FURTHER ASPECTS OF THE PHARMACOLOGY OF *PARA*-AMINOSALICYLIC ACID

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Received July 16, 1252.

THE place of *p*-aminosalicylic acid as a therapeutic agent against tuberculosis is now well established and recent reports[1] have amply confirmed the earlier findings that it has a marked effect in delaying the emergence of streptomycin resistance. In contrast to the large number of papers which have appeared upon the tuberculostatic effect of this drug, there appear to have been very few dealing with the more general pharmacology of the substance and this present series of experiments is an extension of the work described in a previous communication.[2]

It has been generally accepted that aqueous solutions of *p*-aminosalicylic acid should not be used clinically more than about 24 to 48 hours after preparation on account of the rapid darkening in colour which occurs at room temperature. So far as we are aware, however, no pharmacological investigation of these old solutions has been carried out and it appeared to be of interest to determine in what way, if any, these solutions differed in their properties from freshly prepared solutions.

In addition, recent work,[3,4,5,6] suggesting the possibility of a stimulant effect of salicylates on the anterior pituitary gland, particularly a report[7] that *p*-aminosalicylic acid produced a marked eosinopenia in rats, was of considerable interest and it seemed desirable to repeat this work to obtain more data on this important point. A report had also appeared[8] that *p*-aminosalicylic acid had a trypanocidal action in mice and rats and here again it was felt that further evidence would be helpful.

EXPERIMENTAL METHODS

(I) *Investigation of Old Solutions*

(*a*) *Long term feeding experiments.* 20 per cent. w/v aqueous solutions of sodium *p*-aminosalicylate were prepared and allowed to stand at room temperature, exposed to light, for 1 week, 1 month and 6 months respectively. Fresh volumes of these solutions were prepared at weekly intervals during the entire course of the experiment so that the period of ageing was relatively constant throughout the course of the work. These solutions were administered at a dose of 1·0 mg. of sodium *p*-aminosalicylate/g. by stomach tube daily to adult male Wistar rats. The animals were fed on rat cakes (a mixture of Thompsons diet and Parkes diet 41), bread and milk daily, with cooked liver and green food once weekly. Some control groups received fresh 20 per cent. solution and others normal saline solution. The animals were weighed twice a week and growth curves constructed. Blood counts were carried out at intervals of 28 days. During and at the end of the experiments, histological specimens were prepared of the thyroid glands. The tissues were fixed in Susa and

stained with hæmatoxylin and eosin. The thyroid glands were weighed after fixation.

(*b*) *Effect on blood pressure and respiration.* Experiments to determine the effect of solutions on blood pressure and respiration were carried out on rabbits and cats anæsthetised with chloralose and ether. Blood pressure was recorded from the carotid artery and respiration by a tambour from the tracheal cannula. Experiments on the perfused rabbit heart were carried out by the usual Langendorff technique, using the apparatus described by Baker.[9]

(*c*) *Blood levels.* Blood levels in rats were estimated by the method of Newhouse and Klyne.[10]

(*d*) *Therapeutic effect.* The therapeutic activity of fresh and old solutions was determined by the mouse survival test.[2]

(II) *Eosinopenic effect.*

Swiss albino mice, males, weighing from 18 to 24 g. were used. From the start of the experiment each animal was housed separately at a room temperature of 27° C. Blood was obtained by amputating the tail at 0·5 cm. from the distal end. A fairly rapid flow of blood was thus obtained. Eosinophils were enumerated by the method of Speirs and Meyer[11] using Teepol as detergent in place of Alconox. Blood was taken from each mouse prior to subcutaneous and intraperitoneal injection of the drug and normal saline solution. At 2 and 4 hour intervals further samples were taken. Most workers have found considerable experimental variation in eosinophil counts and, to ascertain the accuracy of our own counts, 3 different samples of blood were taken from each of 10 mice and 4 hæmocytometer chambers counted from each sample, that is 12 counts were made on blood from each mouse. The results obtained are shown in Table I.

TABLE I

EOSINOPHIL COUNTS

Mouse	Mean hæmocytometer count and standard error (mean of 12 values)
1	40·6 ± 2·46
2	70·3 ± 7·69
3	48·0 ± 6·17
4	32·2 ± 2·18
5	129·4 ± 10·34
6	33·4 ± 3·21
7	44·4 ± 3·26
8	51·0 ± 6·04
9	50·6 ± 5·66
10	78·3 ± 7·96
	Average 57·8 ± 5·49

The figures in Table I should be multiplied by 6·25 to give eosinophils/cu. mm.

It will be seen that, under these conditions, the standard error is about 10 per cent., but as it was not practicable to carry out 12 counts on each mouse as routine, only one count was made on each blood sample and the test was used as a quantal response. Consideration of the work of Spiers and Meyers (*loc. cit.*) and Mushett[12] suggests that this method is capable of being used as a variable-response type of assay and work is in hand on this problem.

(III) *Trypanocidal activity.*

Swiss albino mice and Wistar albino rats were infected with *Trypanosoma equiperdum* by intraperitoneal injection of suspensions

of the organism in glucose saline solution. These suspensions were obtained by decapitating a mouse heavily infected with *T. equiperdum* and allowing it to bleed into glucose saline. Chemotherapy was commenced either immediately following infection or after the infection had developed for 2 days. The effect of treatment was judged by blood smears and survival time of the animals compared with controls.

RESULTS

(I) *Chronic Toxicity of Old Solutions*

The results of typical experiments are shown in Figures 1 and 2, from which it will be seen that, after a period of 2 to 3 months, all the groups of rats receiving the drug either fresh or aged, were growing less rapidly than the controls. This retardation of growth becomes quite marked after a further period of 1 to 2 months and suggested the effect which is obtained by giving small doses of thiouracil or other anti-thyroid drug to rats. Consequently some of the experimental animals were killed and the thyroid glands examined histologically. There was a marked hyperplasia of the gland shown by almost complete loss of colloid material and by some thickening of the epithelium (Fig. 3).

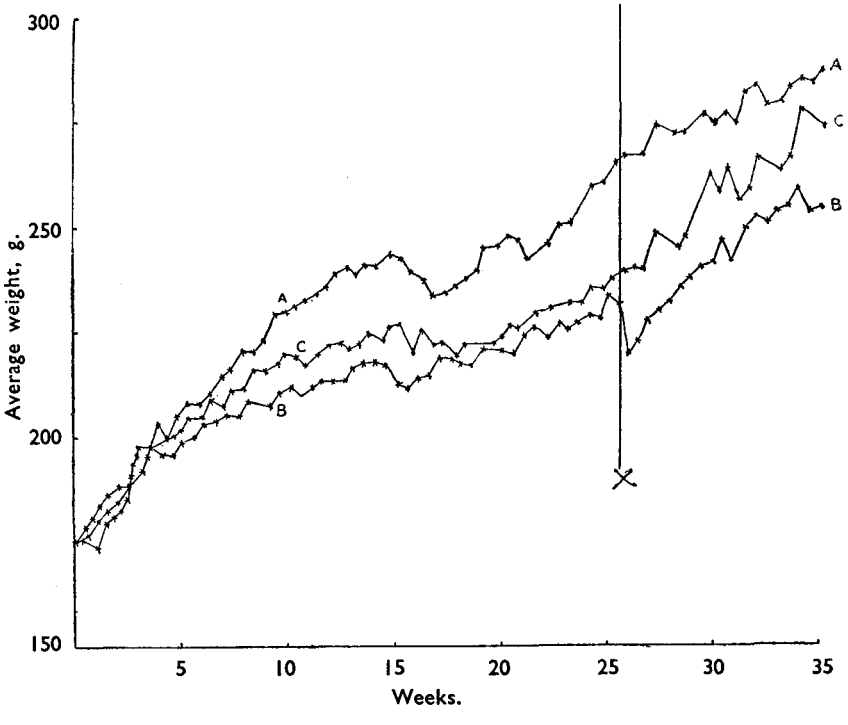

FIG. 1. The effect of sodium *p*-aminosalicylate in a dose of 1 mg./g./day on growth of rats (10 animals per group). A, Control group. B, Fresh solution of sodium *p*-aminosalicylate. C, 1 month old solution of sodium *p*-aminosalicylate. X, Sodium *p*-aminosalicylate solution replaced by normal saline solution.

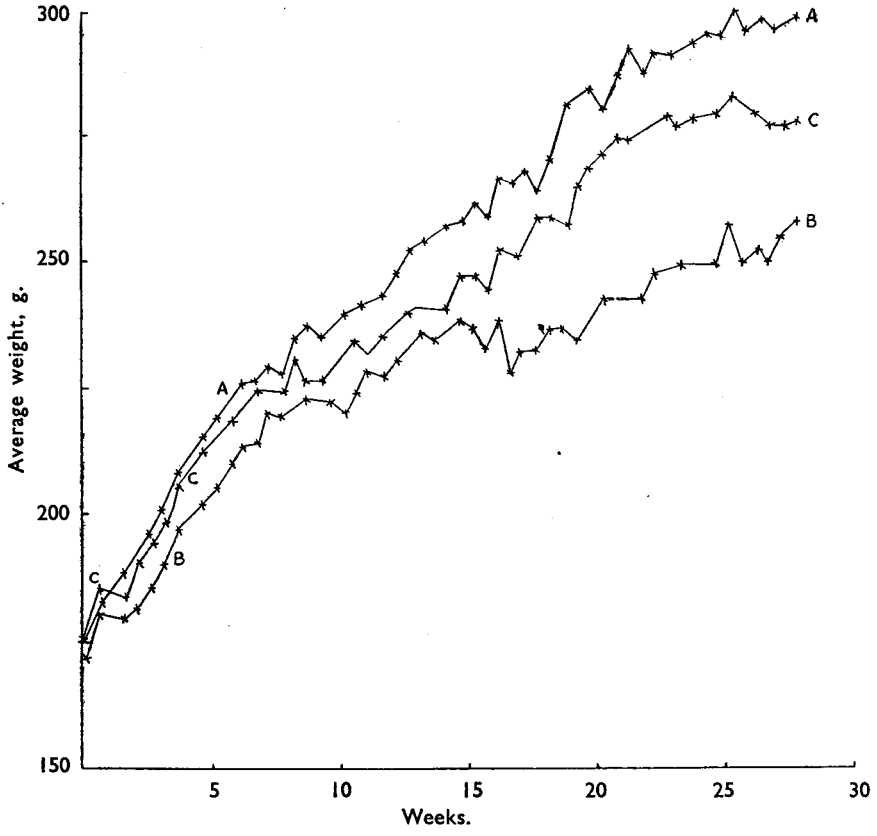

FIG. 2. The effect of sodium *p*-aminosalicylate on growth of rats (10 animals per group). A, Control group. B, Fresh solution, 1 mg./g./day. C, 6-months old solution, 1 mg./g./day.

p-Aminosalicylic acid treatment of the remaining animals was then replaced by treatment with normal saline solution and an immediate rapid increase in rate of growth occurred. This is shown in Figure 1 where the vertical line indicates cessation of treatment. This result suggests therefore that, as in the case of thiouracil, the action on the growth of rats is easily reversible.

Repetition of the above experiments with larger groups of rats confirmed the hyperplastic effect on the thyroid gland. The weight of the thyroid gland was not increased following administration of sodium *p*-aminosalicylate and typical values are shown in Table II.

From all our experiments there seems little evidence that the solutions kept for periods up to 6 months have any greater effect than the freshly prepared solution. This suggests that the thyroid hyperplasia observed is due solely to the sodium *p*-aminosalicylate present. Nevertheless, it was felt desirable to test the action of *m*-aminophenol which is the degradation product of *p*-aminosalicylic acid most likely to be present in these old

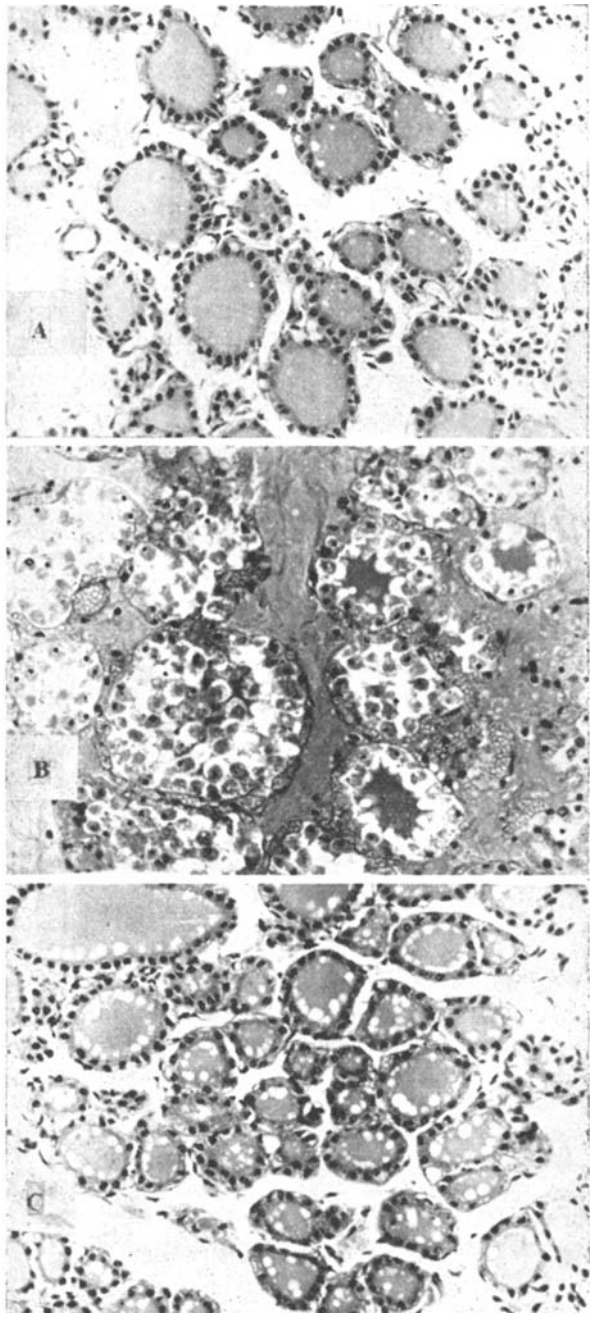

FIG. 3. Thyroid gland of rats (× 300). A, Normal. B, After receiving fresh solution of sodium *p*-aminosalicylate, 1 mg./g./day for 22 weeks. C, After receiving *m*-aminophenol, 0·15 mg./g./day for 20 weeks.

TABLE II

THYROID WEIGHTS

Rat	Dose (oral)	Weight of thyroid mg./100 g. of body weight	Mean thyroid weight mg./100 g. of body weight
42L	1 mg./g. of fresh *p*-amino-salicylic acid solution for 14 weeks	5·73	7·58
42R		6·29	
52L		10·54	
52R		11·92	
4P		5·15	
41L		6·00	
41R		7·30	
41LIR		7·7	
82R	1 mg./g. of 1 month old solution for 14 weeks	8·59	8·67
91LIR		7·53	
92L		10·68	
92R		8·41	
7P		8·6	
72R		9·8	
71LIR		7·0	
12R	Nil	9·50	8·83
22R	Nil	5·29	
32L	Nil	12·67	
32R	Nil	8·57	
IP	Nil	6·83	
IIL	Nil	10·80	
11R	Nil	11·00	
11LIR	Nil	6·00	

solutions. Analytical examination of these solutions showed that they contained the following percentage of *m*-aminophenol:—6 months old solution—0·6 per cent., 3 months-old—0·3 per cent., 1 month old—0·2 per cent., and 1 week old—0·1 per cent. of *m*-aminophenol compared with 0·06 per cent. in a fresh sodium *p*-aminosalicylate solution. For these determinations we are indebted to Mr. B. W. Mitchell, using methods described by Seaman *et al.*[13] and Pesez.[14]

Some preliminary acute toxicity tests showed that the subcutaneous LD50 of *m*-aminophenol to mice was approximately 0·2 mg./g. and a long term feeding experiment in rats was therefore carried out using oral doses of 0·075 and 0·15 mg./g. daily for 20 weeks. At the end of this period, histological examination showed the rats to have completely normal thyroid glands (Fig. 3) and the growth curve on the lower dose showed no departure from the control curve. However, on the higher dose some retardation of growth was apparent. It seems clear therefore, that the action on the thyroid gland is not due to contamination with *m*-aminophenol.

To avoid the uncertainty regarding the composition of supplements to the diet in the foregoing experiments, it was decided to carry out another experiment in which the animals were fed on Thompson cubes only. It was somewhat surprising to find in this experiment no evidence of growth retardation (Fig. 4) but moderate thyroid hyperplasia. This latter effect, however, was definitely less marked than in the previous experiments. One explanation of this result may be the possible presence of thyroxine in the fish-meal constituent of the diet in sufficient amount to inhibit the growth retarding effect of *p*-aminosalicylic acid but insufficient completely to inhibit the hyperplastic effect on the thyroid. A somewhat similar suggestion has been advanced by Lawson and Searle.[15] A second possible

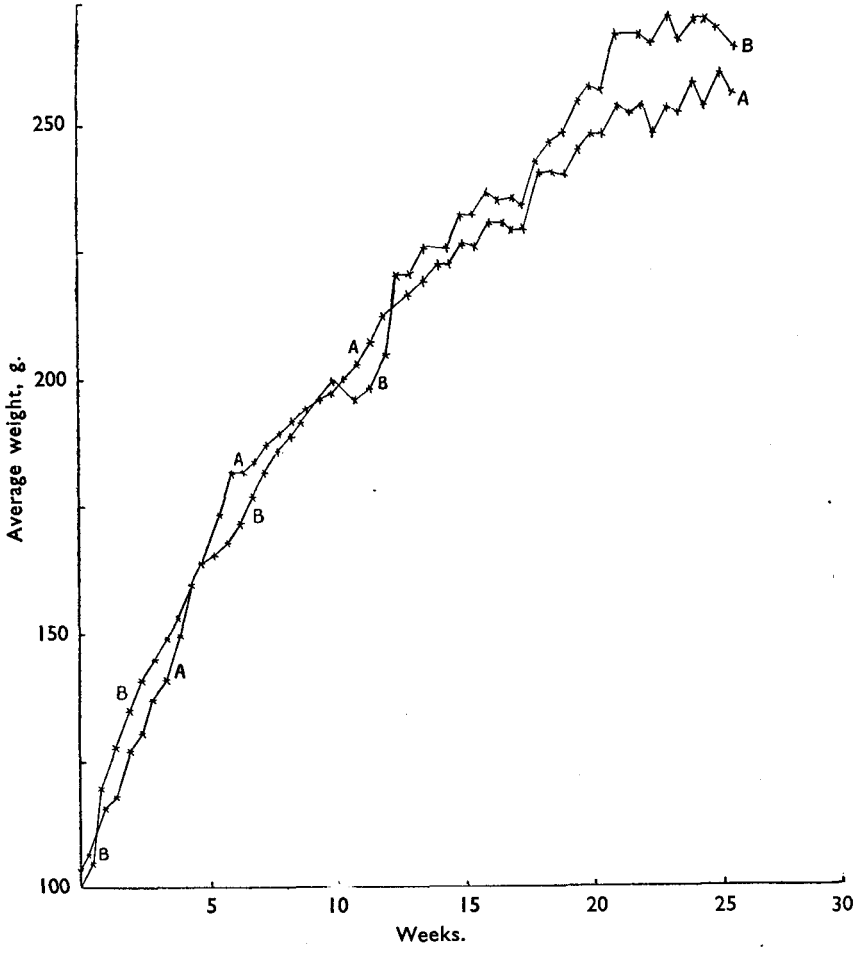

FIG. 4. The effect of fresh sodium *p*-aminosalicylate solution on growth of rats fed on Thompson's cube diet only. A, Controls. B, Fresh solution, 1 mg./g./day.

explanation of the result of this experiment may be that the supplements of cabbage, liver, bread and milk, etc., given in the previous experiments contain an anti-thyroid factor, which increases the effect of *p*-aminosalicylic acid. This suggestion is supported to a certain extent by the work of Chesney *et al.*[16] and Kennedy[17] on the anti-thyroid factor present in Brassicas.

This effect of sodium *p*-aminosalicylate solutions on the thyroid gland of rats is now the subject of further investigations, in which the oxygen consumption and radio-iodine uptake of the animals is being measured. The results will form the substance of a future communication.

(II) *Effect on Blood Pressure and Respiration*

(*a*) *Blood pressure.* A 6-months old 20 per cent. solution of sodium

p-aminosalicylate in a dose of 100 mg./kg. produced a definite increase in blood pressure in both rabbits and cats. This pressor effect was maintained for about 20 to 30 minutes. A similar dose of freshly prepared sodium *p*-aminosalicylate produced only a slight and transient increase in pressure (Fig. 5). This pressor effect was thought to be due to the *m*-aminophenol present in the old solution, but a dose of *m*-aminophenol equal to that present in the old solution did not produce this pressure increase. The injection of a solution containing the same dose of *m*-aminophenol together with fresh sodium *p*-aminosalicylate (100 mg./kg.) also produced no pressor effect (Fig. 4). These results, therefore, appear to exclude *m*-aminophenol as the pressor agent present in old solutions. The mechanism of this pressor effect was further investigated. The adrenolytic agents, benzylimidazoline (priscol) was given in a dose of 7·8 mg./kg. This reversed the normal pressor response to adrenaline but had no effect on the pressor action of sodium *p*-aminosalicylate (Figs. 6 (*a*) and 6 (*b*)). This result suggests that the observed effect of old solutions is not due to a sympathomimetic action.

In the perfusion of the isolated rabbit heart, both freshly prepared and old solutions of sodium *p*-aminosalicylate increased the amplitude of the heart beat without noticeably increasing the rate (Fig. 7). In some experiments, the old solutions appeared to have a more lasting effect than the fresh solutions but this was not an invariable finding. Hence, it appears that the increased pressor effect of old solutions may be partly but not entirely due to their cardiac effect. It is interesting to note that in a few of the heart perfusion experiments some initial decrease in amplitude was noticed with *p*-aminosalicylic acid solutions both fresh and old. This may correlate with the occasional slight initial fall in blood pressure observed in the anæsthetised rabbit and cat.

(*b*) *Respiration.* When 15 mg./kg. of *m*-aminophenol is injected intravenously into the chloralosed cat there is an increase in respiration rate from 31 to 49 inspirations per minute. With the 6 months old sodium *p*-aminosalicylate solution, injection of a dose containing the same amount of *m*-aminophenol produces a decrease in the depth but no change in the rate of respiration. The effect of *p*-aminosalicylic acid on the respiration is not marked and appears not to be due to the presence of *m*-aminophenol.

(III) *Effect of Sodium* p-*Aminosalicylate on Circulating Eosinophils in Mice*

Following subcutaneous injection of fresh sodium *p*-aminosalicylate in a dose of 1·0 mg./g., no decrease in the eosinophil level was observed at two and four hours after administration. Van Cauwenberg (*loc. cit.*) reported that in an intraperitoneal dose of 0·05 mg./g., *p*-aminosalicylic acid produced a significant eosinopenia in rats. We repeated our experiments in mice using this dose and route of injection. In this instance, the results showed an increase in eosinophils at 2 hours and a decrease at 4 hours; this latter effect was not significant. Administration of adrenocorticotrophic hormone in a dose of 5 μg./g. produced a very marked eosinopenia (Table III).

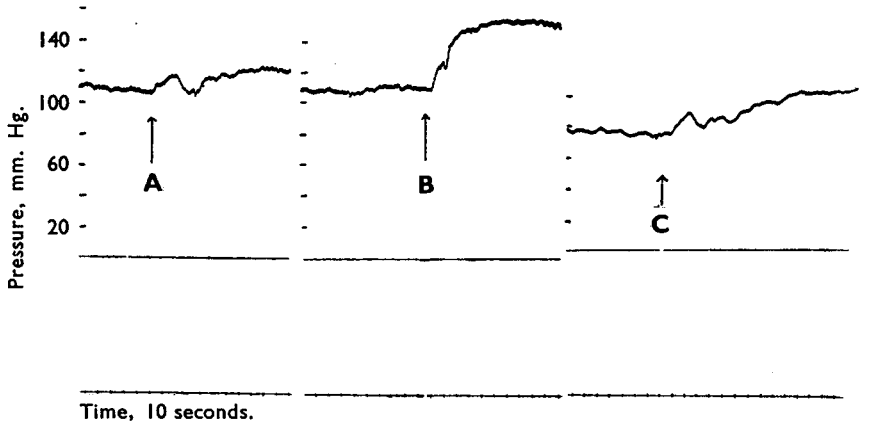

FIG. 5. Effect of fresh and 6 months old sodium *p*-aminosalicylate solution and *m*-aminophenol on the carotid blood pressure of the anæsthetised rabbit. A, Fresh sodium *p*-aminosalicylate solution, 200 mg./kg. (2·5 ml.). B, 6 months old sodium *p*-aminosalicylate solution, 100 mg./kg. (1·25 ml.). C, Equivalent of 6 months old sodium *p*-aminosalicylate solution (15 mg. of *m*-aminophenol and fresh sodium *p*-aminosalicylate solution).

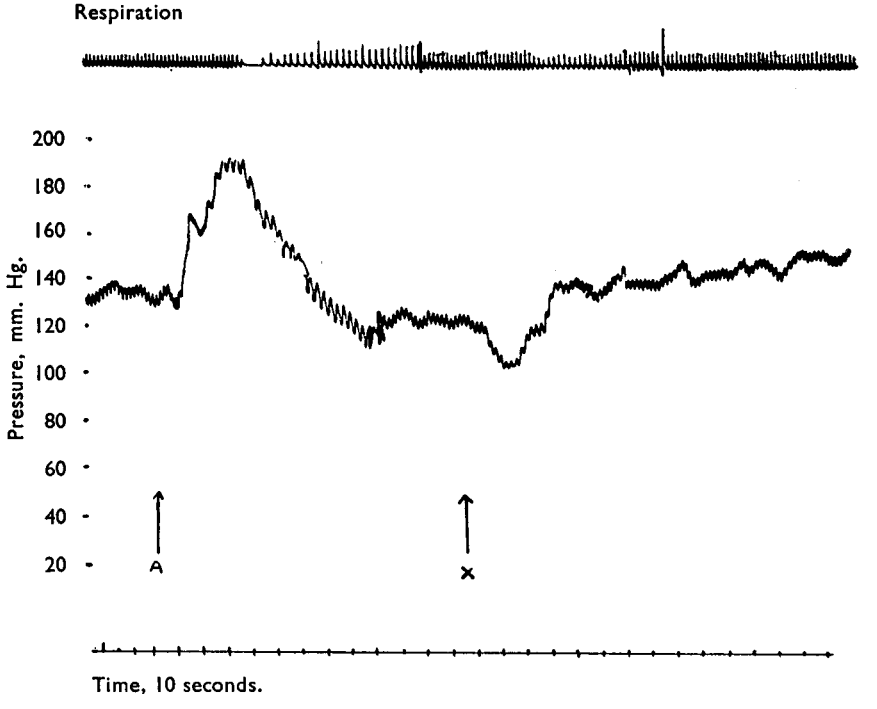

FIG. 6a. The effect of priscol on the pressor activity of a 6 months old solution of sodium *p*-aminosalicylate (cat under chloralose and ether). A, 10 μg. of adrenaline. X, 100 mg./kg. of 6 months old solution of sodium *p*-aminosalicylate. (See Fig. 6b.)

Under the conditions of our experiments, therefore, we were not able to observe any eosinopenic effect of sodium *p*-aminosalicylate in normal mice, suggesting that it has no adrenocorticotrophic hormone-like action. This finding agrees with a recent report by Cronheim *et al.*[18] that *p*-aminosalicylic and *p*-hydroxysalicylic acids were the only compounds out of a number of salicylic acid derivatives which failed to produce a decrease in the adrenal ascorbic acid level in normal rats.

TABLE III

EFFECT OF SODIUM *p*-AMINOSALICYLATE ON EOSINOPHIL COUNT

Number of mice	Compound administered	Dose	Hours after administration	Eosinophils per cu. mm.			
				Initial	Final	Percentage difference	*t*
10	Sodium *p*-aminosalicylate	1·0 mg./g. subcutaneously	2	690	641	− 6·1	1·1
10	,, ,,	1·0 mg./g. subcutaneously	4	468	882	+17·5	—
10	,, ,,	0·05 mg./g. intraperitoneally	2	449	561	+27·8	—
10	,, ,,	0·05 mg./g. intraperitoneally	4	638	569	−10·8	1·9
10	Adrenocorticotrophic hormone	5 μg./g. subcutaneously	2	241	71·3	−70·4	3·46

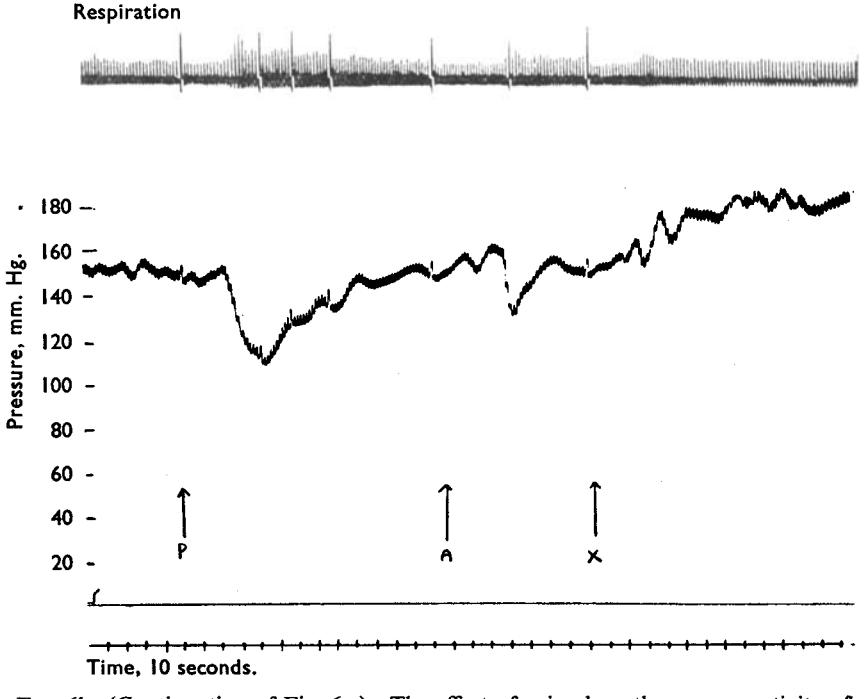

FIG. 6b. (Continuation of Fig. 6a.) The effect of priscol on the pressor activity of a 6 months old solution of sodium *p*-aminosalicylate (cat under chloralose and ether). P, priscol (5 mg./kg.). A, 10 μg. of adrenaline. X, 100 mg./kg. of 6 months old solution of sodium *p*-aminosalicylate.

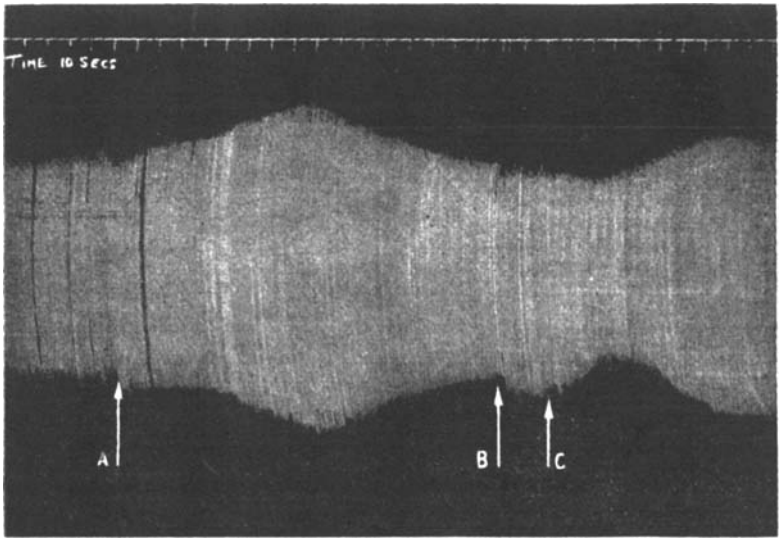

FIG. 7. Effect of fresh and 6 months old sodium *p*-aminosalicylate solutions on the perfused rabbit heart. A, 0·25 ml. of 20 per cent. solution of sodium *p*-aminosalicylate, 6 months old. B, Stop. C, 0·25 ml. of 20 per cent. solution of sodium *p*-aminosalicylate, freshly made.

(IV) *Trypanocidal Activity*

Subcutaneous and intraperitoneal injection of maximal doses of the sodium salt into mice and rats infected with *T. equiperdum* afforded no evidence for any trypanocidal activity. The survival times of treated and control groups were identical. An experiment in which drug was given after the development of infection was equally ineffective. A positive control group of mice treated with a subcutaneous dose of 0·04 mg./g. of sulpharsphenamine showed a very significantly increased survival time.

The possibility existed that the trypanocidal effect reported by Pick (*loc. cit.*) was due to impurities in the drug used. We therefore investigated the trypanocidal action of possible impurities, and, to this end,

TABLE IV

TRYPANOCIDAL ACTIVITY (*Trypanosoma equiperdum*)

Compound administered	Number of animals used	Daily dose mg./g.	Route	Survival time days
Sodium *p*-aminosalicylate	10 mice	1·0	Intraperitoneal	4·0
"	8 rats	1·0	Subcutaneous	4·75
m-Aminophenol	5 mice	0·03	Intraperitoneal	4·6
"	5 mice	0·05	Intraperitoneal	5·4
o-Aminophenol	5 mice	0·03	Intraperitoneal	5·3
"	5 mice	0·05	Intraperitoneal	5·2
5-Aminosalicylic acid	10 mice	0·5	Intraperitoneal	5·4
Sulpharsphenamine	10 mice	0·04	Subcutaneous	>10·0
Nil	10 mice	—	—	4·0
Nil	4 rats	—	—	4·0

maximal doses of *m*-aminophenol, *o*-aminophenol, and 5-aminosalicylic acid[19] were each used. No effect was obtained with any of these compounds and our results are summarised in Table IV.

(V) *Toxicity, Blood Levels and Therapeutic Activity*

(*a*) The acute toxicity of 1 month- and 6 months-old sodium *p*-aminosalicylate solutions was compared with that of fresh solution by intravenous injection into albino mice. Results were observed over a period of 3 days. The results are given in Table V. A graphical determination of the approximate LD50 gave the following results: fresh solution, 3·8 mg./g.; 1 month-old solution, 2·6 mg./g.; 6 months-old solution, 2·3 mg./g. These results suggest that a fairly rapid increase in toxicity takes place during the first months of keeping, followed by little or no increase in toxicity during the succeeding 5 months.

TABLE V

ACUTE TOXICITY

Intravenous dose mg./g.	Number of mice dead		
	Fresh solution	1 month old solution	6 months old solution
2·0			3/10
2·3			3/5
2·4			5/10
2·5		5/10	15/20
2·8			9/15
3·0	0/5	8/10	4/5
3·5	0/5	6/10	5/5
3·8	9/15		
4·0	12/20		
4·2	7/10		

(*b*) It was felt to be of interest to determine the blood levels in some of the rats which had been receiving 1 mg./g. of the sodium salt daily for 14 weeks since these levels might indicate whether any storage took place under these rather extreme conditions. Some preliminary results are given in Table VI, the set of figures for the respective solutions being in each case the average values from a group of 3 rats at the stated intervals after dosage. A similar set of figures is included for a similar group of rats which had received

TABLE VI

BLOOD LEVELS

Number of rats	Daily dose (oral) mg./g.	Period of dosage	Solution used	Mean blood levels μg./ml. (Hours after dosage)		
				17 hours	20 hours	23 hours
3	1·0 mg./g.	14 weeks	Fresh	28·7	3·7	8·0
3	1·0 mg./g.	14 weeks	One month old	23·9	8·3	8·1
3	1·0 mg./g.	One day	Fresh	1·9	—	—

only one dose of 1 mg./g. of sodium *p*-aminosalicylate. It will be seen that, in the group of rats receiving prolonged treatment, there is a moderate level in the blood 17 hours after the daily dose but this rapidly decreases during the next few hours to a negligible level. After a single dose, the amount present after 17 hours is negligible. Hence, it appears that even after prolonged administration, excretion occurs fairly normally but there is some evidence of accumulation in the tissues.

(*c*) A therapeutic test carried out on 1 month old and 6 month old solutions, using 12 mice per group in comparison with a similar size group treated with freshly prepared solutions showed that there was very little difference between the tuberculostatic activities of any of the solutions. A similar size group of infected untreated mice showed the usual high mortality during the test period.

Discussion

Probably the most interesting result of the present series of experiments has been the demonstration that *p*-aminosalicylic acid has a marked effect on the thyroid gland, causing disappearance of the colloid material and some increase in the epithelial tissue. This effect has been referred to as "hyperplasia" on account of its partial resemblance to the effect produced by anti-thyroid agents, such as thiouracil, but with *p*-aminosalicylic acid there is none of the increase in thyroid weight which occurs with thiouracil. Indeed the histological picture more closely resembles that produced by thiocyanate and it seems possible that part of the effect at least may be similar to that of thiocyanate in blocking the synthesis of thyroid hormone. Further work now being carried out with I^{131} and estimation of oxygen consumption may help to elucidate further this action. While the present work was in progress, two references appeared to the effect of *p*-aminosalicylic acid on the thyroid gland, viz., a reference given by Suter[20] and a report by Kjerulf-Jensen and Wolffbrandt.[21] The former merely mentions *p*-aminosalicylic acid as having a weak anti-thyroid action (1/100th that of thiouracil) but the latter workers have obtained marked hyperplasia and increased weight in the rat thyroid after only 10 days treatment. He also obtains similar results with *m*-aminophenol. These effects of *p*-aminosalicylic acid are reversed by thyroxine but not by sodium iodide. Although our own results confirm qualitatively those of Kjerulf-Jensen and Wolffbrandt with *p*-aminosalicylic acid, we find a prolonged test period is required to demonstrate the anti-thyroid effect. Also, we have been quite unable to demonstrate any anti-thyroid effect with *m*-aminophenol. At present, we have no explanation to suggest for these differences in results.

It seems probable that this effect of *p*-aminosalicylic acid on the thyroid may explain the occurrence of enlarged thyroid and mild myxœdema which has been reported during its clinical use.[22,23,24]

There appears to be no evidence in our experiments of any marked difference between the effects of fresh and old solutions so far as the effect on the thyroid gland of rats is concerned. Some difference between these solutions is noticeable as regards their toxicity which appears to increase somewhat rapidly during the first few weeks after preparation and then to increase only very slowly during the succeeding months. In this connection it is perhaps of interest to note that decomposition to *m*-aminophenol is not very extensive even after 6 months keeping, in spite of the fact that the colour of these solutions becomes increasingly darker with age. Indeed the colour of the 6 months solution is almost black. In addition, the increase in toxicity does not run parallel to and cannot be

accounted for quantitatively by the slow but steady increase in *m*-aminophenol. Hence, it appears that neither the increase in colour nor toxicity is due to the formation of *m*-aminophenol. These conclusions are supported by Koelzer and Giesen,[25] who appear to be the only other workers who have examined the properties of old solutions. The figures given by these workers for the toxicity of *p*-aminosalicylic acid and *m*-aminophenol agree almost exactly with our own, but they state that they observed no change in the histological picture after administration of *p*-aminosalicylic acid for up to 30 days. However, they do not state specifically that they examined specimens from the thyroid glands.

Old solutions appear to differ markedly from freshly prepared solutions by reason of the pressor effect of the former. This pressor effect is not blocked by priscol and therefore does not appear to be of sympathetic origin; some part of the effect may be due to a direct cardiac stimulation but there appears to be some other component, possibly a vaso-constrictor action, which we have not yet recognised.

Although there appears to be little diminution in the therapeutic activity of old solutions, the increase in toxicity and pressor effect which takes place on keeping, suggests that the general aversion to using old solutions is well-founded.

We have been unable to confirm earlier reports that *p*-aminosalicylic acid has an eosinopenic and a trypanocidal effect.

SUMMARY

1. Prolonged administration of solutions of sodium *p*-aminosalicylate, both fresh and old (varying in age from 1 week to 6 months), produces retardation in the growth of rats and hyperplasia of the thyroid glands. This effect is easily reversible by cessation of the drug.

2. The toxicity of the solutions to mice increases fairly rapidly during the first month of keeping and then more slowly.

3. Old solutions have a marked pressor effect in rabbits and cats, not reversed by adrenolytic agents. This pressor effect is almost negligible with fresh solutions.

4. The presence of *m*-aminophenol in the old solutions does not appear to be responsible for the increase in toxicity and development of a pressor effect.

5. After daily administration to rats for 14 weeks, there is little evidence that the normal rapid excretion of the drug is affected.

6. No confirmation has been obtained of earlier reports that it has an eosinopenic and trypanocidal effect.

We are indebted to Dr. J. Beattie for much helpful advice, for a great deal of the histological work, and for the photomicrographs. Our thanks are due to Mrs. E. Painter for valuable assistance and to Dr. M. Seiler for the therapeutic experiments. We would also like to thank the Directors of Herts Pharmaceuticals Ltd., for permission to publish this work.

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DISCUSSION

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

DR. F. HARTLEY (London) asked whether the authors could give any information about the possible difference in toxicity between salts having different degrees of hydration. Arguments had been advanced as to the relative merits of the anhydrous calcium salt and the trihydrate. Would the toxicity of the anhydrous salt increase in the course of time as the result of absorption of water? Had the authors made any measurements on the calcium salts? Did they consider it likely that such salts would exhibit any difference in toxicity?

MR. J. JACOBS (Sunderland) asked whether the authors could give any reason for the instability of the solutions. Had any toxic effects been observed clinically when old solutions were used?

MR. T. D. WHITTET (London) referred to the fact that one firm issued *p*-aminosalicylic acid in the form of a solution of the sodium salt. This was preferred to a freshly made solution, about which the patients complained but, after the latter had been kept for several weeks, it had been reported as satisfactory by the patients.

DR. E. I. SHORT (London) asked whether the authors had measured the tissue distribution of the drug in the chronic toxicity test. After a single dose she had observed an accumulation of the salt in the liver. Was there any information on liver damage in the chronic toxicity test?

MR. E. M. BAVIN, in reply, said that he had no information as to the stability of the sodium or calcium salts in various degrees of hydration. All materials examined were in solution, and the periods of time referred

to in the paper were the ages of the solutions and not the age of the solid material from which those solutions were made. Attempts were being made to identify the toxic principle, and using the blood pressure effect as a criterion it appeared that the toxic principle could be extracted by butanol or ethanol. On the question of toxic effects, in clinical practice the well known effects of nausea, vomiting, dizziness and so forth were used, but the toxicity they were assessing was measured by the mortality in animals. On the distribution of the salt in the tissues, some Scandinavian workers, using the fluorescence test, had shown that there was a concentration of the salt in the lung and in the liver. They had not observed any significant liver damage in their animals.