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EFFECT OF SODIUM SALICYLATE AND 2:4-DINITROPHENOL ON THE DISTRIBUTION OF GALACTOSE IN THE EVISCERATED NEPHRECTOMISED RAT

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Abstract—Sodium salicylate and 2:4-dinitrophenol have been administered to rats, and the effect of these compounds on the uptake of D-galactose has been examined in the subsequently eviscerated nephrectomised animals. Sodium salicylate and 2:4-dinitrophenol appear to increase the penetration of the intracellular water by this sugar. This observation is discussed in relation to the effect of these compounds on oxidative phosphorylation.

THE use of salicylate in controlling the hyperglycaemia of diabetes mellitus in humans is well documented.[1, 2] Hecht and Goldner[3] observed that administration of aspirin lowers the fasting blood sugar concentration and increases the glucose tolerance in both normal and diabetic patients, the effect being most evident in the diabetics. Salicylate has been shown also to produce a reduction of the hyperglycaemia in partially pancreatectomised animals[4] and in alloxan-diabetic animals.[5]

In an attempt to elucidate this action of salicylate, its effect on the uptake of glucose by isolated tissue preparations has been studied. For example, Randle and Smith[6] showed salicylate and 2:4-dinitrophenol to accelerate the uptake of glucose and xylose by the rat diaphragm *in vitro*, an observation which was later confirmed by Morgan *et al.*[7] using glucose in the perfused isolated rat heart preparation. This effect of salicylate and 2:4-dinitrophenol may be related to their ability to uncouple oxidative phosphorylation in mitochondrial preparations.[8, 9]

When glucose is used as an indicator sugar, the effects observed are the result of a series of reactions involving uptake, phosphorylation, glycogenesis and catabolism. As any of these reactions may be the rate-limiting step, the effect observed due to treatment with different agents would be difficult to attribute to any one reaction. Therefore the use of a sugar in a preparation in which it is virtually not metabolised would allow observations on the uptake of the sugar only. Levine *et al.*[10] and Wick and Drury[11] showed galactose to be transferred to the intracellular water by the same system responsible for the transfer of glucose, with little or no subsequent metabolism. Randle and Smith[6, 12] and Morgan *et al.*[7] studied the transfer of glucose and some non-metabolised sugars in isolated tissue preparations under the influence of salicylate, 2:4-dinitrophenol and anaerobiosis.

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The following experiments were performed in order to examine the effects of salicylate and 2:4-dinitrophenol on the system responsible for the transfer of D-galactose from the extracellular to the intracellular water in eviscerated nephrectomised rats.

METHODS

Female albino rats of the Wistar strain, weighing between 150 and 200 g, were used throughout. Sodium salicylate and 2:4-dinitrophenol were administered in the drinking fluids supplied to two groups of animals for a period of seven days prior to the observation of the galactose distribution. These drinking fluids had the following composition: *Salicylate drinking fluid:* sodium salicylate, 0·25%; sodium bicarbonate, 0·18%; sodium metabisulphate, 0·006% and tap water to 100. 2:4-*dinitrophenol drinking fluid:* 2:4-dinitrophenol, 0·25%; sodium bicarbonate, 0·294%; sodium metabisulphite, 0·006% and tap water to 100. Animals which were to serve as controls were maintained on tap water only. The animals were placed in individual cages and daily measurements were made of the fluid intake to the nearest 5 ml so that the total amount of each compound consumed by each animal was known. The food consumption and body weight of each animal were also measured daily to the nearest 5 g.

All animals were deprived of food 24 hr before the examination of the volume of distribution of galactose. They were then anaesthetised by the intraperitoneal injection of urethane, 0·5 ml/100 g of a 25 per cent w/v solution in normal saline, eviscerated and nephrectomised. The preparation was weighed to the nearest gramme to give the "eviscerated carcass weight". Heparin-filled polythene cannulae were then inserted into the right external jugular vein and the left carotid artery, and heparin, 100 units/100 g eviscerated carcass weight, was injected intravenously as an anticoagulant. The preparation was placed in a perspex air chamber maintained at 28°C by a thermostatically controlled water jacket so as to allow free access to the cannulae. After 20 min, 0·15 to 0·2 ml blood was removed from the arterial cannula. An aqueous solution of D-galactose, 300 mg/ml, was then slowly injected intravenously in a dose volume of approximately 0·25 ml/100 g eviscerated carcass weight. The total amount of galactose administered was recorded. Further blood samples were removed at suitable intervals after the injection.

Galactose was determined by the method of Somogyi, modified by Nelson,[13] using 0·1 ml aliquots of either blood or plasma. The glucose also present was removed by prior incubation at 37°C for 30 min with 0·2 ml of a 20 per cent v/v suspension of washed bakers' yeast. The initial blood sample served to demonstrate the complete removal of glucose by fermentation with yeast.

In some of the experiments the eviscerated nephrectomised animals were injected with a solution containing inulin, 50 mg/ml as well as galactose. The concentration of inulin in the plasma was estimated by the method described by Smith[11] using 0·1 ml plasma. The volume of distribution of galactose and inulin were calculated from the following expression:

$$\text{Volume of distribution per 100 g eviscerated carcass weight} = \frac{\text{Total dose administered (mg)} \times 10{,}000}{\text{mg of sugar in 100 ml of blood or plasma} \times \text{eviscerated carcass weight}}$$

The concentration of salicylate in the plasma at the time of the estimation of the volume of distribution of galactose was determined by the method of Smith and Talbot,[15] using 0·1 ml plasma.

The concentration of 2:4-dinitrophenol in the plasma was not estimated.

Initial experiments showed the recovery of galactose from blood to be 94·4 per cent with a standard error of ±1·9 from 6 determinations. The recovery of inulin from plasma was found to be 104·3 per cent with a standard error of ±1·1 from 10 determinations, and the recovery of salicylate from plasma was found to be 103·4 per cent with a standard error of ±4·2 from 10 determinations.

In order to determine the likelihood of errors being incurred by estimating the galactose content of plasma instead of whole blood, galactose dissolved in normal saline was added to whole blood and mixed thoroughly by rotation at room temperature for 1 hr. By estimating the packed cell volume of this blood, the theoretical content of galactose in the plasma could be estimated, assuming none would be retained by the blood cells. Analysis of this sample showed the recovery of galactose in whole blood to be 88·75 per cent with a standard error of ±1·41 from 6 determinations. Similarly, the recovery of galactose in the plasma separated from this blood sample was found to be 87·74 per cent with a standard error ±0·44 from 6 determinations. Whence it appears that all the galactose added to the blood sample was retained in the plasma, an observation which confirms the findings of Levine *et al.*[10]

RESULTS

Table 1 shows that when rats were maintained on salicylate or on 2:4-dinitrophenol drinking fluid for seven days, there was an increase in the volume of distribution of galactose at varying time intervals following the injection of this sugar. Estimations were made using whole blood.

TABLE 1. EFFECT OF PROLONGED PRETREATMENT WITH SODIUM SALICYLATE AND 2:4-DINITROPHENOL ON THE INITIAL DISTRIBUTION VALUES OF GALACTOSE IN EVISCERATED NEPHRECTOMISED RATS

Treatment	Plasma salicylate (mg/100 ml)	Volume of distribution of galactose (ml/100 g eviscerated carcass weight)			
		5 min after injection	12 min after injection	19 min after injection	26 min after injection
Water	—	30·2 ± 0·9 (35)	40·2 ± 1·0 (34)	45·6 ± 1·4 (34)	49·8 ± 1·7 (33)
Salicylate	19·7 ± 1·7 (33)	33·0 ± 0·7* (33)	42·7 ± 1·0 (33)	50·2 ± 1·3* (33)	52·5 ± 1·6 (33)
2:4-Dinitrophenol	—	38·0 ± 1·5* (16)	49·9 ± 2·3* (15)	57·8 ± 3·0* (15)	56·8 ± 3·1* (12)

Mean values ±S.E. (number of estimations).
* Significantly different from water (control) value ($P < 0·05$).

However, as observations of fluid intake and body weight indicated that the rats may be in a state of dehydration due to the salicylate and 2:4-dinitrophenol drinking fluids (Table 2), it was considered desirable to obtain an index of the volume of the

extracellular water at the time of the examination of the volume of distribution of galactose by the concomitant estimation of the volume of distribution of inulin. The plasma concentration of these two sugars was estimated 90 min after their intravenous injection as Levine *et al.*[10] had shown there to be no further increase in the value for the volume of distribution of galactose in the rat after this time. Although the distribution of inulin in mammalian tissues increases relatively slowly and is unlikely to have

TABLE 2. EFFECT OF SALICYLATE AND 2:4-DINITROPHENOL ON BODY WEIGHT AND FLUID CONSUMPTION OF RATS

Drinking fluid	Change of body weight Days 1–6 (g)	Fluid intake (ml/100 g body weight per day)
Water	0·0 ± 0·0 (5)	21·7 ± 1·4 (5)
Salicylate	−2·0 ± 2·0 (5)	8·9 ± 0·6* (6)
2:4-Dinitrophenol	−36·3 ± 3·2* (4)	5·4 ± 0·1* (4)

Mean values ± S.E. (number of observations).
* Significantly different from water (control) values ($P < 0.001$).

TABLE 3. EFFECT OF PROLONGED PRETREATMENT WITH SODIUM SALICYLATE AND 2:4-DINITROPHENOL ON THE PENETRATION OF THE INTRACELLULAR WATER BY GALACTOSE IN EVISCERATED NEPHRECTOMISED RATS

Treatment	Plasma salicylate (mg/100 ml)	Volume of distribution (ml/100 g eviscerated carcass weight)		Galactose minus inulin
		Galactose	Inulin	
Water	—	26·5 ± 0·9 (4)	14·8 ± 0·3 (4)	11·7 ± 1·2 (4)
Salicylate	18·3 ± 1·6 (5)	30·0 ± 1·1 (5)	14·4 ± 0·7 (5)	15·7 ± 0·6* (5)
2:4-Dinitrophenol	—	28·3 ± 0·8* (5)	12·9 ± 0·4* (5)	15·4 ± 0·8* (5)

Mean values ± S.E. (number of estimations).
* Significantly different from the water (control) value ($P < 0.05$).

reached its maximum value at 90 min, it is nevertheless apparent that the value for its volume of distribution at this time gives a relative, but not an absolute, estimate of the volume of the extracellular water.

Estimation of the volume of distribution of galactose reflects the volume of the total body water available to this sugar (extracellular plus intracellular). As galactose is not restricted to the extracellular water, the difference between the volumes of distribution of galactose and of inulin gives an estimate of the relative volume of the intracellular water penetrated by galactose. Table 3 shows that 2:4-dinitrophenol caused a significant reduction in the volume of distribution of inulin and a significant increase in the

volume of distribution of galactose. Salicylate caused non-significant changes in these values. However, the difference between the volumes of distribution of galactose and inulin demonstrates that the two uncoupling agents produced a significant increase in the penetration of the intracellular water by the galactose.

In order to facilitate the estimation of inulin and salicylate, which can be performed only on plasma, the concentration of galactose was determined in plasma instead of in whole blood. The values for the volume of distribution of galactose at 90 min are, therefore, lower than those indicated for the distribution of galactose at 26 min in whole blood (see Table 1).

DISCUSSION

The results presented above show sodium salicylate and 2:4-dinitrophenol to increase the volume available for the distribution of galactose in eviscerated nephrectomised rats. This observation demonstrates a qualitative similarity in the effect of these two uncoupling agents on the uptake of galactose by the musculature of the eviscerated nephrectomised rat preparation. Similarities in the behaviour of mitochondria under the influence of these compounds are also well documented. They have both been shown to cause uncoupling of oxidative phosphorylation in intact mitochondria[8, 9] and in submitochondrial fractions produced by treatment of mitochondria with digitonin.[16] They also inhibit the spontaneous swelling of mitochondria in hypotonic sucrose media.[16–18] It would appear therefore that salicylate and 2:4-dinitrophenol are capable of exerting qualitatively similar effects in submitochondrial fractions, in intact mitochondria and on the system responsible for the transfer of galactose to the intracellular water of the eviscerated nephrectomised rat. In each instance the data available indicates the difference between the two to be only quantitative. This is in agreement with the findings of Falcone *et al.*[19] who showed salicylate and 2:4-dinitrophenol to have qualitatively similar effects on the [^{32}P] P_i-ATP and P_i-$H_2{}^{18}O$ exchange reactions and also on the stimulation of adenosine triphosphatase in rat-liver mitochondria. 2:4-Dinitrophenol was, in each instance, the more potent of the two.

A possibility exists that the incorporation of sodium metabisulphite and sodium bicarbonate in the salicylate and 2:4-dinitrophenol drinking fluids may have influenced the findings in the rats treated with these fluids. However, as the volume available for the distribution of galactose in the 2:4-dinitrophenol-treated group of rats was greater than that observed in the salicylate-treated group of rats, it would appear that the metabisulphite and bicarbonate did not contribute to the observed results.

Randle and Smith[12] suggested that the energy of the cells is normally engaged in restraining the entry of glucose. Therefore any treatment interfering with the supply of this energy (salicylate, 2:4-dinitrophenol, anaerobiosis) would allow an increased influx of the sugar. Randle[20] put forward an hypothesis to explain this effect of uncoupling agents and anaerobiosis in which he suggested that the transport system may exist in a phosphorylated (inactive) and a non-phosphorylated (active) form, phosphorylation of the system being effected by adenosine triphosphate. Anoxia and uncoupling agents would deplete the tissues of high energy phosphate bond compounds such as adenosine triphosphate. Randle added, however, that at the present time there is no direct evidence for or against this hypothesis. Nevertheless, although direct

evidence is lacking, much circumstantial evidence exists which is compatible with this hypothesis. This evidence may be summarised as follows:

Compounds which have been shown to lower the adenosine triphosphate content of tissues increase the rate of utilisation of glucose and increase both the rate of uptake and degree of intracellular penetration of certain non-utilisable sugars. Anaerobiosis complies with Randle's hypothesis in so far as it deprives the reduced nicotinamide adenine dinucleotide ($NADH_2$)–flavoprotein–cytochrome span in which adenosine triphosphate is formed of the ultimate electron acceptor, oxygen, and so generally suppresses the formation of high energy phosphate bond compounds. The results obtained with salicylate and 2:4-dinitrophenol in this work may be explained similarly by Randle's hypothesis.

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