference does not amount to very much—at any rate, with moderate amounts of alkali—when considered in relation to the time taken by normal urine. Moreover, I have considered the reaction with ordinary Fehling's solution in regard to which I have already shown that the action of the alkali on the urea, &c., has little or no influence. Again, since Dr. Pavy admits that the amount of ammonia generated from kreatinin alone is not sufficient to inhibit the reaction it is obvious that, in solutions such as the above, in which there is no other product present to generate ammonia, the action must be brought about by some other means than by the evolution of ammonia. Therefore I think it is obvious that the above criticisms by Dr. Pavy can hardly be regarded as in any degree tending to invalidate my statement that "kreatinin is the substance in normal urine which most markedly interferes with Fehling's reaction in the presence of small amounts of sugar..... kreatinin directly inhibits the effect of small amounts of sugar when boiled with Fehling's solution by holding the reduced suboxide in solution and not indirectly by generating ammonia; the latter is not present in sufficient quantity to materially affect the reaction."

*Note.*—For the full publications on the subject dealing with the points mentioned above see my paper in the *Biochemical Journal* for February, 1906, and April, 1907; also Dr. Pavy's papers in *THE LANCET* of July 27th (p. 223) and August 3rd (p. 290) and 10th (p. 361), 1907.

## ON FERMENTS AND THEIR MODE OF ACTION.<sup>1</sup>

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THE subject of Ferments has been chosen for our consideration this evening because I wish to point out the mode of action of organised and unorganised ferments and their relationship to one another. It is also not without its bearing upon some forms of disease.

The analogy between fermentations of different kinds and the course of various eruptive fevers was indeed early recognised by ancient medical writers. Thus Robert Boyle, England's great philosopher, writing in the seventeenth century, says<sup>2</sup>: "He that thoroughly understands the nature of ferments and fermentations shall probably be much better able than he that ignores them to give a fair account of several diseases (as well fevers as others) which will perhaps never be thoroughly understood, without an insight into the

<sup>1</sup> A paper read before the Bournemouth Medical Society on Dec. 11th, 1907.

<sup>2</sup> Boyle's Works, Edition 1744, vol. i., p. 476.