RESEARCH PAPERS

THE EFFECT OF SALICYLATES ON THE THYMUS GLAND OF THE IMMATURE RAT

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Salicylic acid and its derivative, acetylsalicylic acid, induced thymic atrophy not only in intact immature rats but also in adrenalectomised immature animals when administered by mouth in the diet, or by subcutaneous injection. A straight line with a negative slope was obtained by plotting the log dose of salicylate against the relative thymus weight. This linear relation was used to compare quantitatively the thymolytic activities of acetylsalicylic acid, ethyl salicylate, and salicylamide with those of salicylic acid. The results of this investigation suggested that salicyl compounds act directly on the thymus gland and not by stimulating the pituitary-adrenal system. No evidence was found to indicate that salicylates potentiate the thymolytic action of adrenal corticosteroids.

SALICYLATES have been used for the treatment of rheumatic diseases since 1876[1]. When corticotrophin and the cortisone-like steroids were found to possess antirheumatic properties, it was assumed that salicylates acted by stimulating the pituitary-adrenal system. Support for this theory regarding the mode of action of the salicylates was obtained by the observation that salicylates induced depletion of adrenal ascorbic acid in the intact rat[2–7], but not in hypophysectomised animals[8–13]. However, the finding that therapeutic doses of salicylate failed to increase the level of 17-hydroxycorticosteroids in both urine[14] and plasma[15–18] cast some doubt on the validity of this hypothesis. A study of the action of salicylates on carbohydrate metabolism led Smith[1] and later Feeney and others[19], to the conclusion that the effect of salicylate on rheumatic diseases was not mediated through the pituitary-adrenal system, but through some other unknown mechanism.

Salicylates may have a direct adrenal corticoid-like action on some peripheral tissues and exert their therapeutic effect in this manner. Since it is well known that involution of the thymus gland is a manifestation of glucocorticoid-like activity[20–23], a study was made of the action of salicylate and other salicyl derivatives on this target organ. Salicylates were found to cause thymus involution in both adrenalectomised and intact immature rats. The results of this investigation have suggested that (*a*) salicyl compounds act directly on the thymus gland of the immature rat, (*b*) do not specifically influence the pituitary-adrenal system, and (*c*) do not potentiate the effect of endogenous adrenal corticoids. The linear relation which exists between the log dose of the salicyl derivative and the relative thymus weight was used to compare the relative thymolytic activity of several of these compounds.

EXPERIMENTAL METHODS

The test animals were 23 to 28 day-old albino rats derived from the Wistar strain. The compounds under investigation were administered either orally or by subcutaneous injection. In the oral test, the salicyl derivatives were mixed thoroughly in the standard laboratory diet*, and when a free acid was studied, sufficient sodium bicarbonate was added to neutralise the acid and thus prevent any change in the acid-base balance of the test animals. In the studies involving subcutaneous administration, the compounds were dissolved in isotonic saline adjusted

TABLE I

THYMOLYTIC ACTIVITY OF VARIOUS SALICYL COMPOUNDS ADMINISTERED ORALLY TO IMMATURE RATS

Compound	Concentration of salicyl compound in the diet per cent	Thymus weight per cent
Control	0	100·0
Sodium salicylate	0·29	89·5*
	0·58	69·4*
Salicylic acid	0·25	88·4*
	0·50	66·0*
Acetylsalicylic acid	0·25	92·5
	0·50	81·7*
Ethyl salicylate	0·35	92·9
	1·00	70·5*
Salicyl alcohol	0·25	96·4
	0·50	91·3
Salicylamide	0·25	107·0
	0·50	101·0
	1·00	105·0
	4·00	87·2*
Sodium *p*-aminosalicylate	0·25	100·5
	2·00	102·1

* Significant decrease in relative thymus weight ($P = 0{\cdot}95$)

to approximately pH 7. A total of 8 to 10 rats was assigned at random to each dose group, with the restriction that the average body weight was the same for all dose groups in any one experiment. The rats were caged in pairs and the quantity of food offered per day was adjusted so that the caloric intake of each pair of animals was approximately the same at all dose levels of the salicyl derivative. The actual amount of food consumed daily by each pair of rats over the 2 to 3 day test period was recorded to provide an estimate of the average dose of the compound under investigation. The animals were allowed access to the food up to the time of death. On the other hand, in the tests in which the salicylates were injected subcutaneously in divided doses over a 2 to 3 day period, the thymus glands were removed and weighed approximately 20 hours after the last injection.

When adrenalectomies were done, the glands were removed by the lumbar route under ether anesthesia. These animals were given 1 per

* Master Fox Starter Meal—Toronto Elevators Ltd.

cent saline to drink and the stock laboratory diet *ad libitum* as well as a daily subcutaneous injection of 0·1 mg. of desoxycorticosterone acetate in aqueous suspension for a period of 1 week before the test. By following this regimen the adrenalectomised rats appeared to be in good physical

TABLE II

EFFECT OF SALICYLATE ON THE THYMUS GLAND OF THE IMMATURE RAT

Salicylate in the diet* per cent	Average total intake per rat. mg.	Relative thymus wt. mg./100 g. of rat	Reduction per cent in thymus wt.
0	0	426·5 ± 16·0	0
0·0625	14	407·6 ± 6·6	4·4
0·125	27	401·3 ± 9·7	5·9
0·25	47	352·3 ± 9·2†	17·4
0·50	67	286·4 ± 13·8†	32·8

* Salicylic acid mixed with an equal quantity of sodium bicarbonate in the diet.
† Significant at 1 per cent level.

condition and maintained their body weight. The rats were checked for adrenal remnants when the thymus glands were removed at the end of the experiment. Previous work has shown that this dose of desoxycorticosterone acetate had no effect on the thymus gland of the immature rat[23].

RESULTS

The data in Table I show the effect of salicylate and other salicyl derivatives on the weight of the thymus gland of the immature intact rat, when administered in the diet for several days. Salicylamide did not induce thymic atrophy when fed at a concentration of 1 per cent of the diet but caused significant involution when the amount reached 4 per cent of the daily food. In contrast, salicylate and acetylsalicylate

TABLE III

RELATION BETWEEN THE LOG DOSE OF SALICYLIC ACID AND THE RELATIVE THYMUS WEIGHT IN THE IMMATURE RAT

Let Y = the relative thymus weight, and X = the log dose of salicylic acid.
Equation of the regression line = $Y = 825{\cdot}7 - 290{\cdot}8\,X$

ANALYSIS OF VARIANCE TABLE

Source of variation	d.f.	s.s.	m.s.	"F"	P
Between dose groups:					
(1) linear regression	1	63850	63850	51·868	0·01
(2) deviation from regression ..	1	2637	2637	2·142	0·05
Within dose groups (error)	27	33226	1231		
Total	29	99713			

decreased the thymus weight significantly when given at a concentration of less than 0·5 per cent of the diet. Although salicyl alcohol only showed a tendency to lower the thymus weight when the diet contained 0·5 per cent of this compound, it is probable that thymus involution would have occurred if a larger quantity had been administered. On the other hand, sodium *p*-aminosalicylate did not reveal any thymolytic activity even at the 2 per cent concentration.

The results in Table II demonstrate that the decrease in thymus weight induced by the oral administration of salicylate for 3 days, is proportional to the dose. By using the values for 0·125 per cent, 0·25 per cent and 0·5 per cent shown in Table II, it was possible to calculate a regression line relating the log dose of salicylic acid and the weight of the thymus gland. The analysis of variance, with regression, given in Table III

TABLE IV

RELATIVE POTENCY OF SALICYLATES IN THE IMMATURE RAT

Compound	Thymus involution assay*			
	Weight basis		Equimolar basis	
	Potency	Fiducial limits†	Potency	Fiducial limits†
Salicylate	100		100	
Acetylsalicylate	70·4	63–78	91·8	82–101
Ethyl salicylate	49·8	40–69	60·0	49–83
Salicylamide	7·7	6–10	7·8	6–10

* Oral administration for 5 days; thymectomy in the sixth day. † P = 0·95.

illustrates that this relation is linear and that the slope of the regression line is highly significant. A modification of the thymus involution assay for adrenal corticoids[23] was employed to determine the potency of several salicyl derivatives relative to salicylate using intact rats and administering the compounds orally in the diet at two dose levels. Each of the values shown in Table IV represents the weighted mean of at least two valid

TABLE V

THYMOLYTIC ACTION OF ACETYLSALICYLIC ACID (ASA) IN THE IMMATURE RAT

ASA per cent in the diet*	Normal rats			Adrenalectomised rats†		
	Average total intake of ASA per rat mg.	Relative thymus wt. mg./100 g. of rat	Reduction per cent in thymus wt.	Average total intake of ASA per rat mg.	Relative thymus wt. mg./100 g. of rat	Reduction per cent in thymus wt.
0	0	419·6 ± 13·1	0	0	550·0 ± 19·5	0
0·25	71·4	368·2 ± 9·2	12·2	72·2	457·7 ± 12·3	16·8
0·50	137·0	302·6 ± 11·3	27·9	125·3	404·4 ± 12·4	26·5

* Acetylsalicylic acid mixed with an equal quantity of sodium bicarbonate in the diet.
† Maintained for 7 days on 1 per cent saline as drinking water and 0·1 mg. desoxycorticosterone acetate daily before feeding the acetylsalicylic acid.

assays by the thymus involution method. On an equimolar basis, acetylsalicylate was about 90 per cent and ethyl salicylate approximately 60 per cent as active as salicylate. In contrast, salicylamide was less than 10 per cent as potent.

The next step in this study was an investigation of the mode of action of salicylate in causing thymic involution in the intact rat. The effect of acetylsalicylate administered orally for 5 days to intact and adrenalectomised rats was investigated and the data in Table V show clearly that this compound had approximately the same action in both groups. This experiment was repeated by administering the acetylsalicylate subcutaneously over a 2-day period. Again it was possible to demonstrate

in both groups atrophic changes in the thymus gland. The data in Table VI indicate that the per cent reduction in thymus weight was somewhat greater in the intact group than it was in the adrenalectomised rats at the higher dose levels of acetylsalicylate. A significant involution was obtained at both the 10 and 20 mg. dose levels in the intact rat, but only at the 20 mg. level in the adrenalectomised animal. This slight

TABLE VI

THYMOLYTIC ACTION OF ACETYLSALICYLIC ACID AFTER SUBCUTANEOUS INJECTION IN THE IMMATURE RAT

	Normal rats			Adrenalectomised rats		
Total dose of ASA* mg.	No. of rats	Relative thymus wt. mg./100 g. of rat	Reduction per cent in thymus wt.	No. of rats	Relative thymus wt. mg./100 g. of rat	Reduction per cent in thymus wt.
0	25	392·2 ± 5·5	0	15	433·5 ± 8·5	0
5	5	378·2 ± 9·8	3·6	5	417·4 ± 19·2	3·7
10	15	368·7 ± 7·8†	6·0	10	415·8 ± 20·9	4·1
20	45	346·0 ± 4·7†	11·8	20	401·1 ± 6·5†	7·5

* Acetylsalicylic acid (ASA) was neutralised with sodium bicarbonate and administered in five subcutaneous injections over a 2-day period.
† Significant reduction in thymus weight.

difference in the effect was not observed when the acetylsalicylate was given orally. Previous experience with the thymus involution assay of adrenal corticosteroids has shown that the small amount of stress which occurs as a result of local irritation at the site of injection can have an effect on the thymus weight. Hence this slight stimulation of the pituitary-adrenal system in the intact rat can reasonably account for this difference in response between the two groups.

TABLE VII

EFFECT OF SALICYLATE ON THE THYMOLYTIC ACTION OF HYDROCORTISONE IN THE IMMATURE RAT

Treatment		Average per cent reduction in relative thymus wt.*	
	Total dose mg.	Intact rats per cent	Adrenalectomised rats per cent
Control	0	0	0
Sodium salicylate	40	24·3 ± 3·1†	16·0 ± 11·3†
Hydrocortisone	0·3	32·3 ± 7·5	31·3 ± 2·5
Sodium salicylate + hydrocortisone ..	40 + 0·3	44·0 ± 1·7	42·8 ± 3·0

* Average of three replicate experiments. † Standard deviation of individual replicates.

A series of three experiments was carried out in both intact and adrenalectomised rats to investigate the possibility that salicylates exert their effect on the thymus gland of the immature rat by potentiating the action of adrenal corticoids. The compounds employed in this study were dissolved in a neutral aqueous medium containing 10 per cent of ethanol and 0·9 per cent of sodium chloride. The total doses described in Table VII were administered in a series of five divided subcutaneous

injections over a 2-day period. Table VII shows the average percentage reduction in the relative thymus weight in the intact and adrenalectomised rats. The standard deviations given in Table VII provide only an estimate of the variation observed between the replicate tests and do not represent the variation in the individual reponses of the rats. These data give no support to the hypothesis that the thymolytic action of sodium salicylate depends on the presence of adrenal corticoids like hydrocortisone because the percentage reduction in the relative thymus weight obtained when the compounds were given together was less than the sum of the individual effects.

Discussion

According to Hart[24] salicylamide is considerably less toxic than acetylsalicylic acid in the rat when repeated doses are given at daily intervals. Evidence to support this observation was obtained by Seeberg, Hansen and Whitney[25] who reported that while salicylamide was readily absorbed from the gastrointestinal tract of the rat, the serum concentration was lower than that of other salicyl derivatives when the compounds were administered at equal dose levels. In addition salicylamide was found to be less irritant than acetylsalicylic acid to the gastric mucosa of the rat.

Our results on the thymolytic activity of salicylamide relative to salicylate tend to confirm these findings. It is possible that the concentration of salicylamide in the blood at doses comparable to those of salicylate may have been too low to cause involution of the thymus gland. It was also noted that the rats would not tolerate levels of salicylate in the diet much beyond 0·5 per cent, whereas salicylamide could be fed at a concentration of 4 per cent of the diet without any significant impairment of the daily food intake.

However, the supposedly low serum concentration of salicylamide and its less irritant properties may not be entirely responsible for the relatively low thymolytic activity. The results shown in Tables I and IV suggest that compounds with a free carboxyl group such as salicylic acid and its derivatives, with the exception of sodium *p*-aminosalicylate, were the most active in causing thymic atrophy. Neither salicylamide nor salicyl alcohol are true salicylates because they lack a carboxyl group in the position ortho to the hydroxyl group. While the formation of the sodium salt had no effect on the thymolytic activity, esterification of salicylic acid with ethanol reduced the potency by approximately 40 per cent.

The data presented in Tables V, VI and VII suggest that salicylate acted directly on the thymus gland of the immature rat. Salicylate did not appear to primarily induce thymus involution by stimulating the pituitary-adrenal system, although it is reasonable to assume that handling and irritation at the site of injection caused some endogenous production of adrenal corticosteroids. The results given in Table VII show that the percentage reduction in the relative thymus weight obtained when sodium salicylate and hydrocortisone were injected together was less than the sum of the individual effects. Therefore it can be concluded that

salicylate did not act by potentiating the thymolytic action of hydrocortisone. The results presented in Table VII suggest that a slight antagonism may even exist between the two compounds., This could be interpreted as evidence of a possible competition between hydrocortisone and salicylate for certain receptor sites on the cells of the thymus gland.

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