The Effect of Salicylate on Glycosuria, Blood Glucose and Liver Glycogen of the Alloxan-Diabetic Rat

By M. J. H. SMITH, B. W. MEADE
AND J. BORNSTEIN (Fellow of the National Health and Medical Research Council of Australia)
Department of Chemical Pathology, King's College Hospital Medical School, London, S.E. 5

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Large doses of salicylates have been reported to prevent diabetic glycosuria in man and the drug was used in the treatment of diabetes during the latter part of the last century (Gross & Greenberg, 1948).

The recent report of Ingle (1950) that aspirin reduced the glycosuria of rats made mildly diabetic by partial pancreatectomy stimulated us to investigate this effect in more detail. In the present work the effect of salicylate on the glycosuria, blood glucose and liver glycogen of rats made severely diabetic with alloxan was studied. The blood glucose and liver glycogen of normal rats treated with salicylate were also investigated because Lutwak-Mann (1942) reported a marked decrease in liver glycogen in such animals.

EXPERIMENTAL

Animals. Rats of the Wistar strain weighing 150–200 g. were maintained on a diet consisting of crushed commercial cubes, 70 g. made to 100 ml. with milk. Each rat received 50 ml. daily. The alloxan-diabetic rats each received intravenously 50 mg. of alloxan/kg. body weight and no diabetic rat was used whose blood glucose was less than 350 mg./100 ml.

Analytical methods. Urinary glucose was determined by the method of Benedict (1911) and liver-glycogen content by that of Good, Kramer & Somogyi (1933). The glucose in the final stage of the glycogen estimation and also the blood glucose were measured by the method of Nelson (1944).

General arrangement of experiments

Effect of salicylate on the glycosuria of alloxan-diabetic rats. Eight diabetic rats were placed in metabolism cages and 24 hr. urine collections made. After an initial control period, each rat received a daily subcutaneous injection of 100 mg. of sodium salicylate suspended in arachis oil. After a second control period, each rat received a daily subcutaneous injection of an arachis oil suspension of 100 mg. of sodium gentisate. After a further recovery period each rat received 10 mg. of cortisone (cortone acetate, Merck). The rats were weighed daily and a fasting blood glucose was estimated on alternate days.

Effect of salicylate on blood glucose of diabetic rats. Six diabetic rats, maintained under fasting conditions for 12 hr. before and during the experiment, were given a solution containing 100 mg. sodium salicylate in 1 ml. distilled water by subcutaneous injection. Blood glucose was determined on tail-vein samples immediately before and 4, 7 and 24 hr. after injection. Five diabetic rats who received no salicylate were kept under identical conditions and blood glucose measured at the same time intervals.

Effect of salicylate upon the liver glycogen content of diabetic rats. Seven groups of five or six diabetic rats were killed by stunning at different intervals and glycogen estimated in 80–100 mg. portions of liver taken as nearly as possible from anatomically similar lobes immediately after death. The first group was killed and three of the remaining groups given a solution containing 100 mg. sodium salicylate in distilled water by subcutaneous injection. One salicylate-treated and one untreated group were killed 4, 7 and 24 hr. after the start of the experiment. Blood-glucose determinations were made at the start of the experiment and immediately before death in these animals.

Effect of salicylate on liver glycogen and blood glucose of normal rats. The previous experiment was repeated with normal rats which had received extra glucose in the preceding 24 hr. period (cf. Lutwak-Mann, 1942).

RESULTS

The effects of salicylate on the glycosuria of alloxan-diabetic rats. The mean 24 hr. glucose outputs are shown in Fig. 1, where it is seen that injection of

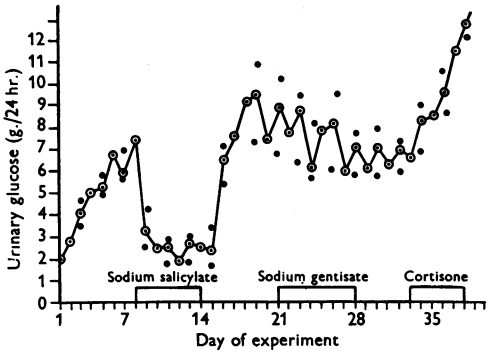

Fig. 1. Mean 24 hr. glucose and ranges for a group of eight alloxan-diabetic rats. Sodium salicylate, 100 mg. in 1 ml. arachis oil daily; sodium gentisate, 100 mg. in 1 ml. arachis oil daily; cortisone, 10 mg. daily as saline suspension of cortone acetate (Merck).

salicylate markedly reduced the glycosuria. The closely related gentisic acid (2:5-dihydroxybenzoic acid), which has been reported (Meyer & Ragan, 1948) to exert similar therapeutic actions to sali-

cylate, had no significant effect, whereas cortisone exacerbated the glycosuria. When the injection of salicylate was stopped the 24 hr. excretion of glucose increased to higher levels than were observed during the initial control period. A similar effect was reported by Ingle (1950). No significant differences in blood-glucose concentrations were observed during the whole experiment. During the initial control period there was a slow but steady loss of body weight, but no further decrease in weight occurred after the animals had received salicylate. The injection of salicylate did not cause any reduction in daily food intake.

The effect of salicylate on the fasting blood glucose of alloxan-diabetic rats. Table 1 shows that there was no significant difference between the two groups of rats at the start of the experiment, but that a significant fall in blood glucose occurred in alloxan-diabetic rats treated with salicylate and this fall is significantly different from any deviations observed in the untreated diabetic animals.

Three of the salicylate-treated rats died during the course of the experiment and one of these had a blood-glucose value of 44 mg./100 ml. immediately before death. This suggests that hypoglycaemia may have been a major factor in the death of these animals.

The effect of salicylate on the liver glycogen of alloxan-diabetic rats. A comparison between the blood-glucose concentrations at the start of the experiment and those determined immediately before death showed a significant fall in the salicylate-treated, but not in the control rats, a similar effect to that observed in the previous experiment. Table 2 shows that despite this fall in blood glucose no significant change occurred in the liver-glycogen

Table 1. *The effect of salicylate on the fasting blood glucose of alloxan-diabetic rats*

Time of estimation (hr.)	Blood glucose with standard deviations (mg./100 ml.) *A* Salicylate	No. of rats	*B* Untreated	No. of rats	Comparison of blood glucose results for groups *A* and *B* n	t	P
0	572±133	6	509±88	5	9	1·0330	0·3
4	355±48	6	574±118	5	9	4·2135	0·01
7	273±124	6	615±52	5	9	5·7098	0·001
24	155±91	3	434±134	5	6	3·2998	0·02

Within group *A* for 0 and 4 hr. $n=5$, $t=5·0348$, $P=0·01$
0 and 7 hr. $n=5$, $t=6·0080$, $P=0·01$
0 and 24 hr. $n=2$, $t=3·1923$, $P=<0·1>0·05$

Table 2. *The effect of salicylate on the liver glycogen of alloxan-diabetic rats*

Time killed (hr.)	Liver glycogen content with standard deviations (g./100 g. wet wt.) Untreated	No. of rats	Salicylate	No. of rats
0	1·04±0·83	5	—	—
4	0·77±0·57	6	0·67±0·52	6
7	0·33±0·18	5	0·64±0·32	5
24	1·25±1·45	6	0·76±0·72	6

Table 3. *The effect of salicylate on the blood glucose and liver glycogen of normal rats*

Time killed (hr.)	Blood glucose with standard deviations (mg./100 ml.) No. of rats	Untreated *A*	No. of rats	Salicylate *B*	Liver glycogen with standard deviations (g./100 g. wet wt.) No. of rats	Untreated *A*	No. of rats	Salicylate *B*
0	4	81±13	—	—	6	7·48±2·66	—	—
4	5	90±21	5	81±24	6	5·66±2·9	7	0·65±0·48
7	5	91±20	8	98±25	6	4·24±1·01	8	0·70±0·33
24	5	93±23·5	6	87±24	6	7·80±0·38	7	4·48±3·73

At no point in the comparison of blood-glucose results was a value of P less than 0·6 obtained.

Comparison of the liver glycogen results:

For groups *A* and *B* 4 hr. $n=11$, $t=4·5471$, $P=0·001$
7 hr. $n=12$, $t=8·0638$, $P=0·001$
24 hr. $n=11$, $t=1·9773$, $P=0·1$

Within group *A* 0 and 4 hr. $n=10$, $t=1·1239$, $P=0·3$
0 and 7 hr. $n=10$, $t=2·8379$, $P=0·02$
0 and 24 hr. $n=10$, $t=0·2654$, $P=0·8$

content. In the comparison of the liver-glycogen results from untreated and salicylate-treated rats no value of P less than 0·3 was obtained.

The effect of salicylate on the blood glucose and liver glycogen of normal rats. Table 3 shows that there was no significant change of blood glucose in the salicylate-treated rats, but that a marked fall in liver glycogen occurred 4 and 7 hr. after the salicylate injection. This depletion appeared to be temporary and partial replacement of the glycogen had occurred in 24 hr. Our results are in agreement with those reported by Lutwak-Mann (1942), except that this worker found in some experiments that 24 hr. after treatment, glycogen in the livers of salicylate-treated rats was greater than that of the controls.

DISCUSSION

The present work shows that in diabetic rats salicylate reduces the glycosuria, lowers the blood glucose, but causes no change in liver-glycogen content. Cortisone exacerbated the glycosuria in these animals and this is of interest because of reports that the therapeutic effects of salicylate are primarily mediated through the pituitary and adrenal glands, a hypothesis which has been criticized (Meade & Smith, 1951). It must be concluded that in diabetic rats depression in the urinary and blood glucose by salicylate is not due to its deposition as liver glycogen. Other possible mechanisms to account for the disappearance of this glucose are: first, inhibition of gluconeogenesis; second, increased deposition of muscle glycogen; third, increased glucose use in the tissues; and fourth, conversion to fat.

In the non-diabetic rat salicylate caused no change in blood glucose, but a significant reduction of liver glycogen and similar mechanisms may be involved. The possibility that the glucose may be converted to glucuronic acid which is used for conjugation with salicylate is unlikely because of Lutwak-Mann's (1942) conclusion that the ability of rat liver to form a conjugated glucuronide from salicylate was negligible.

It is proposed to extend this work to investigate the possibilities discussed above.

SUMMARY

1. The effect of salicylate on the glycosuria, blood glucose and liver-glycogen content of the alloxan-diabetic rat and on the blood glucose and liver glycogen of the normal rat have been studied.

2. Salicylate reduces the glycosuria and blood glucose in the diabetic rat, but causes no change in the liver-glycogen content. In the normal rat salicylate causes no alteration in the blood glucose, but a depression of the liver-glycogen content.

3. Possible mechanisms of these changes are discussed.

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REFERENCES

Benedict, S. R. (1911). *J. Amer. med. Ass.* **57**, 1193.
Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
Gross, M. & Greenberg, L. A. (1948). *The Salicylates*, p. 108. New Haven: Hillhouse Press.
Ingle, D. J. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 673.
Lutwak-Mann, C. (1942). *Biochem. J.* **36**, 706.
Meade, B. W. & Smith, M. J. H. (1951). *Lancet*, **2**, 224.
Meyer, K. & Ragan, C. (1948). *Science*, **108**, 281.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.

The Inhibition of Aconitase by 'Inhibitor Fractions' Isolated from Tissues Poisoned with Fluoroacetate

By W. D. LOTSPEICH, R. A. PETERS and T. H. WILSON
Department of Biochemistry, University of Oxford

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Fluoroacetate has been shown to inhibit the oxidation of citrate in animal tissues *in vivo* (Liébecq & Peters, 1948; Martius, 1949). Furthermore, Buffa & Peters (1949) have shown that animals poisoned with fluoroacetate show large accumulations of citrate in many of their tissues (their paper may be consulted for references to the earlier literature). This observation has been confirmed by Potter & Busch (1950), Lindenbaum, White & Schubert (1951) and Kandel, Johnson & Chenoweth (1951). Despite many attempts, no isolated enzyme has been shown to be inhibited by fluoroacetate. This and other work has led to the hypothesis, originally proposed to explain *in vitro* experiments by Liébecq & Peters (1948) and Martius (1949), that fluoroacetate is metabolized in a similar manner to