THE ACTION OF URINARY ANTISEPTICS

By ANSON JORDAN, B.A., M.B., B.C. (Cantab.)

From the Pharmacological Laboratory, Cambridge

(Received August 19th, 1910)

The following work was undertaken with the object of investigating the action of the more important reputed urinary antiseptics, under varying conditions, especially with regard to different organisms and to the reaction of the urine.

For the purpose of the experiments the following drugs were selected :—

(1) Urotropin. This drug was chosen since it has the reputation of being the most powerful urinary antiseptic we possess.

(2) Sandal-wood oil and its derivative Santalol-salicylate, or 'Santyl,' were taken as being generally regarded as the most efficacious of the oils, balsams and resins.

(3) Salicylic acid was selected as the representative of the Coal-tar group.

The micro-organisms were chosen from groups which commonly affect the urinary tract; those which only rarely occur were not used, while two important varieties—the Tubercle Bacillus and the Gonococcus—could not be employed because of the difficulty and uncertainty of cultivating them. I therefore limited myself to the study of :—

(1) The Putrefactive Organisms.

(2) *Staphylococcus pyogenes aureus*.

(3) *Bacillus coli communis*.

The latter organism is of special interest since pure infections of the urinary tract with it are frequent.

METHODS

I made use of my own urine throughout the experiments. It was tested before and after the work and found to be normal to the ordinary clinical tests, i.e., as to reaction, specific gravity, and absence of albumen, sugar and deposits. The diet, exercise and mode of life were kept as far as possible constant at the time, so that the results are

strictly comparable. The complete twenty-four hours' specimen was collected from morning to morning, and samples of that of the previous twenty-four hours used in each day's work. In the determination of acidity it was found convenient to take as an arbitrary standard a 'degree of acidity' which may be defined as the 'amount of acid in 10 c.c. of a urine of specific gravity 1020, which will exactly neutralise 1 c.c. of decinormal soda, using phenol-phthalein as the indicator.' The urine was diluted ten times with distilled water to make the colour less obvious. The first definite change of tint was taken as the end-point. The degree of acidity is then found by taking the amount, in cubic centimetres, of decinormal soda required for 10 c.c. of the given urine and dividing it by the number, beyond 1,000, in the specific gravity of the specimen, and multiplying by 20. Thus urines of different specific gravities are reduced to a common level. For example, suppose a urine of specific gravity 1015 required 2·8 c.c. of decinormal soda, then the acidity is $\frac{2\cdot8 \times 20}{15} = 3\cdot7$. By this means, as was pointed out by Joulie,¹ the acidity is given in terms of the total solids and not of the bulk of fluid.

I am, of course, aware that this direct titration of alkali does not give the true acidity of such a mixture of acid salts as the urine, nor the amount of any one acid constituent, but it is the only simple and accurate method of determining the urinary acidity,^{2,3,4} and it gives reliable comparative results. In alkaline urine the degree of alkalinity was found by adding excess standard acid and titrating as above.

The acidity and alkalinity was varied by taking acid sodium phosphate or potassium citrate, respectively, by the mouth. In certain experiments with sandal-wood oil and with salicylic acid the acidity was increased or diminished by adding directly to the urine known amounts of NaH_2PO_4 or NaHCO_3 . By this method specimens of different acidities can be obtained more readily than by the more laborious method of taking each drug by the mouth and sterilizing each specimen of urine; and, moreover, it was found experimentally that this method was satisfactory; that is, the same results were obtained as if the drug had been taken by the mouth.

The various drugs were taken by the mouth for periods of three to five days, and specimens of urine were not tested until the drug had been taken for at least twenty-four hours. In those experiments dealing with putrefaction the urine was incubated in chemically clean but unsterilized tubes at body temperature, and thus allowed to putrefy. In experiments

with other organisms in pure culture it was necessary to obtain sterile urine and to infect it with the organism in question. Boiling might conceivably alter the composition of the urine, and so is inadmissible; and as it is almost impossible to obtain sterile urine by the method of cleansing the meatus, &c., and passing into sterile vessels, as has been recommended, since the urine is always contaminated with the *Diplococcus urethrae*, it was sterilized by passing through a Pasteur-Chamberland filter fitted with an arrangement for collecting large quantities.

THE EFFECT OF VARIATIONS IN THE ACIDITY ALONE

A number of experiments were made in which the different organisms were grown in urines of varying acidity and alkalinity. Measured by the standard already given, it was found that the average acidity of the

FIG. 1. THE VARIATIONS IN THE NORMAL URINARY ACIDITY AND THE EFFECT OF DRUGS IN ALTERING IT.

twenty-four hours' specimens of urine over a period of five weeks was 3·9, the extreme variations being between 3 and 5; the average of the morning specimens being 4·3 and the extreme variations from 3 to 5·5. By taking reasonable doses of acid sodium phosphate the acidity was raised to 8·9, while with potassium citrate the urine was readily made alkaline to the extent of 2·5 degrees. The results are shown in detail graphically in the accompanying curve (fig. 1). The results correspond closely to those of Hutchison.²

When a urine of average acidity is allowed to putrefy in the manner described above, it becomes alkaline in twenty-four hours, and is giving off free ammonia in two to three days. Urine which is alkaline when passed putrefies rapidly, while the putrefaction of urine of high acidity is considerably delayed.

A number of experiments were made with a view to determining the effect of the degree of acidity upon putrefaction (fig. 2).

FIG. 2. THE EFFECT OF VARIATIONS IN THE ACIDITY UPON THE RATE OF ALKALINE FERMENTATION.

Experiment I.—Specimens of urine incubated at body temperature.

(a) 24-hours' urine, Jan. 14-15.

Sp. Gr. 1016. 2.8 c.c. of decinormal soda required for 10 c.c. Acidity = 3.5.

Result—Alkaline in less than 24 hours, ammoniacal in 36 hours.

(b) 24 hours' specimen, February 11-12.

Acidity = - 2.5 (alkaline).

Result—Ammoniacal in 24 hours.

(c) Morning specimen, February 3-4.

Acidity = 8.9.

Alkaline in 2 days, and ammoniacal in three.

(d) Specimen to which acid sodium phosphate had been added, artificially raising the acidity to 20.

Result—Alkaline in 4 days, and ammoniacal in 5 days.

To stop putrefaction altogether the acidity must be raised to 80 or 90, i.e., seven or eight times as high as it is possible for it to be raised by giving drugs by the mouth.

In the experiments with *Staphylococcus pyogenes aureus*, tubes of filtered urine were taken, shown to be sterile by incubating for twenty-four hours without change, and sown with *Staphylococcus* from a young agar culture. The tubes were then incubated at body temperature. In this way the *Staphylococcus* is found to grow very readily in urine and is seen as a deposit of minute whitish granules which tend to adhere to the sides of the tube. It breaks up urea, giving rise to ammoniacal fermentation, but the characteristic foul smell of advanced putrefaction is generally absent. Its growth is favoured by alkalinity, and is delayed by acidity in the same manner as is putrefaction. The time of the first appearance of free ammonia, as in the case of putrefaction, was taken as the gauge of the rate of growth, and similar experiments to the putrefactive ones were performed. The results are illustrated in fig. 2. The only differences of importance are that the *Staphylococcus* is a little more active and the experiments more constant than those with the mixed organisms of putrefaction.

The *Bacillus coli* was grown in sterile urine in the same way as the *Staphylococcus*. It grows very readily and appears as a fine cloudy deposit which renders the urine uniformly turbid. On shaking or stirring, peculiar glistening swirls are seen, and these are not present in cloudy putrefying urine, nor have I observed the appearance in connection with any deposit or substance in urine except with bacteria of the Coli group and with *Bacillus typhosus*. The *B. coli* is sometimes seen in urine joined end to end, forming a long spirillum-like structure

which in a hanging drop worms its way among the normal motile bacilli. Sown back to agar the bacillus assumes its normal form, and these curious forms appear to occur more frequently in old growths in urine. In urine made artificially many times more acid or more alkaline than occurs in the body, the *B. coli* will grow; and, despite what is said to the contrary, no marked differences are observed in its rate of growth whether the urine be acid or alkaline. The *B. coli* does not cause alkaline fermentation, and in consequence it is impossible to obtain any such simple indication of its rate of growth as with the other organisms. I therefore made the simple observations that either (1) the organism grew readily, (2) grew with difficulty, or (3) grew not at all. Until limits of acidity and alkalinity beyond those which occur in the body were reached, it was impossible to appreciate any differences in the rate of growth. It is possible that the statement often made that its growth is inhibited by alkaline urine, is due to an erroneous assumption from the fact that the bacillus, when growing alone, tends to occur in acid urine only, such being the case because urine is normally acid, and *B. coli* has no tendency, as have most of the other bacteria which grow in it, to make it alkaline.

THE EFFECT OF DRUGS

(1) *Urotropin*

Hexamethylenetetramine, commonly known by the trade name of urotropin, was discovered by Butlerow in 1860 as a chemical substance, and introduced as a drug in 1894 by Nicolaier. It was first suggested as a solvent for uric acid in the same way as was piperazine. The use of both these drugs as solvents of uric acid in the body is now discredited, but the action of urotropin as a urinary antiseptic is established. The fact that urotropin is synthesised from formaldehyde, and readily yields that substance on boiling with acids, suggested that its action was due to the liberation of formaldehyde in the urine. Nicolaier⁵ in his early papers on the subject showed that the disinfecting power of urotropin in the urine varied a good deal with temperature and other conditions, and he expressed the view that the action of the drug was due to the formation of formaldehyde. Caspar⁶ demonstrated formaldehyde in the urine of certain patients taking urotropin. Citron⁷ considered that the formaldehyde was present as a loose soda compound. Cotyl and Salus⁸ came to the conclusion that the antiseptic action was

due to urotropin itself, and not to formaldehyde. Cammidge⁹ failed to find formaldehyde in the urine, and concluded that urotropin does not act by its formation. An excellent summary of the whole subject to the year 1905 will be found in a series of papers by Guiard.¹⁰

Experiments with Urotropin.—

(1) Bacteriological Experiments as to the antiseptic power of the drug.

Samples of 24 hours' specimens of urine, obtained as follows, were used :—

A. Urotropin, 10 grs. t.d.s. taken.

Acidity + 1.5.

B. Urotropin, 10 grs. with acid sodium phosphate, 30 grs. t.d.s.

Acidity + 7.0.

C. Urotropin, 10 grs. with potassium citrate, 60 grs. t.d.s.

Acidity — 1.5 (alkaline).

From these three specimens some samples were allowed to putrefy, while others were sterilized by the filter and sown with the organisms in pure culture as in the previous experiments on acidity.

Table of Results

In all cases the urine was incubated at body temperature.

Urine of Acidity	Putrefies in	Staphylococcus renders Ammoniacal in	B. coli.
C. Acid — 1.5 (alkaline)	2 days	3 days	Grows freely.
A. Acid + 1.5	4 „	4 „	Grows not freely.
Acid + 5.0	12 „	10 „	No growth in 14 days
B. Acid + 7.0	No change after 14 days' incubation in any case.		

Further experiments with urines of intermediate degrees of acidity were done, the acidity being varied by adding acid sodium phosphate or sodium bicarbonate directly in the manner already described. In all cases the same dose of urotropin was taken by the mouth. The results are shown, with those tabulated, in the subjoined curve (fig. 3).

These experiments demonstrate that the antiseptic power of urotropin in alkaline or neutral urine is almost nil, that this power rises rapidly as the acidity increases, and when the latter reaches about 4 (i.e. slightly above the normal) it becomes absolute and the urine remains indefinitely sterile. Furthermore, no differences of any importance were here observed in the cases of the different organisms: all were affected in much the same way. These experiments can only be explained by the

assumption that in the urines of higher acidity there is present an antiseptic of considerable power which does not exist in the urines which are alkaline or of low acidity.

Probable percentage of Urotropin which has yielded Formaldehyde.

FIG. 3. TO SHOW THE EFFECT OF ACIDITY UPON UROTROPIN.
(AGAINST PUTREFACTION.)

The degree of antiseptic power of these acid urines was to some extent ascertained as follows:—It was found that when urotropin urine of acidity 7 (specimen B) was added in varying proportions to normal urine of acidity 3·9, one part of this urotropin urine with nine parts of the normal urine was sterile when incubated. Now in these experiments 30 grains of urotropin was taken in the twenty-four hours, and about 50 ozs. of urine was secreted during that time. Urotropin is shown by the bromine water test to appear in the urine about twenty minutes after its administration, and to persist for about twenty-four hours after this has ceased. Not all, but probably the greater part, of the 30 grains is therefore contained in the 50 ozs. of urine, so that the strength of the substance secreted in the urine will be about 1 in 1,000 or less, and this further diluted to the extent of 1 in 10 is, as we have seen, sufficient to prevent putrefaction. I found that if carbolic acid be added to urine to stop putrefaction, about 1 in 800 is required for the purpose, so that the antiseptic substance in acid urotropin urine must be at least ten times as powerful as carbolic acid. Moreover, Mosso and Paleotti¹¹ have shown that, if formaldehyde be added directly to urine, it stops putrefaction when of a strength of 1 in 10,000, and has a definite antiseptic effect when present 1 in 50,000, so that it is obvious that the substance present in acid urotropin urine corresponds closely in antiseptic power with formaldehyde.

It remains to demonstrate the presence of formaldehyde in urotropin urine, and, if possible, to determine the conditions of its formation. It is upon a failure to do this that the workers, who have come to the conclusion that urotropin does not act by the formation of formaldehyde, have based their views. Cammidge⁹ showed that 5 per cent. of urotropin in neutral solution was required to exert the same antiseptic power as 0·08 per cent. of formaldehyde. It is obvious which of these figures corresponds most nearly to the maximum possible strength of the substance present in a urotropin urine with antiseptic power, yet Cammidge concluded from a failure to detect it chemically that no formaldehyde was present. He used as tests for formaldehyde the reduction of Fehling's solution, the resorcin test, and Schiff's rosanilin reaction. I repeated some of his experiments with results corresponding to his. He does not appear to have realised in how small an amount the formaldehyde would be, if present, for he mentions the fact that urotropin urine never smells of formaldehyde as evidence against its presence. A solution, of 1 in 10,000, which would exert quite a powerful

antiseptic action, as we have seen, would, of course, have not the slightest smell. Moreover, none of Cammidge's tests, if tried upon a 1 in 10,000 solution of formaldehyde, are positive. There is, however, an extremely delicate test which will readily detect 1 in 200,000 of formaldehyde, or even less. This is the phloroglucin test. A 1 per cent. solution of phloroglucin with 25 per cent. of alcohol is made with distilled water, and a little strong caustic soda solution added. A few drops of this gives a bright cherry-red colour with solutions of formaldehyde. In testing for small amounts the solution should be added slowly, and the colour appears in a few seconds and fades after a time (probably by reason of the combination of the formaldehyde and the soda). The test solution must be fresh. With this test I have never failed to detect formaldehyde in acid urotropin urine. A distinct red colour is produced on adding the test fluid directly to the urine which is not produced in normal urine or in alkaline urine containing urotropin. The demonstration may be made more obvious by distilling the urine 'in vacuo' at body temperature and testing the distillate, when a bright cherry-red colour is produced. Boiling is of course inadmissible, since formaldehyde might then be produced by the heat.

Experiments to elucidate the conditions under which formaldehyde is formed in urotropin urine:—

Broth Culture Experiments with Urotropin.

Broth cultures to which drugs had been added as described were infected with the *Staphylococcus* and incubated for 24 hours.

Drugs added to culture						Result
(1)	Urotropin 1 in 1000 with trace of NaH_2PO_4	...	...	...	...	No growth
(2)	Urotropin 1 in 300 with trace of NaH_2PO_4	...	...	...	...	No growth.
(3)	Urotropin 1 in 100 alone	...	...	...	...	Grew slightly.
(4)	Urotropin 1 in 20 alone	...	...	...	...	No growth.
(5)	Urotropin 1 in 20 with trace of NaHCO_3	...	...	...	...	Grew slightly.

These experiments demonstrate that outside the body the addition of a trace of an acid salt to urotropin will enormously increase its antiseptic power. They also show that in neutral solution a strength of 1 in 20 will inhibit growth, but that this property is destroyed by the addition of alkali; an explanation of this fact is given later.

Chemical Experiments. By means of the phloroglucin reaction not only can very small amounts of formaldehyde be detected, but, in addition, from the depth of colour a rough indication of the strength of the solution can be obtained. For this purpose the test is applied and

the colour matched by adding the same amount of phloroglucin solution under identical conditions to solutions of formaldehyde of different known strengths. When the colour is identical, the strength of the formaldehyde may be assumed to be nearly so. In this way a rough quantitative estimate is obtained.

Experiments. A solution of urotropin in distilled water of strength 1 in 1,000, i.e., about the strength in which it occurs in urine, was made up, and samples of it were incubated at body temperature for twenty-four hours.

(a) In neutral solution it was found that the slightest trace of formaldehyde was liberated, probably about 1 in 200,000.

(b) In solution with a small amount of acid sodium phosphate, or a trace of a mineral acid, a strong reaction was given, the colour indicating a strength of about 1 in 10,000.

(c) In solution with a small amount of sodium bicarbonate or other alkali no reaction is given, even after prolonged incubation. This, of course, explains the differences in the behaviour of tubes 4 and 5 in the last described broth-culture experiments.

(d) Lastly, if a solution is acidified, incubated, and gives the reaction indicating about 1 in 10,000 formaldehyde, and to it is then added sufficient sodium bicarbonate to make it alkaline, together with a slight trace of ammonia, after twenty-four hours' incubation it fails to show the least trace of formaldehyde, all the urotropin has been reconstituted.

It will be seen that these chemical experiments are in every respect consistent with those in which the actual effects of urotropin as a urinary antiseptic were investigated, provided that it be accepted that its powers are due to formaldehyde. Urotropin in solution is a comparatively unstable substance. In acid solution it tends to disintegrate into formaldehyde and ammonium compounds. In alkaline solution the reaction tends the other way. Various factors—the dilution, the time allowed, and above all the temperature—will affect these reactions, but from a pharmacological point of view one is only concerned with these factors as they occur in the body when urotropin is being given as a drug, namely, with a dilution of 1 in 1,000 or less, and with effects taking place during a number of hours at a temperature of about 37° C. If these conditions are observed, it is found that urotropin in neutral

solution disintegrates, yielding formaldehyde to a very small extent, namely, about 0·05 per cent. to 0·1 per cent. In alkaline solution no dissociation occurs. In acid solutions the percentage of urotropin which breaks up increases considerably, in a degree related to the acidity, so that with an acidity corresponding to that of acid urine about 10 per cent. is split up into formaldehyde.

It has been shown that these processes occur in urine when urotropin has been taken, and that the antiseptic power of the drug runs parallel with, and can only be explained by, the amount of formaldehyde present. So that the object in giving urotropin as a urinary antiseptic is to exhibit formaldehyde in the urine, and to keep this in view constitutes, I believe, the key to the correct use of the drug.

(2) *Sandal-wood Oil*

Sandal-wood oil has largely superseded copaiba and cubebs, and is generally reputed to be the most efficient of the essential oils. It contains two substances allied to the sequiterpenes, santalol and santalal. In the last few years these substances, or combinations of them, have been put upon the market under the names of gonosan, santyl, etc. Santyl, which is santalol-salicylate, is highly praised from a clinical standpoint,¹² and in consequence some experiments were done with it to compare its efficiency with that of the crude oil.

Sandal-wood oil, like most other essential oils, is excreted in the urine partly unchanged and partly as a compound of glycuronic acid. Its reputation as a urinary antiseptic is not great, though it is acknowledged to be of value in the treatment of gonorrhoea. It would seem that here it must act as an antiseptic, though some writers regard its action rather as an 'astringent' to the mucous membrane, because in cases of gonorrhoea which are benefited by sandal-wood oil, other and more powerful antiseptics are said to be of little avail. This astringent speculation is nonsense; none of the essential oils exhibit astringent properties. Urotropin has not been given a full trial; Leedham-Green¹³ found it useful in certain stages of the complaint.

Experiments with sandal-wood oil. The sandal-wood oil was taken in gelatine capsules in doses of 20 m. three times a day, and specimens of the resulting urine were used. The acidity of the specimens was varied by the method of direct addition (v. s.).

(1) Putrefaction :—

Acidity	Time, in days, for first appearance of free ammonia
2 (alkaline)	1 day
+ 3.5	4 days
+ 10	7 days

Some cloudiness in 24 hours in all these tubes.

(2) Growth of *B. coli* in sterilised sandal-wood oil urine :—

Acidity	Result
- 2 (alkaline)	} Growing freely in 24 hours.
+ 3.5	
+ 10	
+ 15	Growing freely in 48 hours.

(3) Growth of *Staphylococcus* in sterilised sandal-wood oil urine :—

Acidity	Time for first appearance of free ammonia
- 2 alkaline)	3 days
+ 3.5	9 days
+ 10	14 days
+ 15	Growing but not alkaline in 14 days

It will be seen that the action of sandal-wood oil against putrefaction is very feeble. It about doubles the time the urine takes to putrefy. In the case of the *Bacillus coli* there is practically no antiseptic action at all, this organism growing quite well in sandal-wood oil urine. With the *Staphylococcus*, however, the case is entirely different; here there is quite a powerful antiseptic effect. In alkaline urine sandal-wood oil is far more potent against the *Staphylococcus* than is urotropin, while though in urines of higher acidity it does not entirely prevent the growth of the organism as does urotropin, it has yet a marked and powerful effect.

This selective action of the drug upon a particular organism was so unexpected that to exclude the possibility of error all these experiments with the *Staphylococcus* were repeated, using controls of normal urine of corresponding acidities, but similar results were obtained and there seems no question of the accuracy of the observations.

It was noticed that in the sandal-wood oil urine, which was undergoing putrefaction, while there was a delay in the appearance of free ammonia the cloudiness of commencing putrefaction appeared fully as early as it does in normal urine. It may be suggested that this is due to a restraining influence of the drug on the putrefactive cocci rather than the bacilli, if, as seems likely, we assume that the cocci are the chief

agents in breaking up urea. Moreover, the fact of the value of sandal-wood oil against the gonococcus would be explained if we assume this selective action to extend to cocci generally. However this may be, there seems to be no doubt that sandal-wood oil has a considerable degree of power in preventing the growth of *Staphylococcus*, while towards the other organisms it is almost inert.

Experiments with Santalol Salicylate ('Santyl'). To obtain some idea of the comparative values of sandal-wood oil, and of its derivative santalol salicylate ('santyl'), some specimens of urine after taking the latter drug were allowed to putrefy and the results compared with those obtained with the crude oil.

Experiments.—

Doses of santyl of 30 m. t.d.s.

24 hours' urine used.

Acidity.	Time for first appearance of free ammonia
— 2 (alkaline)	2 days.
+ 2 (acid)	3 days
+ 5 (acid)	5 days.
+ 10 (acid)	8 days.

These results are slightly better than those with the crude oil, but since the dose was nearly double that of the latter it must be concluded that santalol salicylate has a rather feeblar action than has sandal-wood oil; this can, however, be made up for by the fact that the drug can be given in larger doses, and it is less unpleasant to take.

(3) *Salicylic Acid*

Salicylic acid was chosen as the representative of those coal-tar products which have a urinary antiseptic action. Of these, salol—phenyl salicylate—is reputed to be the most powerful, but since it has a double action, due to its two constituents, it seemed not to be so suitable for experimental purposes.

Salicylic acid is excreted in the urine as salicyluric acid and as sodium salicylate. In an acid urine some of this sodium salicylate is split up, yielding the free acid. As a pure antiseptic it probably does not matter much in what form salicylates are taken, but the acid itself has the power of increasing the urinary acidity to some extent, and it was among many drugs used for this purpose before the introduction of acid sodium phosphate. With a view to getting some idea of this secondary action, I took salicylic acid, instead of any of its derivatives.

Experiments with Salicylic Acid. Salicylic acid in doses of 20 grains three times daily. Twenty-four hours specimens of urine used. No appreciable increase in the acidity of the twenty-four hours urine resulted. The twenty-four hours specimen with which the pure culture experiments were made had an acidity of 3·5, i.e., slightly below the average. Hutchison,² who investigated this point, found that the acidity was increased. Possibly, had the drug been taken for a longer period, the average acidities would have shown some increase, but my result shows that this action is very feeble and quite insignificant.

For investigating the antiseptic action experiments were done of the same kind as with the other substances.

Putrefaction.—

Acidity		Time taken to become alkaline		Time taken to become ammoniacal
— 3·5 (alkaline)	...	1·2 days	...	2 days
+ 3·5	...	4 days	...	5 days
+ 10	...	8 days	...	10 days

Some specimens of higher acidities were clear at the end of ten days.

Experiments with the Staphylococcus.—

Acidity		Time taken to become alkaline		Time taken to become ammoniacal
— 2 (alkaline)	...	2 days	...	3 days
+ 3·5	...	3 days	...	4 days
+ 10	...	Not alkaline at the end of 10 days.		

Experiments with the Bacillus Coli. In urines of all acidities up to 10 the bacillus was growing well in twenty-four hours, in the urines of higher acidity it was forty-eight hours before any growth was apparent, and the organism was not growing well then.

It will be noticed that the antiseptic power of salicylic acid in the urines of high acidity is somewhat greater than can be accounted for by the increase in the acidity alone; this may be due to an alteration of the proportions between salicylic acid and salicyluric acid.

CONCLUSIONS

1. Using a defined arbitrary standard, the average acidity of normal urine was about 4. With the largest doses of acid sodium phosphate (NaH_2PO_4) which can be conveniently tolerated, this was raised to 9. The urine was readily made alkaline with potassium citrate.

2. Putrefaction will occur readily in, and the *Staphylococcus* or *Bacillus coli* will grow easily in, urines of all degrees of acidity and alkalinity which can be produced in the body. The acidity exercises a restraining influence upon the putrefaction, or the growth of *Staphylococcus*, which is proportional to its degree; thus, between the limits of alkalinity on the one hand, and the highest degree of acidity which can be produced on the other, the time of ammoniacal fermentation is increased about five-fold. The *B. coli* was found to grow about equally well in urines of all degrees of acidity and alkalinity within the above limits. It grows as well in alkaline as in acid urine.

3. *Urotropin* acts as a urinary antiseptic by the formation of formaldehyde. In an alkaline urine no formaldehyde is formed, and in consequence the antiseptic action is nil. In neutral or faintly acid urines a small amount, probably not exceeding 1 per cent., is split up, and the action is feeble. The percentage of formaldehyde, and with it the antiseptic power, increases with the acidity until with an acidity of 4.5 (i.e., slightly above normal) no organism will grow in the urine, which therefore remains indefinitely sterile; at this point there is reason to believe that about 10 per cent. of the urotropin has been converted into formaldehyde. The process of conversion into formaldehyde is a simple chemical one, which occurs in all solutions of urotropin under conditions of dilution, temperature, acidity, and time which are identical with those obtaining in the body. It was found that urotropin acted equally well with the different organisms used.

4. The antiseptic action of *sandal-wood oil* in the urine was found to be quite feeble against putrefactive organisms or the *B. coli*, but it showed a marked selective action upon *Staphylococcus*, against which it has quite a specific antiseptic power.

5. The power of *Salicylic Acid* to increase the urinary acidity was very slight. As an antiseptic it has quite a definite action, though not a very powerful one. Against the putrefactive organisms or the *B. coli* it is much more efficient than sandal-wood oil; it has, however, no selective action upon any organism, and in consequence is inferior to sandal-wood oil for the *Staphylococcus*.

A diagram (fig. 4) is given in which the comparative values of the drugs are represented. It will be seen that urotropin acting in a urine of reasonably high acidity is for all purposes far and away the most powerful. If the urine is alkaline, neutral, or only faintly acid,

salicylic acid is the most efficient of these three substances against either putrefactive organisms or the *B. coli*, while sandal-wood oil is the more efficient for the *Staphylococcus*. The action of neither of these drugs, however, can be compared to that of urotropin in an acid urine.

FIG. 4. THE EFFECT OF THE DEGREE OF URINARY ACIDITY UPON THE ANTISEPTIC POWER OF THE DIFFERENT DRUGS AGAINST PUTREFACTION.

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