The Effect of Salicylate on the Glycosuria and Hyperglycaemia Induced by Cortisone in the Normal Rat

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Salicylate has been reported to reduce the glycosuria and blood glucose of alloxan-diabetic rats (Smith, Meade & Bornstein, 1952). Bilateral adrenalectomy also decreases the severity of the diabetes in these animals, and inhibition of the action of adrenal corticoids was considered a possible mechanism of the effect of salicylate. The influence of salicylate on the glycosuria and hyperglycaemia induced by cortisone in normal rats force-fed a high-carbohydrate diet was therefore investigated. The changes in blood glucose and liver glycogen of adrenalectomized rats produced by cortisone, in the presence and absence of salicylate, were also studied.

EXPERIMENTAL

Animals. Male rats of the Wistar strain, maintained on a diet of commercial cubes to a weight of approximately 300 g., were fed a high-carbohydrate diet consisting of: Ostermilk no. 2 (Glaxo), 6 g. (out-of-date hospital stock); Hepovite (Evans Medical Supplies), 1.5 g.; glucose, 9 g.; water to 30 ml.

Increasing amounts of the high-carbohydrate diet were given for a period of 4 days when full feeding was instituted; each rat then received 15 ml. of the diet by stomach tube at 10 a.m. and 6 p.m. Male rats of the same colony, weighing 150–200 g., were used for the experiments on liver-glycogen content.

Analytical methods. Urinary glucose was determined by the method of Benedict (1911) and urinary non-protein nitrogen (N.P.N.) by a micro-Kjeldahl procedure (Harrison, 1949). Liver-glycogen content was measured by the method of Good, Kramer & Somogyi (1933). The glucose in the final stage of the glycogen estimation, and also the blood glucose, was measured by the method of Nelson (1944).

General arrangement of experiments

Effect of salicylate on the glycosuria and hyperglycaemia induced by cortisone in the normal rat. Eight rats maintained on the high-carbohydrate diet were placed in metabolism cages and 24 hr. urine collections made, feeding by stomach tube being performed at 10 a.m. and 6 p.m. daily. After an initial control period each rat received a subcutaneous injection of 2.5 mg. cortisone (cortone acetate, Merck) immediately before force-feeding; after 3 days the dose of cortisone was increased to 5 mg. to ensure an adequate glycosuria. Salicylate was administered as a subcutaneous injection of an arachis-oil suspension of 100 mg./ml. of

sodium salicylate. The rats were weighed daily and blood glucose was measured on tail-vein samples in each rat immediately before and at intervals of 2, 4, 6 and 8 hr. after the morning feed on one day of the control period, one day of the period in which cortisone only was given and one day when cortisone and salicylate were being administered.

Effect of cortisone, alone and with salicylate, on the liver-glycogen content of adrenalectomized rats. The rats were bilaterally adrenalectomized and maintained for 4 days after operation on a diet consisting of commercial rat cubes, bread and milk, and were given 0.45% (w/v) saline to drink. The 48 adrenalectomized rats were divided into seven groups. Three groups, each containing six animals, were killed by stunning at 0, 4 and 6 hr., two groups were injected with 5 mg. of cortisone/rat at 0 hr. and killed at 4 hr. (seven rats) and 6 hr. (11 rats) respectively; in the two remaining groups of six animals each rat received 5 mg. of cortisone and 100 mg. of sodium salicylate at 0 hr., one group was killed at 4 hr. and the other at 6 hr. No preliminary fast was employed, food being removed at 0 hr. The liver-glycogen content of these animals at 0 hr. was within the normal range, whereas a 24 hr. fast reduced it to practically zero (cf. Long, Katzin & Fry, 1940). No preliminary fast was used in the above experiments because the injection of salicylate into rats having a very low liver-glycogen content killed them within 2 hr. Blood-glucose estimations were made immediately before death, and glycogen was estimated in 80–100 mg. portions of liver taken as nearly as possible from anatomically similar lobes immediately after death.

RESULTS

The effect of salicylate on the glycosuria induced by cortisone in the normal rat. The mean 24 hr. glucose outputs of four of the rats are shown in Fig. 1, where it is seen that injection of salicylate markedly reduced the glycosuria which increased again when salicylate was stopped. During the initial control period there was no loss in body weight but a steady loss occurred when cortisone was given. A decrease of approximately 50 g./rat in body weight occurred during this period and was accompanied by an increase in the urinary N.P.N. excretion. In the control period the average daily urinary N.P.N. was 0.26 g. whereas when cortisone was given the rats excreted a daily average of 0.38 g. in the urine. No further decrease in weight occurred when the animals received salicylates but the average urinary N.P.N. (0.37 g.) was not significantly decreased. Four of the

original eight rats died from acute infections during the experiment and are therefore not included in the final results. Similar toxic effects of cortisone in rats, particularly the marked loss in body weight and death from intercurrent infection, have been reported by Ershoff (1951) and Ingle (1952).

Fig. 1. Mean 24 hr. urinary glucose for a group of four normal rats fed on a high-carbohydrate diet and injected with cortisone and sodium salicylate. Cortisone: 5 or 10 mg. as saline suspension of cortisone acetate (Merck) daily; sodium salicylate: 100 mg. in 1 ml. arachis oil daily. The arrows indicate the days on which the blood-sugar curves in Fig. 2 were obtained.

The effect of salicylate on the hyperglycaemia induced in the normal rat by cortisone. Blood glucose was measured in each rat on tail-vein samples immediately before, and 2, 4, 6 and 8 hr. after, the 10 a.m. feed on the 3 days indicated by the arrows in Fig. 1. The results are shown in Fig. 2 where it is seen that the administration of cortisone caused a hyperglycaemic curve whereas the concurrent injection of salicylate markedly reduced the effect.

The effect of cortisone with and without salicylate on the liver-glycogen content and blood glucose of adrenalectomized rats. The liver-glycogen results are shown in Table 1. These results show that fasting caused

Fig. 2. Mean blood-sugar curves after feeding the rats described in Fig. 1. The animals were fasted for 16 hr. before being given the same amounts of the high-carbohydrate diet by stomach tube. ●, control period, day 7; ▲, cortisone period, day 14; ■, cortisone-salicylate period, day 19.

a significant depletion of glycogen at 6 hr. in the untreated group, whereas cortisone caused a significant deposition of glycogen at 6 hr. The administration of salicylate with the cortisone caused marked depletion of the glycogen at 4 and 6 hr. Comparison of the three groups at 4 hr. showed no significant difference between the untreated and cortisone-treated groups, but the liver glycogen of the salicylate-cortisone-treated group was significantly lower than both. At 6 hr. the liver glycogen of

Table 1. *The effect of cortisone with and without salicylate on the liver glycogen of adrenalectomized rats* (The liver-glycogen contents are given as g. glycogen/100 g. liver.)

(The liver glycogen contents are given as g. glycogen/100 g. liver.)						
	A Untreated		B Cortisone-treated		C Cortisone-salicylate-treated	
Time killed (hr.)	Liver glycogen (%±s.d.)	No. of rats	Liver glycogen (%±s.d.)	No. of rats	Liver glycogen (%±s.d.)	No. of rats
0	2.33±0.55	6	—	—	—	—
4	1.77±0.81	6	3.30±2.9	7	0.5±0.12	6
6	1.45±0.70	6	3.99±2.0	11	0.6±0.2	6
Within groups	...		...		...	
	A			B		
	n	t	P	n	t	P
0 and 4 hr.	10	1.5092	0.2	11	0.8977	0.4
0 and 6 hr.	10	3.1196	0.02	15	1.9970	0.1
	C			B/C		
	n	t	P	n	t	P
	10	7.8829	0.001	11	2.6054	0.05
	10	7.2046	0.001	15	4.1246	0.001
Comparison of liver-glycogen results for groups A, B and C						
	A/B			A/C		
	n	t	P	n	t	P
4 hr.	11	1.3881	0.2	10	4.2561	0.01
6 hr.	15	3.0494	0.01	10	3.3650	0.01

Table 2. *The effect of cortisone with and without salicylate on the fasting blood glucose of adrenalectomized rats*

Time killed (hr.)	A Untreated		B Cortisone-treated		C Cortisone-salicylate-treated	
	Blood glucose (mg./100 ml. $\pm$ s.d.)	No. of rats	Blood glucose (mg./100 ml. $\pm$ s.d.)	No. of rats	Blood glucose (mg./100 ml. $\pm$ s.d.)	No. of rats
0	125 $\pm$ 11	6	—	—	—	—
4	116 $\pm$ 9	6	116 $\pm$ 2	7	102 $\pm$ 26	6
6	106 $\pm$ 12	6	123 $\pm$ 19	11	95 $\pm$ 41	6

Within groups	A			B			C		
	n	t	P	n	t	P	n	t	P
0 and 4 hr.	10	1.6080	0.2	11	2.2733	0.02	10	2.0055	0.1
0 and 6 hr.	10	2.9909	0.01	15	0.2355	0.8	10	1.7254	0.2

Comparison of blood-glucose results for groups A, B and C

	A/B			A/C			B/C		
	n	t	P	n	t	P	n	t	P
4 hr.	11	0.3069	0.8	10	1.2864	0.3	11	1.6166	0.2
6 hr.	15	1.9972	0.1	10	0.6377	0.6	15	1.9728	0.1

the salicylate-cortisone-treated group was again lower than the other groups and the cortisone-treated group had a significantly raised liver glycogen compared with the untreated group. Cortisone therefore caused deposition of liver glycogen at 6 hr., and the concurrent administration of salicylate not only produced depletion of existing glycogen at 4 and 6 hr., but also prevented the deposition of glycogen by the cortisone at 6 hr.

The blood-glucose results are shown in Table 2. This table shows that fasting reduced the blood glucose at 6 hr. in the untreated group but not in the other two groups. Comparison of the three groups at 4 hr. revealed no significant differences, but at 6 hr. the cortisone-treated group had a significantly higher blood glucose than the other two groups. These results suggest that cortisone prevented the reduction in blood glucose at 6 hr. caused by fasting, but this did not occur when salicylate was given concurrently.

DISCUSSION

The present work shows that in the adrenalectomized rat salicylate prevents the deposition of new glycogen by cortisone and therefore inhibits the increased glycogen synthesis caused by cortisone.

In the normal rat, force-fed a high-carbohydrate diet, salicylate reduced the glycosuria and hyperglycaemia induced by cortisone. The increased urinary-nitrogen excretion when cortisone was given showed that increased gluconeogenesis had occurred, and a possible explanation of the effects of salicylate was an inhibition of this process. This was considered unlikely because the concurrent administration of salicylate almost completely suppressed the glyco-

suria but caused no significant reduction in the urinary-nitrogen output. In addition Ingle (1941) has shown that in rats maintained on a similar diet and injected with cortisone, only about 1 g. of the daily urinary glucose could be derived from protein. This worker has suggested that cortisone inhibits the utilization of glucose by the tissues, and an interference with this action may provide an explanation of the effects of salicylate on the glycosuria and hyperglycaemia.

Similar considerations apply to the effect of salicylate in reducing the blood glucose and glycosuria of alloxan-diabetic rats (Smith *et al.* 1952) because Stetten, Welt, Ingle & Morley (1951) using [14 C] glucose have reported that in these animals the rate of glucose production was only barely increased whereas the rate of oxidation of glucose was markedly reduced.

The antagonistic effects of cortisone and salicylate are also of interest because of the suggestion that the therapeutic effects of salicylates in rheumatic fever are primarily mediated by the pituitary and adrenal glands, leading to the production of adrenal corticosteroids (Hetzel & Hine, 1951). If this hypothesis is correct then salicylates and adrenal corticosteroids, of which cortisone may be considered a typical example, should have the same general effects in the tissues.

SUMMARY

1. The effects of salicylate on the glycosuria and hyperglycaemia induced by cortisone in the normal rat, and of cortisone with and without salicylate on the blood glucose and liver glycogen of the adrenalectomized rat have been studied.

2. Salicylate reduces the glycosuria and hyperglycaemia induced by cortisone in the normal rat. Cortisone causes deposition of liver glycogen in the adrenalectomized rat while the concurrent administration of salicylate produces not only depletion of existing glycogen but also prevents the deposition of new glycogen by the cortisone.

3. The implications of these effects of salicylate are discussed.

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Chemical Studies of Peripheral Nerve During Wallerian Degeneration

4. PHOSPHATASES

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When a peripheral nerve is cut, that portion of the nerve distal to the point of section soon loses its ability to transmit a nerve impulse. This is associated with a series of changes characteristic of Wallerian degeneration. The axon of each nerve fibre is destroyed, followed by the physical and later by the chemical destruction of the myelin sheath. There is also a cellular proliferation associated with an increase in the concentration of nucleic acid. Accompanying the destruction of the myelin sheath there is a decrease in the concentration of myelin lipids and neurokeratin (see Young, 1942; Abercrombie & Johnson, 1946; Johnson, McNabb & Rossiter, 1949, 1950; Logan, Mannell & Rossiter, 1952, for references).

With the exception of acid phosphomonoesterase, which was studied by Heinzen (1947) and Bodian (1947), chemical determinations of the activity of enzymes in peripheral nerve during Wallerian degeneration have been confined to those enzymes responsible for the destruction or the synthesis of acetylcholine. In this paper, changes in the activity of a number of phosphatases are described. During the degeneration, there is an increase in the rate of hydrolysis of phenylphosphate at pH 4.9 (acid phosphomonoesterase) and of adenosine-5-phosphate at pH 7.5 (5-nucleotidase), a fall in the rate of

hydrolysis of phenylphosphate at pH 9.9 (alkaline phosphomonoesterase) and little change in the rate of hydrolysis of adenosinetriphosphate (ATP) at pH 7.2 in the presence of Ca^{++} (adenosinetriphosphatase).

When the nerve is crushed (axonotmesis) rather than sectioned (neurotmesis), a procedure that interrupts the axons and myelin sheaths, but leaves the connective tissue sheaths intact, it degenerates as before. After a short latent period, the degenerating peripheral segment is re-innervated by axon tips from the intact central stump. There is thus a regeneration superimposed upon the degeneration. During the phase of degeneration the changes in the activity of the phosphatases are similar to those following nerve section. During the phase of regeneration the myelin lipids and neurokeratin slowly reappear and the concentration of nucleic acid falls (Burt, McNabb & Rossiter, 1950; Logan *et al.* 1952). There is an increase in the activity of alkaline phosphomonoesterase and adenosinetriphosphatase. While the activity of these two enzymes is increasing, the activity of acid phosphomonoesterases and 5-nucleotidase is falling. A preliminary report of some of these experiments has already appeared (Hollinger & Rossiter, 1952).