# STUDIES IN THE PHARMACOLOGY OF 4-BENZAMIDOSALICYLIC ACID AND ITS SALTS

BY E. M. BAVIN and BARBARA JAMES

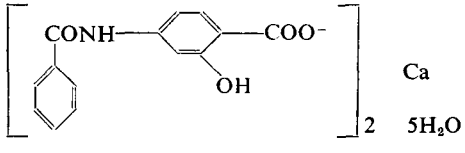
*From Smith and Nephew Research Ltd., Hunsdon Laboratories, Ware, Herts*

Received July 13, 1953

RECENT reports[1,2] have shown that *p*-aminosalicylic acid delays to a remarkable extent the development of streptomycin resistance by *Mycobacterium tuberculosis*, and investigations are in progress to determine whether it has the same effect on the development of isoniazid resistance. The combined use of streptomycin and *p*-aminosalicylic acid is now regarded as essential when the former drug is given in the chemotherapeutic treatment of tuberculosis and most workers feel that this is the main use for *p*-aminosalicylic acid, in spite of its own definite tuberculostatic effect. The nauseous taste of *p*-aminosalicylic acid, however, still remains a difficulty, especially since the drug has to be given in large doses daily over a long period, and a number of patients find it almost impossible to continue the treatment in spite of its unquestioned value. Many attempts have been made to overcome this difficulty, mainly by the use of different pharmaceutical presentations of the drug such as cachets, tablets and granules but these forms are necessarily somewhat more costly.

Recently we have been able to study a derivative of *p*-aminosalicylic acid which, because of its almost complete lack of taste, may be one answer to the difficulty referred to above.

This derivative is the calcium salt of 4-benzamidosalicylic acid with the formula (I).




I

Its laboratory number is H.P. 170.

The free acid was first reported by Rosdahl[3] and independently by Seymour and co-workers[4,5], the latter group showing it to possess *in vitro* tuberculostatic activity inferior to *p*-aminosalicylic acid[5]. A chance observation, recently, however, showed that the calcium salt was very insoluble and, presumably because of this insolubility, was almost tasteless. It occurred to us that if this calcium compound was decomposed in the body to liberate free *p*-aminosalicylic acid, it might provide a more tolerable method of administering this latter drug. Theoretically, the compound could liberate 47·6 per cent. by weight of *p*-aminosalicylic acid. It was decided therefore, to make a study of some aspects of the pharmacology of calcium 4-benzamidosalicylate with particular reference

to its absorption, plasma levels and excretion. The toxicity and tuberculostatic effects of the compound were also investigated.

## Experimental Methods

*Toxicity.* Acute and chronic toxicity tests were carried out on white Swiss male mice between 20 and 30 g. in weight, the drug being given by gastric tube in aqueous gum acacia suspension. Chronic toxicity tests were also carried out on albino male rats, to which the drug was administered mixed with the diet (Diet 41).

*Plasma, Urine and Tissue levels.* (*a*) *p*-Aminosalicylic acid was determined in plasma and urine by the method of Newhouse and Klyne[6].

(*b*) A method was evolved for the determination of 4-benzamidosalicylic acid in biological tissues based on the separation from *p*-aminosalicylic acid by chloroform extraction at an acid *p*H, hydrolysis by prolonged boiling with sulphuric acid, followed by diazotisation and coupling with a standard *p*-aminosalicylic acid solution as described by Pesez[7].

The details of the method are as follows:—

*Reagents*

Hydrochloric acid —5 N solution
Sulphuric acid —10 per cent. v/v solution
Sodium hydroxide —2 N solution
Sodium *p*-aminosalicylate—0.15 per cent. w/v solution (*freshly prepared*)
Sodium nitrite —1 per cent. w/v solution
Sodium carbonate —saturated solution
Chloroform.

*Standard solution.* 10 μg./ml. aqueous solution of sodium 4-benzamidosalicylate.

*Method.* Place 1 ml. of oxalated plasma in a small separating funnel, add 9 ml. of distilled water and 1 ml. of 5 N hydrochloric acid. Mix and extract 3 times with 5 ml. quantities of chloroform. The funnel is shaken for 1 minute with each extraction. If the separation is incomplete, the bottom layer is centrifuged and the chloroform layer removed with a pipette. The chloroform extract is evaporated to dryness in a boiling tube (6″ × 1″) on a boiling water bath. 3 ml. of 10 per cent. sulphuric acid are added to the dried residue, which is then heated on a boiling water bath for 4 hours. The boiling tubes are fitted with air condensers consisting of 9″ of glass tubing passing through rubber bungs of a suitable size. Colour is extracted from some makes of rubber bung during hydrolysis and these must be avoided.

The tubes are removed from the water bath. 2 ml. of 5 N sodium hydroxide is added and the tubes are placed in a freezing mixture between 0° C. and −5° C. In quick succession, 0·5 ml. of freshly prepared 0·15 per cent. sodium *p*-aminosalicylate and 0·5 ml. of 1 per cent. sodium nitrite solution are added and the tubes are shaken. The tubes are removed from the freezing mixture after exactly 5 minutes and 3 ml. of saturated sodium carbonate solution is added. The tubes are shaken and allowed to stand

for 15 minutes at room temperature. 1 ml. of water is added to each tube and the colour read on a Unicam spectrophotometer or similar instrument at wavelength 435λ.

The colour is stable for 1 hour after the addition of the carbonate solution. The amount of 4-benzamidosalicylic acid present is calculated from the colour produced by a standard solution containing 10 μg./ml. sodium 4-benzamido-salicylate, which is treated as plasma in the above method. Two blank determinations are carried out. One is a reagent blank, in which plasma is replaced by water, the optical density of which is subtracted from the optical density of the standard solution. The second blank is a plasma blank, taken either before dosage or from a nontreated control, the optical density of which is subtracted from the optical density of the unknown sample.

Concentration of 4-benzamido salicylic acid (μg./ml. plasma) =

$$\frac{\text{Optical density of unknown—Optical density of plasma blank}}{\text{Optical density of standard—Optical density of reagent blank}} \times \frac{257}{279} \times 10$$

Recovery of known amounts of the soluble sodium salt of 4-benzamido-salicylic acid added to plasma are shown in Table I and will be seen to be reasonably satisfactory. Recoveries from urine are also good provided the sample is diluted to contain not more than 40 μg./ml.

TABLE I

RECOVERY OF ADDED 4-BENZAMIDOSALICYLIC ACID FROM PLASMA AND URINE

| Plasma | | Urine | |
|---|---|---|---|
| Content | Found | Content | Found |
| 7 mcg./ml. | 9·6 μg./ml. | 5 μg./ml. | 5·45 μg./ml. |
| 17 " | 20·2 " | 10 " | 9·6 " |
| 21 " | 23·2 " | 20 " | 15·0 " |
| 35 " | 36·2 " | 40 " | 41·8 " |
| 40 " | 38·8 " | 80 " | 63·0 " |
| | | 120 " | 101·0 " |

(*c*) Tissue levels of *p*-aminosalicylic acid and 4-benzamidosalicylic acid were estimated by homogenising the weighed whole tissues in 100 ml. of Krebs-Phosphate-Ringer solution[8] in a high-speed homogeniser and carrying out the estimations in an aliquot of the homogenate. For the investigation of the action of tissue homogenates on the decomposition of 4-benzamidosalicylic acid, the homogenates were prepared as already described and the soluble sodium salt of 4-benzamidosalicylic acid was used as substrate. The mixtures of homogenates and substrate were incubated at 37° C. Controls were employed to exclude any effect by the Ringer solution or any non-enzymatic effect of the tissue.

*Prothrombin times* were estimated by Quick's method[9].

*Clotting times* were estimated by Lee and White's method[10].

*Chemotherapeutic activity* was measured by the mouse corneal technique[11] using the H.37 Rv strain of organism.

## RESULTS

1. *Toxicity.* Single doses of calcium 4-benzamidosalicylate up to 10 g/kg., administered orally to mice produced no mortality. This low

toxicity may be partly due to the insolubility of the drug and the resulting deficient absorption, particularly with high doses. A group of 10 mice was given 5 g./kg. per day orally for 1 month without any effect on their rate of growth. Experiments on chronic toxicity were carried out in rats, using daily doses of 0·5, 1·25 and 5·0 g./kg. over a period of 18 weeks. No animals died and on the lowest 2 doses the rate of growth of the animals was identical with that of the control group. On the highest dose, however, there was an early check in the rate of growth which lasted for about 3 weeks and the loss was not recovered during the period of the experiment. (Figure 1).

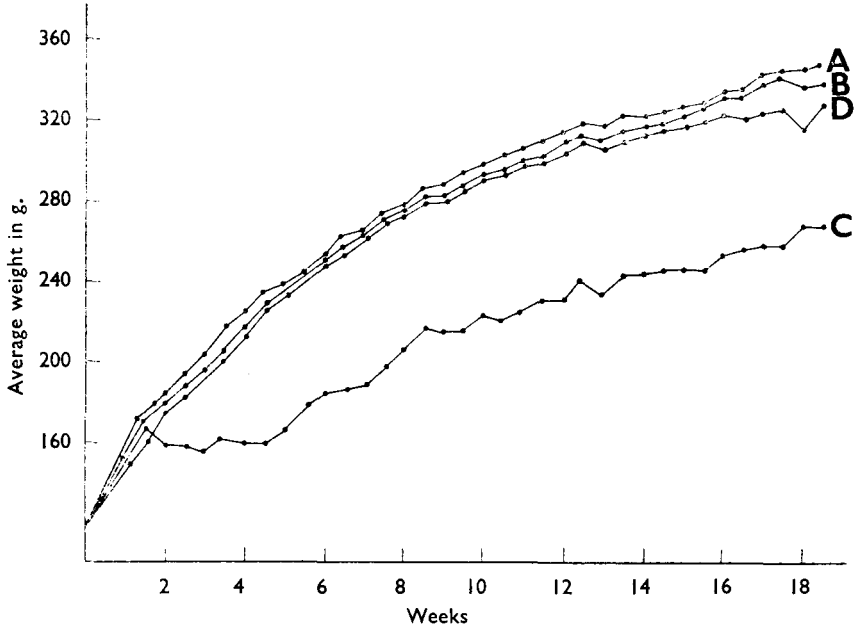



FIG. 1. The effect of calcium 4-benzamidosalicylate on the growth of rats (10 animals/group).

A. 0·5 g./kg./day. C. 5·0 g./kg./day.
B. 1·25 g./kg./day. D. Control group.

Since we had previously found that *p*-aminosalicylic acid produces thyroid hyperplasia[12] in rats, we examined microscopically the thyroids of the rats used on the highest dose in the above chronic toxicity experiments. The glands showed loss of colloid material and proliferation of the cubical epithelium. Little change was observed in the thyroids from rats receiving the lower doses.

The mean weight for the thyroid glands (both lobes) from 6 rats treated with daily doses of 1·25 g./kg. for 9 weeks was 6·66 ± 0·62 mg./100 g. The mean weight of the glands from the control animals was 7·03 ± 0·46 mg./100 g. The difference in weight between the two groups of thyroids was 0·37 mg./100 g. and this was not statistically significant. These results showed that calcium 4-benzamidosalicylate resembled *p*-aminosalicylic

acid in its effect on the structure of the rat thyroid and in not producing any marked hypertrophy of the gland. This action may be due to the effect of the substance itself or to its decomposition to *p*-aminosalicylic acid. Histological examination of heart, lung, liver, kidney, spleen, pituitary and adrenal glands showed no effect. There was no effect on the red and white blood cell counts, clotting and prothrombin times.

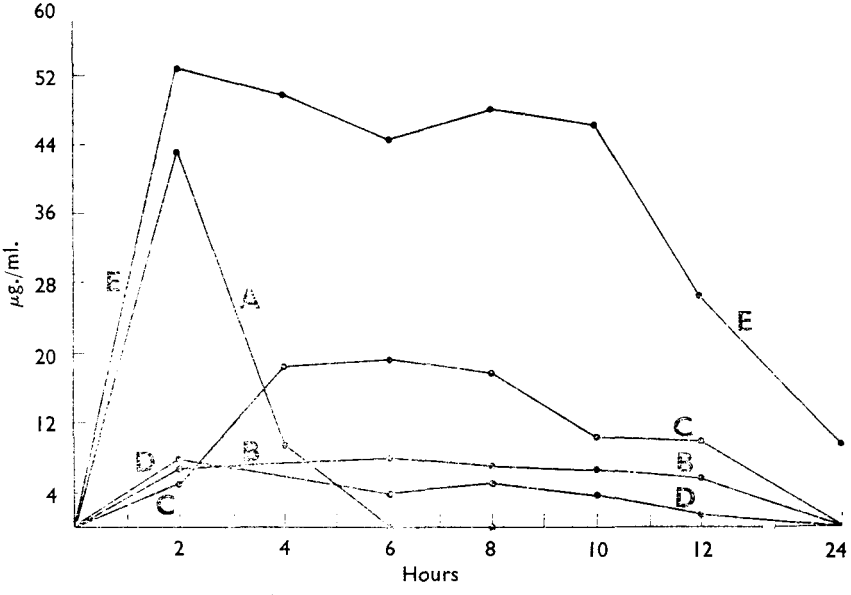



FIG. 2. Plasma levels of *p*-aminosalicylic acid and 4-benzamidosalicylic acid in rabbits after oral dosage.

A. *p*-Aminosalicylic acid level after 0·213 g. kg. of sodium *p*-aminosalicylate.
B. *p*-Aminosalicylic acid level after 0·39 g. kg. of calcium 4-benzamidosalicylate.
C. 4-Benzamidosalicylic acid level after 0·39 g./kg. of calcium 4-benzamidosalicylate.
D. *p*-Aminosalicylic acid level after 1 g./kg. of calcium 4-benzamidosalicylate.
E. 4-Benzamidosalicylic acid level after 1 g./kg. of calcium 4-benzamidosalicylate.

II. *Plasma Levels.* Figure 2 gives the mean plasma levels of *p*-aminosalicylic acid and 4-benzamidosalicylic acid obtained in 3 groups of 3 rabbits each. The first group received the normal clinical dose of sodium *p*-aminosalicylate orally (i.e., 15 g./70 kg. = 0·213 g./kg.); the second group received the stoichiometric equivalent of calcium 4-benzamidosalicylate (i.e. 0·392 g./kg.); and the third group received 1·0 g./kg. of calcium 4-benzamidosalicylate. As will be seen, the first group rapidly attained a fairly high peak plasma level of *p*-aminosalicylic acid, but this fell to zero within 6 hours. In contrast the second group showed that much lower but much more prolonged plasma level of *p*-aminosalicylic acid, accompanied by a higher and prolonged plasma level of 4-benzamidosalicylic acid. The third group showed higher values for 4-benzamidosalicylic acid with little difference in the *p*-aminosalicylic acid levels.

The result of this experiment showed that calcium 4-benzamidosalicylate

was absorbed from the gastro-intestinal tract, presumably in the form of free 4-benzamidosalicylic acid or its soluble sodium salt, and then broken down in the body to yield *p*-aminosalicylic acid at a steady rate over a period of about 24 hours.

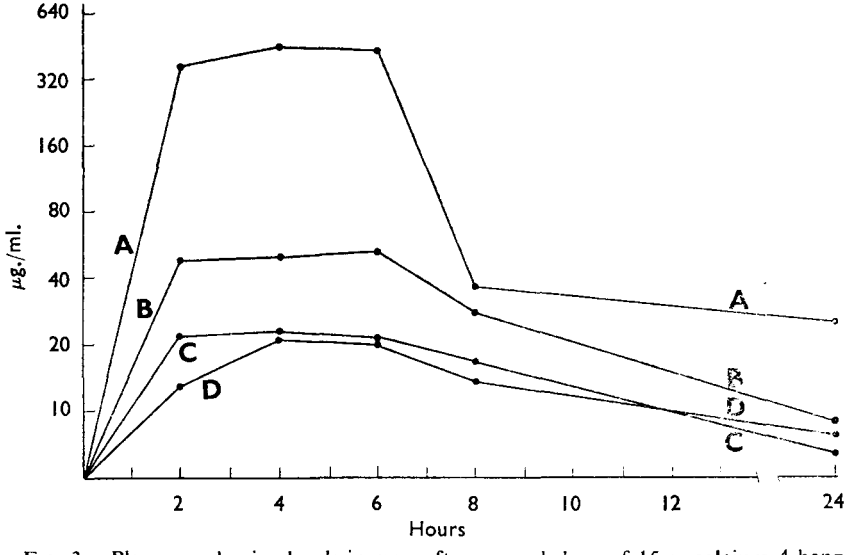



FIG. 3. Plasma and urine levels in man after an oral dose of 15 g. calcium 4-benzamidosalicylate in milk.

A. *p*-Aminosalicylic acid level in urine.
B. 4-Benzamidosalicylic acid level in urine.
C. *p*-Aminosalicylic acid level in plasma.
D. 4-Benzamidosalicylic acid level in plasma.

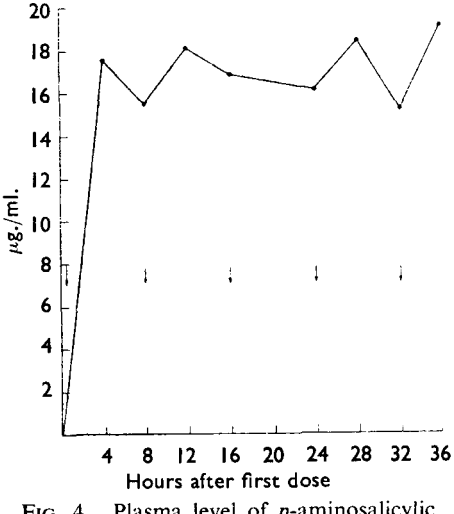



FIG. 4. Plasma level of *p*-aminosalicylic acid in man after divided doses of calcium 4-benzamidosalicylate. Arrows denote doses of 5 g.

In Figures 3 and 4 are shown plasma levels obtained in human volunteers. Figure 3 gives the results obtained with a single dose of 15 g. of calcium 4-benzamidosalicylate and Figure 4 shows the effect of repeated doses of 5 g. of the compound at intervals of 8 hours. Both figures show approximately the same maximum level of *p*-aminosalicylic acid of about 20 μg./ml. but the effect of the repeated doses is clearly to maintain the plasma content of *p*-aminosalicylic acid at an approximately steady level for the period of administration of the compound. Figure 3 also shows that a very high

concentration of *p*-aminosalicylic acid and free 4-benzamidosalicylic acid is attained in the urine. These urinary concentrations are about 25 times and twice the blood plasma levels of the respective constituents.

Plasma levels were also investigated in three groups of mice which had received 2 per cent. and 1 per cent. of calcium 4-benzamidosalicylate in their diet for a period of 1 week. Comparison was made with a similar group of mice receiving 1 per cent. of sodium *p*-aminosalicylate for a similar period. It was felt that the information from this experiment would be of value in itself and would also be of use in assessing the result of chemotherapeutic tests on the compound. The results are given in Table II obtained 2 hours, 4 hours and 6 hours after giving the medicated diet. This Table shows that, even when given at twice the dose, calcium 4-benzamidosalicylate produces somewhat smaller plasma levels than does *p*-aminosalicylic acid, although this difference appears to become less a few hours after administration.

TABLE II

*p*-AMINOSALICYLIC ACID LEVELS IN PLASMA OF MICE AFTER RECEIVING CALCIUM 4-BENZAMIDOSALICYLATE IN THE DIET FOR ONE WEEK

| Group | *p*-Aminosalicylic acid μg./ml. | | |
|---|---|---|---|
| | 2 hours | 4 hours | 6 hours |
| 2 per cent. .. .. .. .. .. | 30 | 4·1 | 14·1 |
| 1 per cent. .. .. .. .. .. | 0 | 0 | 4·7 |
| 1 per cent. sodium *p*-aminosalicylate .. | 55·5 | 12·5 | 17·4 |

III. *Urinary excretion.* Preliminary experiments on rats and rabbits showed that after a single dose of 2 g. of calcium 4-benzamidosalicylate a marked and prolonged urinary excretion occurred of both *p*-aminosalicylic acid and 4-benzamidosalicylic acid. The result of a typical experiment on a rabbit is given in Table III.

TABLE III

4-BENZAMIDOSALICYLIC ACID AND *p*-AMINOSALICYLIC ACID LEVELS IN URINE OF RABBITS AFTER A DOSE OF 2 g. OF CALCIUM 4-BENZAMIDOSALICYLATE

| | Time | *p*-amino-salicylic acid μg./ml. | 4-benzamido-salicylic acid μg./ml. |
|---|---|---|---|
| Rabbit 1 .. .. | 0 to 3 hours | 276 | 12·8 |
| " " .. .. | 3 to 24 " | 394 | 24·4 |
| Rabbit 2 .. .. | 0 to 24 " | 254 | 18·1 |

Similar results were obtained in human volunteers, and are illustrated in Figure 3. From this it appears that the urinary excretion of *p*-aminosalicylic acid and 4-benzamidosalicylic acid following one dose of calcium 4-benzamidosalicylate is not fully complete in 24 hours. This is in marked contrast to the very rapid excretion which occurs when sodium *p*-aminosalicylate is given orally. The unusually high concentration of *p*-aminosalicylic acid found in the urine was of particular interest in relation to the possible clinical use of calcium 4-benzamidosalicylate in genito-urinary tuberculosis. This is discussed at greater length below.

This same finding of high urinary levels of *p*-aminosalicylic acid also suggested that the kidney might play an important part in the breakdown of 4-benzamidosalicylic acid and this possibility was investigated as part of a general study of the action of tissue homogenates on the compound.

IV. *Tissue Levels.* A group of 3 rats was treated with daily oral doses of 1·25 g./kg. of calcium 4-benzamidosalicylate for a period of 1 week and the animals were then killed 16 hours after the last dose. The levels of *p*-aminosalicylic acid and 4-benzamidosalicylic acid in the liver, lung, spleen and kidney were found to be very variable between 0 and 150 μg./g., with a tendency for the *p*-aminosalicylic acid content of the lung to be lower than that of the other tissues.

V. *Tissue Homogenates.* The effect of homogenates of various tissues on sodium 4-benzamidosalicylate after incubation for 24 hours is shown in Table IV. It will be seen that all the tissues produced hydrolysis of the

TABLE IV

DECOMPOSITION OF SODIUM 4-BENZAMIDOSALICYLATE BY TISSUES

| Tissue | *p*-Aminosalicylic acid (μg./ml. of homogenate) | | | | |
|---|---|---|---|---|---|
| | Normal tissue and 100 μg./ml. of sodium 4-benzamido-salicylate | Boiled tissue and 100 μg./ml. of sodium 4-benzamido-salicylate | Normal tissue and 50 μg./ml. of sodium *p*-amino-salicylate | Krebs Ringer and 100 μg./ml. of sodium 4-benzamido-salicylate | Krebs Ringer and 50 μg./ml. of sodium *p*-amino-salicylate |
| Liver .. .. .. | 31·0 | 0 | 30·7 | 0 | 30·4 |
| Lung .. .. .. | 27·0 | 0 | 34·8 | 0 | 36·3 |
| Kidney .. .. | 57·5 | 0 | 37·0 | — | — |
| Blood .. .. | 15·2 | — | 23·0 | — | — |

compound, but the kidney appeared to be the most active in this respect. This result lends support to the suggestion made above as to the origin of the urinary *p*-aminosalicylic acid. The lack of effect by boiled tissue suggests that the decomposition is brought about enzymatically, but whether the enzyme responsible for the hydrolysis is some form of peptidase or an enzyme of less specific action is not at present known. The experiments of incubating tissue homogenates with *p*-aminosalicylic acid showed that some loss of *p*-aminosalicylic acid occurred, possibly due to decarboxylation, during 24 hours' incubation. Theoretically, the figures in the first column of the Table should be corrected for this loss.

VI. *Chemotherapeutic effect.* The result of a typical chemotherapeutic test is given in Table V. It will be seen that calcium 4-benzamidosalicylate is less active than *p*-aminosalicylic acid, weight for weight, and from an approximate calculation, it appears that it may have 50 per cent. of the activity of *p*-aminosalicylic acid. This agrees quite closely with the percentage of *p*-aminosalicylic acid theoretically obtainable from calcium 4-benzamidosalicylate and suggests that the tuberculostatic effect is due to the liberation of *p*-aminosalicylic acid and not due to an activity *per se* of the compound. This suggestion is supported by the plasma levels of *p*-aminosalicylic acid and 4-benzamidosalicylic acid in rabbits and mice shown in Figure 2 and Table II. If the molecule of 4-benzamidosalicylic

acid is itself tuberculostatic, the high plasma levels of this acid attained in these experiments would have led to an expectation of a high tuberculostatic activity, when tested *in vivo* in other groups of mice. The low plasma level of *p*-aminosalicylic acid obtained simultaneously with the higher levels of 4-benzamidosalicylic acid further reinforces the argument

TABLE V

CHEMOTHERAPEUTIC EFFECT OF CALCIUM 4-BENZAMIDOSALICYLATE COMPARED WITH *p*-AMINOSALICYLIC ACID IN MICE

| Group | Number of animals | Compound | Percentage level in diet | Percentage without lesions |
|---|---|---|---|---|
| 1 | 10 | Calcium 4-benzamidosalicylate | 0·5 | 50 |
| 2 | 10 | " " | 1·0 | 60 |
| 3 | 8 | " " | 2·0 | 87·5 |
| 4 | 6 | *p*-Aminosalicylic acid | 0·25 | 50 |
| 5 | 12 | " " | 0·5 | 66·7 |
| 6 | 9 | " " | 1·0 | 88·9 |
| 7 | 10 | Untreated | — | 10·0 |

that the lower activity of calcium 4-benzamidosalicylate, as compared with *p*-aminosalicylic acid, is due solely to the liberation of small concentrations of *p*-aminosalicylic acid *in situ.*

## DISCUSSION

The present investigation has shown that the calcium salt of 4-benzamidosalicylic acid is decomposed, at least partially, in the animal body to form *p*-aminosalicylic acid and benzoic acid. The mechanisms of this reaction is obscure at the moment, but it is apparently enzymatic in character and brought about by most of the tissues of the body, predominantly by the kidney. It is hoped to investigate this reaction more closely by the use of specific enzymes.

The liberation of *p*-aminosalicylic acid occurs slowly and steadily and it is possible to maintain comparatively steady, rather low, plasma levels of *p*-aminosalicylic acid over a protracted period, particularly when divided doses are administered. It is perhaps worth recording that a recurrent maximum plasma level of *p*-aminosalicylic acid of 20 $\mu$g./ml. was reached under various conditions of dosage. This effect may be merely fortuituous or may indicate the existence of some kind of chemical equilibrium. Further work may throw more light on this point.

The fact that high urine levels of *p*-aminosalicylic acid occur after treatment with calcium 4-benzamidosalicylate, combined with the probability that the tuberculostatic action of the compound is due solely to *p*-aminosalicylic acid liberated, suggested that calcium 4-benzamidosalicylate might be of value in genito-urinary tuberculosis. Clinical trials of the substance in this condition have been inaugurated through the kindness of Mr. J. G. Gow and are still in progress. Two recent reports[13,14], however, suggest that calcium 4-benzamidosalicylate is of considerable value as a substitute for *p*-aminosalicylic acid in the treatment of genito-urinary tuberculosis. The advantages reported are lack of toxicity and taste and prolonged *p*-aminosalicylic acid blood levels.

It is also reported that it appears to delay the development of streptomycin resistance equally as well as *p*-aminosalicylic acid. With these facts

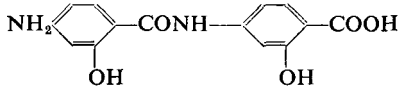
in mind, the clinical trials of calcium 4-benzamidosalicylate in pulmonary tuberculosis were commenced and the results will be reported in due course. Interim observations suggest that the drug is well received by the patient because of the absence of nausea following its use.

During our work on this compound, we considered the possibility that the *in situ* liberation of 0·36 g. of benzoic acid per g. of drug might have toxic effects or alternatively the detoxication of this amount of benzoic acid by glycine conjugation might disturb the amino-acid balance. However, it was clear from the animal experiments and later, from the clinical trials that the overall toxicity of calcium 4-benzamidosalicylate is very low. Experiments to investigate more fully the fate of the benzoic acid part of the molecule are planned.

The fate of the calcium ion has, also, not yet been fully investigated. Preliminary work has shown that urinary calcium is not greatly increased after administration to volunteers and presumably most of the calcium is excreted *via* the fæces.

It seems that calcium 4-benzamidosalicylate resembles *p*-aminosalicylic acid in causing a loss of colloid material from the rat thyroid gland and the possibility must, therefore, be considered of occasional cases of myxœdema occurring during clinical use. These cases, however, have been mild and relatively rare during treatment with *p*-aminosalicylic acid, and it seems likely that same will be the case with calcium 4-benzamidosalicylate.

The facts that the molecule appears to be, itself, tuberculostatically inactive and that only half of the molecule is converted into *p*-aminosalicylic acid suggested that a modification of the structure of the compound might be advantageous. Consequently, consideration was given to the use of 4-(4-amino-2-hydroxy-benzamido)-salicylic acid (II).




II

This will be seen to be a compound of two molecules of *p*-aminosalicylic acid, instead of one molecule of *p*-aminosalicylic acid and one molecule of benzoic acid as occurs in calcium 4-benzamidosalicylate, and has already been shown to have an *in vitro* activity equal to that of *p*-aminosalicylic acid[15]. If decomposition occurs in the body on the same lines as with calcium 4-benzamidosalicylic acid, this substance should yield approximately twice as much *p*-aminosalicylic acid, and, presumably, should therefore, have a correspondingly increased therapeutic activity. Examination of the calcium salt of this acid showed that it resembled the calcium salt of 4-benzamidosalicylic acid, in insolubility and tastelessness and an investigation of this substance (H.P 354) is now in progress.

## Summary

1. The calcium salt of 4-benzamidosalicylic acid (H.P. 170) is a very insoluble, almost tasteless compound, which decomposes in the body to

liberate *p*-aminosalicylic acid. Plasma levels of *p*-aminosalicylic acid are low but prolonged, and urine levels are much higher and equally prolonged.

2. It has a very low toxicity to mice and rats. It produces loss of colloid material from the rat thyroid and thus resembles *p*-aminosalicylic acid.

3. It has a tuberculostatic activity of approximately 50 per cent. of that of *p*-aminosalicylic acid. This activity is probably entirely due to liberated *p*-aminosalicylic acid.

4. It is decomposed to *p*-aminosalicylic acid by homogenates of several tissues. The action is probably enzymatic.

5. It has been found to be of value as a substitute for *p*-aminosalicylic acid in the treatment of genito-urinary tuberculosis. It appears to delay the emergence of streptomycin resistance.

6. A method is described for the determination of 4-benzamidosalicylic acid in biological fluids.

We are indebted to Mr. D. E. Seymour and Mr. J. G. Gow for much helpful discussion during the course of this work, and our thanks are also due to Mr. B. W. Mitchell for valuable help and advice with the analytical methods and Dr. M. Seiler for the chemotherapeutic experiments. We would also like to record our indebtedness to Mr. P. Wagner and (the late) Mr. J. M. Sells for much careful laboratory work.

Our thanks are also due to the Directors of Smith and Nephew Research, Ltd. for permission to publish this work.

## References

1. Medical Research Council report, *Brit. med. J.*, 1949, **2**, 1521.
2. Medical Research Council report, *ibid.*, 1952, **1**, 1157.
3. Rosdahl, *Svensk kem. Tidskr.*, 1948, **60**, 12.
4. Drain, Martin, Mitchell, Seymour and Spring, *J. chem. Soc.*, 1949, 1498.
5. Goodacre, Mitchell and Seymour, *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 301.
6. Newhouse and Klyne, *Biochem. J.*, 1949, **44**, VII.
7. Pesez, *Bull. Soc. Chim. Fr.*, 1949, 918.
8. Umbreit, Burris and Stauffer, *Manometric Techniques and Tissue Metabolism*, Burgess Publishing Co., Minneapolis, 1949, p. 119.
9. Quick, Brown and Bancroft, *Amer. J. med. Sci.*, 1935, **190**, 501.
10. Lee and White, *ibid.*, 1913, **145**, 495.
11. Rees and Robson, *Brit. J. Pharmacol.*, 1950, **5**, 77.
12. Bavin and James, *J. Pharm. Pharmacol.*, 1952, **4**, 856.
13. Gow, *Brit. med. J.*, 1953, **1**, 95.
14. Ross, Gow and Hill, *ibid.*, 1953, **1**, 901.
15. Bavin, Drain, Seiler and Seymour, *J. Pharm. Pharmacol.*, 1952, **4**, 844.

## DISCUSSION

The paper was presented by MR. E. M. BAVIN.

DR. G. E. FOSTER (Dartford) said it was not clear what constituted the mechanism of the reaction in the colorimetric assay.

MR. T. H. ELLIOTT (Singapore) asked why the authors had not considered the use of *p*-phenylene-ethylenediamine which could be used in acid solution, was relatively stable, and was likely to give more satisfactory recoveries.

MR. E. M. BAVIN, in reply, pointed out that the mechanism of the reaction was explained in Pesez's paper (reference 7). If the hydrolysis broke down the calcium benzamidosalicylate to *p*-aminosalicylic acid, the latter was merely a coupling agent. There was nothing peculiar about *p*-aminosalicylic acid as a final coupling agent, and one could use any other phenol. *m*-Aminophenol could have been estimated by the use of *p*-phenylene-ethylenediamine or by other methods, but the method used was chosen because of previous experience of it. He agreed that the recoveries shown in Table I were not good, but it had been found that the method used gave quite satisfactory results from the clinical point of view.