THE ACTION OF AMMONIUM BENZOATE ON THE URINE.

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It has long been known that when benzoic acid or benzoates are taken by the mouth the benzoic radicle synthesizes with glycocoll to be excreted in the urine as hippuric acid, and in 1877 Bunge and Schmiedeberg showed experimentally that in dogs this synthesis takes place in the kidney. When such urine putrefies the hippuric acid is decomposed and again yields benzoic acid, which unites with the ammonia or other alkalis present to form a neutral salt. The decomposition has been attributed to the action of an enzyme (Schmiedeberg) and to bacteria (Minkowski, van der Welde and Stokvis, Wiechowski, Seo). Dakin, investigating the fate of sodium benzoate in the human organism, found that 5 to 10 grams. taken by the mouth per diem underwent almost complete conversion into hippuric acid and was eliminated as such, and he held that his results confirmed and extended those of Lewinski, but Raiziss and Dubin maintained that with large amounts "free" or non-conjugated benzoic acid appears in the urine, and they made a tentative suggestion that this might be due to an overtaxing of the synthetic power of the kidney, whereby some benzoic acid passes unchanged.

Based upon these researches, the pharmacology of benzoates has been presented in text-books in a rather stereotyped form. In the case of ammonium benzoate, we find attributed to it the power of rendering normal urine more acid in reaction and antiseptic, although no explicit information is offered as to how these results are brought about. Experimental workers to-day are divided in opinion as to whether the continuous administration of sodium or ammonium benzoate does yield non-conjugated benzoic acid in the urine. Following the technique of Raiziss and Dubin I have found that non-conjugated benzoic acid does occur after the continuous administration by mouth of ammonium benzoate, the benzoic acid being present in the form of an alkaline salt.

The present research was undertaken to investigate (1) the effect of ammonium benzoate on the reaction of the urine; (2) whether after the administration of ammonium benzoate non-conjugated benzoic acid is ever found in the urine; (3) the action of ammonium benzoate as a urinary antiseptic.
The Effect of Ammonium Benzoate on the Reaction of the Urine.—One finds that great difference of opinion exists as to the effect of some drugs upon the reaction of the urine, and this is true of ammonium benzoate. The discrepancy is due in some measure to the reliance placed upon the estimation of acidity by titration with caustic soda using phenolphthalein as an indicator. Such titration does not give the true acidity, but merely the excess of acid over base within the working limits of the special indicator used, and varies greatly if the daily volume of urine is fluctuating. The hydrogen-ion-concentration, however, as expressed in the logarithmic notation of Sörensen ($pH$) is a true index of the real acidity, independent, within wide limits, of the concentration of the fluid to be tested. But while it is chemically accurate to compare the acidity of a decinormal solution of hydrochloric acid with that of a decinormal solution of phosphoric acid by titration with decinormal alkali, the application of this comparison to urine is a different matter. The normal urinary acidity is brought about by the preponderance of acid salts in a mixture of what is chiefly the phosphatic salts of sodium. Now, the hydrogen-ion-concentration of a given volume of decinormal hydrochloric acid is usually expressed as $pH$ 1.04 (strongly acid), while that of a solution of varying quantities of disodium hydrogen phosphate and potassium dihydrogen phosphate, for example, is only found within the limits of $pH$ 4.4 to $pH$ 9.0 (weakly acid to weakly alkaline), this difference between the two solutions being due to the fact that a solution of hydrochloric acid dissociates very completely, while that of a weak acid like phosphoric acid, or its salts, has a relatively small dissociation constant. This latter more nearly represents the state of affairs in the urine, where weak acids are present and the amounts combined with bases are large. While in the past we have been comparing the acidities of different urines by the amount of decinormal soda required for their neutralisation, it will be found that there is often no relation between the figures thus obtained and the hydrogen-ion-concentration. For example, taking three consecutive days in a test case, the figures shown in Table I. were obtained.

Estimated by titration with an alkali, this case would be said to be fluctuating in acidity, and if these three specimens were used as controls, their average reading would greatly misrepresent the true nature of the acidity, which, according to the
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hydrogen-ion-concentration, is constant all the time. Another point to be noted is that phenolphthalein as an indicator only gives an end-point well on the side of alkalinity (pH 8.3). The following readings illustrate the effect of this: 1080 c.c. urine, alkaline to litmus, gave a total acidity equivalent to 151 c.c. decinormal hydrochloric acid, while the pH was 7.3 (alkaline).

During the present research these factors were kept in view. In an endeavour to find out whether ammonium benzoate given by the mouth can render a urine acid in reaction over 2000 specimens were examined. The subjects chosen were invariably persons who were receiving no medicinal treatment, the majority being hospital patients on ordinary diet. In every case the urine was examined for at least seven days before the drug was given, and all samples were tested by litmus, titrated with decinormal soda, and the hydrogen-ion-concentration estimated

| Day | Amount of Urine in 24 hours | Total Acidity as measured in c.c. N/10 NaOH | Hydrogen-ion-concentration |
|-----|----------------------------|---------------------------------------------|-----------------------------|
| 1   | 795.6 c.c.                 | 254.6                                       | 5.1                         |
| 2   | 738.7 "                    | 421.3                                       | 5.1                         |
| 3   | 511.0 "                    | 97.1                                        | 5.1                         |

by the colorimetric method of Clark and Lubs; the urinary ammonia also was determined in some cases.

It was found that by titration with decinormal caustic soda no constant results were obtained. In cases where the urine was of normal acidity ammonium benzoate caused little or no increase: in weakly acid or in alkaline urines it produced a rise in the total acid output (in terms of N/10 NaOH). Such increases were not consistently maintained beyond the second or third day after drug treatment was commenced. Two constant features noted were an increase in the daily volume of the urine and an increase of urinary ammonia. The hydrogen-ion-concentration readings, on the other hand, were very constant both in individual cases and as a whole. The urine of the average hospital patient was found to be within the limits of pH 5.0 to pH 5.6, and after giving ammonium benzoate in 20-grain doses thrice daily almost all urines came to an acidity of pH 5.0 to pH 5.4. In no case did the hydrogen-ion-concentration rise beyond the limits of normality.
Taking the results as a whole, it was found that ammonium benzoate taken by mouth in normal persons cannot be regarded as a potent acidifier of normal urine. There is no doubt that it can often rapidly acidify an alkaline urine such as is found in many cases of chronic cystitis or after large doses of bicarbonate of soda. A urine artificially made alkaline with citrate or bicarbonate of sodium will doubtless return to its normal acidity if left to nature, but ammonium benzoate can restore the normal level in a much shorter time. On the other hand, ammonium benzoate does not merit the place given to it by some for its power of increasing the degree of acidity of normal urines. I found that while it is easy to make a urine alkaline or to render an alkaline urine acid, it is very difficult to increase the acidity over the normal limit, the body being able apparently to adjust itself to deal with the drugs exhibited. This is especially true of benzoates. In the light of our present knowledge of what determines the acidity of a normal urine, the probable explanation of the effect of ammonium benzoate is that the acid products of its excretion use up base, thus increasing the preponderance of acid salts.

(2) The Excretion of Benzoic Acid in the Urine after Ammonium Benzoate.—In estimating the amount of benzoic acid in urines a combination of the method of Folin and Flanders and that of Raiziss and Dubin was adopted. In the former, used generally to estimate the hippuric acid content, the whole of the hippuric acid is converted into benzoic acid, which, extracted by means of chloroform, is titrated by a standard solution of sodium ethylate. The latter is a somewhat similar method for estimation of unsynthesised benzoic acid, with toluol as the solvent. With careful technique the accuracy of these methods, as tested with pure benzoic acid dissolved in chloroform or toluol, was found to be very satisfactory (\( \frac{1}{2} \) per cent. to 1 per cent.). The plan adopted was as follows:

(1) Folin-Flander method gives \( \text{Total benzoic acid.} \)

(hippuric acid converted to benzoic acid and unconjugated benzoic acid unchanged)

(2) Raiziss-Dubin method gives \( \text{Unconjugated benzoic acid.} \)

Subtract (2) from (1) gives \( \text{Hippuric acid in terms of unconjugated benzoic acid.} \)
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From these figures we can calculate (a) any increase of hippuric acid (i.e., conjugated benzoic acid), and (b) the quantity of unconjugated benzoic acid.

Starling states that hippuric acid is a frequent though not a constant constituent of the urine, and varies in amount from 0.1 gram. to 1.0 gram. per diem, although after a rich vegetable diet it may rise to 2.0 grams. In my series the average amount of conjugated benzoic acid (i.e., hippuric acid) excreted daily yielded from 0.3 gram. to 0.9 gram. benzoic acid, equivalent to approximately 0.4 to 1.3 grams. hippuric acid. In normal urines unconjugated benzoic acid was never present.

After the exhibition of ammonium benzoate (20 grains t. i. d.), in every instance there was a sharp rise in the hippuric acid output, which rose at once to at least twice its normal level. The amount excreted rose steadily day by day until at the end of one week it was generally about six times higher than normal. The second point noted was that, when the hippuric acid reached 2.0 grams. or thereabouts, there were traces of unchanged benzoic acid in the urine. On the first and second days the quantities present were generally immeasurable, but thereafter the amount was quite appreciable, varying from 0.1 gram. to 0.8 gram., and represented over the whole test period from 5 per cent. to 8 per cent. by weight of the total benzoate administered. This "free" or unconjugated benzoic acid was obtained constantly in each case (Table II.).

Assuming the view that there is a renal synthesis of benzoic acid and glycocoll to form hippuric acid, it seems from these results that this synthetic power of the kidneys is only able to function within limits dependent upon the amounts of benzoic acid presented to the renal cells. On increasing either the concentration or the rate of passage of the benzoic acid the renal cells seem unable to cope with the work demanded of them and thus a proportion of the benzoic acid is allowed to pass unsynthesized. In my series the limit seemed to be reached when the excretion of hippuric acid exceeded about 3.0 grams. daily (the equivalent of 2.0 grams. benzoic acid). These results bear out the tentative suggestion of Raiziss and Dubin regarding the possibility of an overtaxing of the kidney.

(3) Ammonium Benzoate as a Urinary Antiseptic.—In this connection the drug was tested in vitro and in clinical cases. After oral administration, a drug like ammonium benzoate has to pass through several chemically active media,
### TABLE II.

|                | Total Benzoic Acid found. | Unconjugated Benzoic Acid | Conjugated Benzoic Acid (Hippuric Acid) |
|----------------|---------------------------|----------------------------|----------------------------------------|
| **CASE I.**    |                           |                            |                                        |
| Before ammonium benzoate | 0.4739  | nil. | 0.4739 |
|                  | 0.3397  | nil. | 0.3397 |
|                  | 0.8860  | nil. | 0.8860 |
|                  | 0.5216  | nil. | 0.5216 |
| After ammonium benzoate gr. xx. t.i.d. | 0.3679 | trace | 0.3679 |
|                  | 1.5595  | nil. | 1.5595 |
|                  | 2.9727  | nil. | 2.9727 |
|                  | 2.7173  | nil. | 2.7173 |
|                  | 3.4004  | nil. | 3.4004 |
|                  | 3.1116  | nil. | 3.1116 |
|                  | 3.4162  | nil. | 3.4162 |
| **CASE II.**    |                           |                            |                                        |
| Before ammonium benzoate | 0.9950  | nil. | 0.9950 |
|                  | 0.3916  | nil. | 0.3916 |
|                  | 0.9590  | nil. | 0.9590 |
|                  | 0.6105  | nil. | 0.6105 |
| After ammonium benzoate gr. xx. t.i.d. | 2.2340 | nil. | 2.2340 |
|                  | 2.5709  | nil. | 2.5709 |
|                  | 2.8120  | nil. | 2.8120 |
| **CASE III.**   |                           |                            |                                        |
| Before ammonium benzoate | 0.6158  | nil. | 0.6158 |
|                  | 0.9606  | nil. | 0.9606 |
|                  | 0.8658  | nil. | 0.8658 |
|                  | 0.9047  | nil. | 0.9047 |
| After ammonium benzoate gr. xx. t.i.d. | 2.1831 | trace | 2.1831 |
|                  | 2.3700  | trace | 2.3700 |
| **CASE IV.**    |                           |                            |                                        |
| Before ammonium benzoate | 0.899   | nil. | 0.899 |
|                  | 0.719   | nil. | 0.719 |
|                  | 0.651   | nil. | 0.651 |
|                  | 0.5015  | nil. | 0.5015 |
| After ammonium benzoate gr. xx. t.i.d. | 2.1889 | trace | 2.1889 |
|                  | 1.6583  | trace | 1.6583 |
|                  | 2.2018  | trace | 2.2018 |
|                  | 1.7260  | trace | 1.7260 |
|                  | 3.1297  | trace | 3.1297 |
|                  | 3.9712  | trace | 3.9712 |

| Ammonium Benzoate given. | Unconjugated Benzoic Acid found. | % by Weight recovered in Urine. |
|--------------------------|---------------------------------|-------------------------------|
| Grains.                  | (Approx.) Grains.               | (Approx.)                     |
| **CASE I.**              |                                 |                               |
| II.                      | 420                             | 28                            | 1.8318 | 5% |
| III.                     | 180                             | 12                            | 1.0203 | 8% |
| IV.                      | 120                             | 8                             | 0.4137 | 5% |
| IV.                      | 420                             | 28                            | 2.628 | 7% |

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the gastric juice, portal tract, general circulation, and finally the selective cells of the kidney. The prospective urinary antiseptic arrives therefore in the urinary channels in a highly diluted condition and usually in a much altered state chemically. Accordingly it appeared that a suitable way of testing the effect of ammonium benzoate would be to give it internally over a period of some days, and then test the urine for bactericidal power, if any, against a previously prepared culture of urinary coliform organisms. In this way the urine was obtained containing the benzoate in precisely the form in which it would appear if given clinically in cystitis.

Method.—An emulsion was made by rubbing several loopfuls of a culture of coliform organisms (prepared from the urine of a case of chronic cystitis) in normal saline. 100 c.c. of the test urine was shaken up with animal charcoal to decolorise as far as possible, and filtered. 10 c.c. of the filtrate in each case was put into a test-tube of the same calibre as the others used in the test. To each was added $\frac{1}{4}$ c.c. of the bacterial emulsion, the tubes were rotated to mix the contents, and the specimens incubated at 37° C. for 48 to 72 hours. The results are expressed in terms of relative turbidity and of the presence of the glistening swirls characteristic of coliform organisms.

**TABLE III.**

| 48 hours' Incubation. |
|-----------------------|
| **Urine.** | **Controls.** | **Cases after Ammonium Benzoate.** |
| | a. | b. | c. | d. | e. | f. | g. | h. |
| $\rho$H | 5.4 | 6.7 | 5.1 | 4.9 | 5.0 | 6.1 | 5.4 | 6.2 |
| Growth | xx | xxx | ? | —— | —— | —— | —— | x |

| 72 hours' Incubation. |
|-----------------------|
| **Urine.** | **Controls.** | **Cases after Ammonium Benzoate.** |
| | | a. | b. | c. | d. | e. | f. | g. | h. |
| $\rho$H | 5.2 | 5.1 | 7.3 | 4.8 | 5.2 | 5.2 | 6.0 | 6.2 |
| Growth | xxx | xx | xxx | —— | ? | x | xx | xx |

x = Growth of coliform bacilli (turbidity).
—— = Inhibition (clear urine).

After 48 hours' incubation the control urines were markedly turbid and showed vigorous growth of *B. coli*. After ammonium benzoate had been given over a period of one week (gr. 20 t. i. d.), some urines remained quite clear, others showed varying degrees...
of turbidity, and on grading them it was found that on the whole the higher the acidity (pH) the clearer was the urine. Subcultures made from the clear specimens gave fewer and smaller growths than those from turbid urines. In cases where staphylococci and streptococci were used instead of B. coli, no appreciable difference was noted after the drug was given, but this point was not investigated further. After 72 hours' incubation very rarely did any urine remain clear. One concluded that in some urines ammonium benzoate given by mouth in therapeutic doses retarded the growth of coliform organisms, but that this inhibitory power must be slight.

Clinically, it was found that ammonium benzoate produced or maintained an acid urine, and where the urine in B. coli cystitis was acid, I found it gave more relief to symptoms to alkalise with bicarbonate of soda before administering benzoate. Some cases of alkaline cloudy urines rapidly became clear with relief of symptoms, but in obstinate cases of chronic cystitis, especially in women, ammonium benzoate only prepared the way for urotropin, the combination of these two drugs giving good results.

In treating cystitis with ammonium benzoate the first effect is the production of a moderate degree of diuresis which is maintained, and this no doubt plays a part by flushing the urinary passages. That ammonium benzoate has an antiseptic action is doubtful, and the drug is certainly not to be relied upon clinically when used alone. To ascribe an antiseptic power to it because of its acidifying properties is fallacious, as the drug cannot produce a urine sufficiently acid to prohibit the growth of organisms. Any effect must be due to the salts of unconjugated benzoic acid in the urine, a 5 per cent. to 8 per cent. solution of which, in my experiments, seemed capable of slightly retarding the growth of B. coli.

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REFERENCES.—Bunge and Schmiedeberg, Arch. exp. Path. u. Pharm., 1877, vi., 233. Dakin, H. D., Journ. Biol. Chem., 1910, vii., 103. Folin and Flanders, Journ. Biol. Chem., 1912, xi., 257. Lewinski, Arch. exp. Path. u. Pharm., 1908, lviii., 397. Minkowski, Arch. exp. Path. u. Pharm., 1883, 397. Raiziss and Dubin, Journ. Biol. Chem., 1915, xxi., 331. Schmiedeberg, Arch. exp. Path. u. Pharm., 1881, xiv., 288. Seo, Arch. exp. Path. u. Pharm., 1908, lviii., 75. Starling, Principles of Human Physiology, 1920, p. 1169. Wiechowski, Beitr. z. Chem. Physiol., 1906, vii., 204.