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THE GLYCOGENOLYTIC ACTION OF SODIUM SALICYLATE

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Salicylate, in the form of salts of salicylic acid and of its acetyl derivative, has been used in the treatment of rheumatic fever for many years, and in one form or another it is probably the most widely used of household remedies ; yet our knowledge of its pharmacology remains deficient. This is not from lack of interest in the salicylates, for Gross and Greenberg (1948) have recently listed over 4,000 relevant publications. However, the literature does not afford a clear conception of the relation between the numerous established actions of the drug ; it is not even certain which actions are of therapeutic importance and which are purely side-effects. It seemed worth while, therefore, to look into some of the less obvious actions of salicylate. Of these perhaps the most striking is that observed by Lutwak-Mann (1942), that the administration of sodium salicylate caused almost complete disappearance of liver glycogen in the rat ; she concluded that this was evidence of interference with enzymatic processes by salicylate.

Such an action might be of therapeutic importance, but it would be hazardous to draw this inference from Lutwak-Mann's (1942) data, because large doses of salicylate were used, and because the mortality resulting from such doses made it impracticable to employ fasted animals, so that only fed rats with initially high liver glycogen concentrations were studied.

The primary aim of the present investigation was to re-examine the effect of salicylate on liver glycogen, to see whether the effect occurred at dosage levels more comparable with those used therapeutically, and to determine if it occurred in fasted as well as in recently fed animals.

The blood sugar was also estimated, since hyperglycaemia might accompany the expected glycogenolysis, although no related change in blood sugar concentration was found by Lutwak-Mann (1942). However, it had previously been reported that salicylate caused hyperglycaemia in the dog (Barbour and Herrmann, 1921) and in man (Morris and Graham, 1931).

METHODS

Swiss albino mice aged 3½ to 5 months were used. Glycogen was estimated, after hydrolysis by the method of Good *et al.* (1933), by ceric sulphate titration (Miller and van Slyke, 1936) ; blood sugar was determined by the method of Miller and van Slyke (1936).

The mice were dealt with in batches of 9–12 of the same sex per day ; two days before use they were given white bread in addition to the usual diet of rat cake. Two series of experiments were performed: in the first food was given until the beginning of the experiment ; in the second all food was withdrawn 18 hr. previously.

The mice were paired off for weight. One of each pair was given an intraperitoneal injection of 10 mg. sodium salicylate in 0.05 ml. water ; the other, control, animal received an intraperitoneal injection of 0.05 ml. normal saline. They were killed by a blow on the head at 1, 2, or 4 hr. after injection, and in each series an untreated group was killed at zero hr. 0.2 ml. blood for the estimation of glucose was obtained, after decapitation, from the neck, and liver was removed for the determination of glycogen content.

RESULTS

The data are presented to show (i) whether the animals were fed or fasted before the experiment, (ii) their sex, (iii) the time after injection at which they were killed, and (iv) whether they were controls or were salicylate-treated. The results were analysed by application of the t-test to the differences between means. Non-significant values of P will be omitted, and although those between 0.05 and 0.02 will be given, it is recommended that the minimum acceptable level of significance be taken at $P=0.02$.

TABLE I

MEAN LIVER GLYCOGEN CONCENTRATIONS OF FED AND OF FASTED SALICYLATE-TREATED AND CONTROL MICE, KILLED AT DIFFERENT TIMES AFTER INJECTION

The number of observations in each group is shown in parenthesis after each mean. P=0·05 is regarded as not significant.

Sex	Time Killed, (Hr. after Injection)	Fed			Fasted		
		Mean Liver Glycogen Concentration, mg./g.		P	Mean Liver Glycogen Concentration, mg./g.		P
		Control	Treated		Control	Treated	
Male	0	30·7 (16)	—		5·9 (20)	—	
	1	13·9 (15)	8·7 (15)	0·05	5·7 (20)	3·0 (20)	0·001
	2	13·9 (15)	5·2 (15)	0·01	3·8 (19)	2·3 (19)	0·02
	4	6·9 (14)	3·7 (14)	—	4·4 (19)	2·7 (19)	0·02
Female	0	39·8 (16)	—		8·1 (20)	—	
	1	26·4 (15)	12·9 (15)	0·001	8·5 (18)	4·4 (18)	0·001
	2	25·1 (14)	13·0 (14)	0·01	5·3 (20)	3·2 (20)	0·02
	4	8·3 (14)	6·6 (14)	—	5·7 (20)	4·8 (20)	—

Liver Glycogen

The liver glycogen results are presented in Table I. In both fed and fasted series the mean liver glycogen concentrations were significantly higher in the control females than in the control males. Thus, for example, in the fed series the male mean liver glycogen concentration at 1 hr. is 12.5 mg./g. less than the female ($0.01>P>0.001$).

The controls which had been fed till the beginning of the experiment show, in both sexes, sharp falls in mean liver glycogen concentration from 0 to 1 hr. These did not continue from 1 to 2 hr., but were followed by further falls from 2 to 4 hr. Such features may be interpreted as the result of the withdrawal of food, although possibly the fall from 0 to 1 hr. in the males is to a greater extent a result of the saline injection, since the male mice were more excitable than the females. In contrast, the salicylate-treated animals of both sexes had even lower mean liver glycogen concentrations at 1 hr., from which there was no rise within the period of the experiment. The differences between the saline and salicylate-treated animals' mean liver glycogen concentrations are significant at 2 hr. in both sexes, and at 1 hr. in the females.

The control mean liver glycogen concentrations of the animals fasted before the experiment gradually fell from 0 to 4 hr. The fasted salicylate-treated groups show sharp falls, in both sexes, from 0 to 1 hr., but not thereafter. The differences between the saline- and salicylate-treated groups' means were maximal at 1 hr. in both sexes, and although these differences were less than in the previous series, they are found to be highly significant on application of the t-test.

In summary, the mean liver glycogen concentrations of the salicylate-treated animals were significantly lower than those of the saline-treated controls, irrespective of sex, or whether fed or fasted immediately before the experiment.

Blood Sugar

The difference between the sexes was not marked in the control blood sugar concentrations, presented in Table II, although, in keeping with

TABLE II

MEAN BLOOD SUGAR CONCENTRATIONS OF FED AND OF FASTED SALICYLATE-TREATED AND CONTROL MICE, KILLED AT DIFFERENT TIMES AFTER INJECTION

The number of observations in each group is given in parenthesis after each mean. P=0·05 is regarded as not significant

Sex	Time Killed (Hr. after Injection)	Fed			Fasted		
		Mean Blood Sugar Concentration, mg./100 ml.		P	Mean Blood Sugar Concentration, mg./100 ml.		P
		Control	Treated		Control	Treated	
Male	0	167·8 (9)	—		114·9 (20)	—	
	1	145·5 (15)	150·9 (15)	—	108·6 (20)	118·2 (20)	—
	2	142·3 (13)	138·7 (13)	—	111·6 (18)	107·7 (18)	—
	4	108·2 (13)	87 ·3(13)	0·05	91·5 (16)	92·9 (16)	—
Female	0	153·5 (15)	—		99·7 (20)	—	
	1	130·4 (14)	162·1 (14)	0·01	105·3 (18)	148·0 (18)	0·001
	2	138·4 (11)	151·3 (11)	—	92·1 (20)	120·0 (20)	0·001
	4	114·8 (12)	108·3 (12)	—	91·1 (16)	102·4 (16)	—

their greater excitability, higher initial values were found in the males.

In both fed and fasted animals the mean blood sugar concentrations of the salicylate-treated males were, in general, almost identical with the corresponding control values; no significant differences were found. It should be emphasized that these results do not prove that salicylate had no effect on the blood sugar level of the male mice; but it is obvious that any significant effect with the doses used, and demonstrable by the use of substantially more animals, would be relatively small.

The salicylate-treated females had significantly higher mean blood sugar concentrations than those of the corresponding controls at 1 hr.; only in the fasted series was this so at 2 hr. The differences were greater and more significant in the fasted series.

To summarize: a significant hyperglycaemia occurred in the salicylate-treated females, whether fed or fasted immediately before the experiment; but there was no significant change in blood sugar concentration in the male mice.

The sex difference in the response of the blood sugar concentration to sodium salicylate was not absolute. The disparity between the sexes lies not in the presence or absence of a certain effect, but in the relative extent of the effect. The sex difference in body weight, and therefore in dose expressed in relation to weight, may have been a contributory factor; but by itself this cannot account for the discrepancy, because the mean doses of the fasted males and fed females were almost identical: the mean doses, in mg. sodium salicylate per g. body weight, were: fed males, 0.32; fed females, 0.36; fasted males, 0.35; fasted females, 0.40.

The explanation of this sex difference is not obvious. So many factors influence the blood sugar concentration that the possibilities are numerous. One factor is taken into account in the present investigation—the rate of reduction of liver glycogen concentration; this did not differ greatly between the sexes. There may well be a sex difference in the rate of metabolism generally, and of the rate utilization of glucose in particular. This possibility merits further investigation.

Discussion

It may be concluded from the present data that sodium salicylate, in single non-toxic doses, caused a reduction of the liver glycogen concentration in young Swiss albino mice, irrespective of sex, or whether fed or fasted immediately before the experiment. This effect was a rapid one, occurring within 1–2 hr. of administration. It should be emphasized that no deaths resulted from the doses used, and that the salicylate-treated animals were indistinguishable in their behaviour from the saline-treated controls. Certain differences in detail between the present results and those of Lutwak-Mann (1942) will be noted; the latter showed a more marked effect, justifying the use of the term "depletion" of liver glycogen; this may have been due to the larger doses used. A more interesting difference is that Lutwak-Mann (1942) recorded the effect of sodium salicylate on rats' liver glycogen as being maximal 4 hr. after injection; her fatalities also occurred at this time. In keeping with the present results, mice succumb to fatal doses of sodium salicylate within about one hour.

That single doses of sodium salicylate did or did not cause a significant hyperglycaemia, depending on sex, is very striking. The importance of this observation is that it strongly suggests that changes in blood sugar level due to salicylate should be regarded as secondary, i.e., as a by-product of the glycogenolysis and other, undefined, effects. It cannot be argued that breakdown of liver glycogen to glucose is sufficient to explain the results, since in the males the reduction in liver glycogen concentration is unaccounted for in the absence of definite hyperglycaemia.

It is tempting to speculate on the mechanism of the glycogenolysis. It may result from the inhibition of an enzyme concerned in the synthesis of glycogen, as Lutwak-Mann (1942) infers. However, the evidence seems insufficient to warrant such a far-reaching conclusion; it remains to be shown that the glycogenolytic effect of sodium salicylate is due to a direct action of the drug on the liver. Furthermore, an acceptable hypothesis should take into reckoning the sex difference in the associated hyperglycaemia. This raises the question of whether the glycogenolytic action of salicylate may legitimately be considered as an isolated phenomenon. Such an assumption may be inadvisable.

Salicylate has been shown to have profound effects on the carbohydrate metabolism of mice. This does not necessarily apply to man. However, the finding of Morris and Graham (1931), that therapeutic doses of salicylate cause hyperglycaemia in man, suggests that salicylate may well have a glycogenolytic action in man.

Although the importance of the present findings is uncertain, it is clear that the classical concept of salicylate, as a drug whose main action is confined to the central nervous system, may be untenable.

Summary

1. The liver glycogen and blood sugar concentrations of young Swiss albino mice were determined 1, 2, and 4 hr. after the administration of 10 mg. sodium salicylate.

2. The liver glycogen concentrations of the salicylate-treated animals were significantly lower than those of saline-treated controls at 1 or 2 hr. after injection, irrespective of sex, or whether fed or fasted immediately before the experiment.

3. Though hyperglycaemia occurred in the salicylate-treated females, there was no significant change in blood sugar concentration in the males.

4. The results are briefly discussed, and it is concluded that the hyperglycaemia should be regarded as a secondary effect, and that salicylate probably has other major actions on carbohydrate metabolism.

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