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Some Hormonal Influences on the Glucose Uptake of Normal Rat Diaphragm *in vitro*

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It has been reported that serum from alloxan-diabetic rats inhibits the glucose uptake *in vitro* of diaphragm from normal rats (Tuerkischer & Wertheimer, 1948). Bornstein & Park (1953) confirmed and extended this observation, showing that hypophysectomy of the alloxan-diabetic animal is followed by a loss of the inhibitory properties of the serum. They have further shown that the simultaneous administration of growth hormone and cortisone to the hypophysectomized alloxan-diabetic rat results in the reappearance of the inhibitor of glucose uptake, although the administration of either hormone alone, or the addition of either or both hormones to the incubation medium, is without effect on the glucose utilization *in vitro* of the isolated rat diaphragm.

The present investigation confirms and extends these observations of Bornstein & Park (1953).

METHODS

Animals. Serum was prepared from the blood of albino rats of an inbred strain weighing 200–300 g. and maintained on a stock laboratory diet (Short & Parkes, 1949). Diabetes was induced in rats fasting for 24–48 hr. by a single intravenous injection of alloxan (45 mg./kg.). The alloxan was synthesized in this Laboratory by the method of Hartman & Sheppard (1943). Only those animals which had a 24 hr. fasting blood-glucose value of at least 250 mg./100 ml. at least 3 weeks after the injection of alloxan were used for these studies. One group of diabetic rats was subsequently hypophysectomized by the parapharyngeal approach and used 10 days later for experiment.

All serum-donor animals, whether operated, treated or control, had free access to water but were without food for 18–20 hr. before they were used. Each rat was lightly anaesthetized with Nembutal, the blood was drawn into a syringe from the dorsal aorta, and serum was prepared. The glucose content of the serum was determined, and each sample was diluted with an equal vol. of buffer (Gey & Gey, 1936). The final glucose concentration of the diluted serum was adjusted to 250 mg./100 ml. by the addition of glucose. In what follows the term serum will refer to serum diluted in this manner.

Hormone preparations. Growth hormone was prepared by a modification of the method of Wilhelmi, Fishman & Russell (1948). For injection the hormone was dissolved in 0.9% NaCl at pH 8.5–9.0.

Procedure. Hypophysectomized alloxan-diabetic rats were treated with growth hormone by intraperitoneal injection at the rate of 200 µg./rat/day. Cortisone acetate was administered as a suspension in 0.9% NaCl by intraperitoneal injection at a rate of 100 µg./rat/day. Normal rats were treated in one of three different ways. In the first experiment growth hormone was given, where indicated, at the rate of 2 mg./rat/day and cortisone at the rate of 1 mg./rat/day, for 3 days. In the second experiment 3 mg. of growth hormone and 5 mg. of cortisone/rat/day were administered for 3 days. In the third experiment the latter dosages of growth hormone and of cortisone were administered for 14 days. In all instances the treated serum-donors remained without food for 18 hr. after treatment and before serum was prepared. Control animals received appropriate treatment with 0.9% NaCl.

Diaphragm tissue was obtained from normal rats weighing 100–130 g. which had fasted for 18–20 hr. The rats were decapitated, the diaphragms quickly and gently excised, divided into halves and allowed to soak for 15 min. in buffer (Gey & Gey, 1936) containing 250 mg. of glucose/100 ml. Each hemidiaphragm was then blotted and transferred to a beaker containing 1 ml. of the incubation medium (either buffer or the previously prepared serum). The tissues were incubated in a Dubnoff metabolic shaker for 90 min. at 37°, with a gas phase of O₂ + CO₂ (93:7). At the end of the incubation the hemidiaphragms were blotted and weighed, and the glucose content of each beaker was estimated

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according to the Somogyi (1952) modification of the Nelson method. The difference between the initial and final glucose concentrations was used to calculate the glucose uptake of the diaphragm in terms of glucose utilized/g. of tissue/hr.

RESULTS

Table 1 shows that the glucose uptake of the isolated normal rat diaphragm is greater when the diaphragm is incubated in normal rat serum than when it is incubated in buffer. When serum from hypophysectomized alloxan-diabetic rats, or from hypophysectomized alloxan-diabetic rats treated with either growth hormone or cortisone, is used as the incubating medium, the glucose uptake of isolated

normal diaphragm is greater than that of similar diaphragm tissue incubated in buffer. With serum from the hypophysectomized alloxan-diabetic rat treated with both growth hormone and cortisone, the glucose uptake of the isolated normal diaphragm is no greater than that found with buffer. Thus it is seen that the glucose uptake with serum from the hypophysectomized-diabetic rat treated with both hormones is similar to that with diabetic serum, and significantly lower than with serum from hypophysectomized alloxan-diabetic animals treated with either hormone alone. This is in general agreement with the observations of Bornstein & Park (1953), although our experiments were carried out under rather different conditions.

Table 1. *Glucose uptake of normal rat diaphragm in different sera in vitro*

Serum was diluted with an equal vol. of Gey & Gey (1936) buffer. Final glucose concn. of the medium was adjusted to 250 mg./100 ml. Hypox = hypophysectomized; GH = growth hormone.

Expt.	Origin of serum	No. of hemidiaphragms in each group	Glucose uptake (mg./g. of wet diaphragm/hr.; mean $\pm$ S.E.M.)	Significance (<i>P</i>) of difference in glucose uptake
A	(Buffer)	17	3.21 $\pm$ 0.26	A - B <0.01 A - D <0.01 A - E <0.01
B	Normal rat	14	4.99 $\pm$ 0.20	A - F <0.01
C	Diabetic rat	13	3.37 $\pm$ 0.35	C - B <0.01 C - D <0.01 C - E <0.01 C - F <0.01
D	Hypox-diabetic rat	28	5.38 $\pm$ 0.28	—
E	Hypox-diabetic rat treated with GH	14	5.09 $\pm$ 0.30	G - B <0.01 G - D <0.01 G - E <0.01 G - F <0.01
F	Hypox-diabetic rat treated with cortisone	15	4.79 $\pm$ 0.30	—
G	Hypox-diabetic rat treated with GH and cortisone	28	3.41 $\pm$ 0.22	—

Table 2. *Glucose uptake of normal rat diaphragm in vitro in sera from normal rats treated with growth hormone (GH) or cortisone or both*

Results are expressed as the mean value of the glucose uptake (mg. of glucose/g. wet wt. of diaphragm/hr.) $\pm$ S.E.M. (no. of observations).

Expt. no.	Glucose uptake in serum from rats treated with			
	Saline	Growth hormone (GH)	Cortisone	GH + cortisone
		GH 2 mg./rat/day, cortisone 1 mg./rat/day, for 3 days		
1	4.32 $\pm$ 0.21 (12)	5.09 $\pm$ 0.47 (6)	4.34 $\pm$ 0.39 (6)	4.93 $\pm$ 0.27 (12)
		GH 3 mg./rat/day, cortisone 5 mg./rat/day, for 4 days		
2	4.73 $\pm$ 0.25 (12)	5.38 $\pm$ 0.28 (12)	5.22 $\pm$ 0.28 (6)	5.05 $\pm$ 0.35 (12)
		GH 3 mg./rat/day, cortisone 5 mg./rat/day, for 14 days		
3	(a) 4.58 $\pm$ 0.12 (11)	—	—	(a) 4.09 $\pm$ 0.18 (11)
	(b) 6.16 $\pm$ 0.19 (11)*	—	—	(b) 4.94 $\pm$ 0.19 (11)*

* Insulin (0.1 unit/ml.) was added to serum.

Table 3. *Fasting serum-glucose values of the rats used in Expts. 1-3 (Table 2)*

Expt. no.	Serum glucose (mg./100 ml.) of animals treated with			
	Saline	Growth hormone (GH)	Cortisone	GH + cortisone
1	87	80	107	74
2	79	84	129	90
3	93	—	—	82

Table 2 shows the glucose uptake of diaphragms from normal rats, incubated in serum from normal intact rats which had been treated with either growth hormone or cortisone or with both hormones simultaneously. In Table 3 are shown the average blood-sugar levels of the donors of the serum for these experiments. In the first and second experiments (Table 2) there is no significant difference between the glucose uptakes of diaphragms in the presence of serum from normal untreated rats, from normal rats treated with either cortisone or growth hormone or from normal rats receiving both hormones simultaneously. Nevertheless, in Expt. 3 (Table 2), involving a longer period of treatment with both hormones, the glucose uptake of the rat diaphragm incubated in serum from normal rats treated with growth hormone and cortisone is significantly lower ($P < 0.05$) than that observed with serum from normal untreated animals. In this experiment the ability of insulin, added *in vitro*, to stimulate glucose uptake was only about one-half of that observed in the presence of serum from control rats (Table 2, Expt. 3).

The fasting blood sugar of the serum-donor animals which were treated with growth hormone or cortisone or both was not markedly elevated (Table 3).

DISCUSSION

The failure of serum from the alloxan-diabetic rat to promote the glucose uptake of the isolated normal rat diaphragm is unlikely to be due to a deficiency of insulin alone. Since the ability of the serum from the alloxan-diabetic rat is restored by hypophysectomy the failure of the diabetic serum to promote the uptake of glucose is likely to result at least in part from the presence of an inhibitor of glucose uptake which disappears after hypophysectomy. In the absence of evidence to the contrary it may be tentatively assumed that the inhibitor of glucose uptake which appears in the serum of hypophysectomized alloxan-diabetic rats when these animals are treated with both growth hormone and cortisone is identical with that in the serum of alloxan-diabetic rats.

No depression of glucose uptake is seen with the serum of normal rats when these are treated with both growth hormone and cortisone unless the treatment is continued for 14 days (Table 2). Even then the depression is small, although it is statistically significant in our experiments. The depression of glucose uptake seen in these experiments may well be due to the appearance in the serum of an inhibitor of the type considered above.

It has been reported that the simultaneous administration of corticotropin and growth hormone (Engel, Viau, Coggin & Lynn, 1952), or cortisone and growth hormone (Bogdonoff, Scherr, Lister, Owen & Engel, 1955), induces diabetes in normal rats force-fed with a high-carbohydrate diet. In our experiments no diabetes had been induced in normal rats by the injection of either growth hormone or cortisone or both, as judged by the fasting blood-sugar values 18 hr. after the last injection. However, it should be emphasized that in these experiments the animals were fed a stock diet *ad lib.* and blood sugars were determined after a fast of 18 hr., in contrast with the experiments of Engel *et al.* (1952) and Bogdonoff *et al.* (1955).

Our results with hypophysectomized-diabetic rats agree with those of Bornstein & Park (1953). The mechanism of action of the supposed inhibitor remains obscure and a discussion of the possible mechanisms involved is not at present of value.

SUMMARY

1. When normal rat diaphragm is incubated with serum from hypophysectomized-diabetic rats the glucose uptake is: (a) greater than in the presence of serum from diabetic rats; (b) similar to that with serum from hypophysectomized-diabetic rats treated with either growth hormone or cortisone; (c) greater than that with serum from hypophysectomized-diabetic rats treated with growth hormone and cortisone simultaneously.

2. When normal rat diaphragm is incubated with serum from normal rats treated with growth hormone and cortisone simultaneously for 14 days, the glucose uptake *in vitro* is slightly but significantly lower than that observed with diaphragm in normal rat serum.

3. These results are compatible with the view that there is present in the serum of diabetic rats an inhibitor of glucose uptake by diaphragm tissue, the presence of which depends upon the availability of both growth hormone and cortisone or related substances. This inhibitor can appear in the serum of the normal rat treated with both growth hormone and cortisone.

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Metabolism and Metabolic Response to Electrical Pulses in White Matter from the Central Nervous System

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The greater part of electrophysiological and metabolic work on the peripheral nervous system has been carried out with medullated fibres, as these preponderate in convenient samples such as the sciatic nerve. On the other hand, most observations on the central nervous system are with grey matter, probably because this preponderates in the cerebral cortex and is most active metabolically. Present observations aim at linking these two types of study by examining cerebral white matter. An opportunity likely to be afforded by cerebral white matter rather than by grey is the possibility of observing phenomena related to transmission along fibre tracts some millimetres in length. For electrical stimulation in directions related to fibre tracts sheets of white matter were desired, and in small animals these were found most conveniently in the subcortical white matter, with which most of the present studies have accordingly been made. Parts of the brain stem, medulla and thalamus have also been included.

A prerequisite was to know which substrates would maintain respiration, the level of phosphocreatine and the respiratory response to electrical pulses in the separated tissue, especially as peripheral nerve is suspected of differing from the cerebral cortex in this respect. A variety of different substrates have therefore been studied with white matter in the way in which grey matter had previously been examined (Kratzing, 1953).

EXPERIMENTAL

Media and tissues. Media were those employed by Kratzing (1953), all containing 2.75 mM-CaCl₂ and glycylglycine as the main buffer at pH 7.4. Tissues were prepared from the brain of guinea pigs as sheets by cutting with a blade and guide (McIlwain, 1951), grey matter 0.35 mm. and

white matter 0.4 mm. in thickness. Subcortical white matter was obtained by exposing the lateral ventricle, placing the guide upon it and cutting and floating the slice to a dish of medium. It was here inspected and any portions of grey matter were cut off. Normally, however, it contained little grey matter, and white matter was left on the uncut portion of the hemisphere. This uncut portion was inverted and the slices of grey matter were cut from it. Medulla was taken from the ventral surface of the lower part of the brain just posterior to the pons; two successive slices parallel to the outer surface were cut and were visibly different from each other in structure. In a few observations with thalamus and midbrain (at the level of the anterior colliculi), these were chopped in the machine of McIlwain & Buddle (1953) at 0.35 mm. intervals. Sciatic nerve, also from the guinea pig, was freed from its outer sheath and adherent fat.

In a given experiment, tissues from two or three different parts were compared in their behaviour towards a chosen substrate. From one animal were obtained six or eight specimens of cerebral cortex, and two specimens of the other parts named above. In most experiments tissues from one guinea pig were prepared in the order: subcortical white matter, grey matter of the cerebral cortex, and medulla; these specimens were trimmed to rectangles about 40–50 mg. in weight which fitted grid electrodes *H* used in vessels *A* (Ayres & McIlwain, 1953). All specimens of these tissues were placed in electrodes whether or not they were to receive pulses. Electrical condenser pulses were from the generator of Ayres & McIlwain (1953), at 100/sec., peak potential 10 v, and time constant 0.4 msec.

Determinations. Respiration was determined manometrically by conventional procedures. At the end of experiments 1 ml. of the fluid was taken for determination of lactic acid according to Barker & Summerson (1941).

In the experiments in which tissue content of phosphates was to be determined (Table 3), the procedure after making manometric measurements was as follows. A vessel was removed from the thermostat, its electrode holder carrying the tissue was rapidly removed to a shallow dish of medium and opened, and the tissue lifted on a mounted bent wire; it was drained from medium for 1 sec. against the dish,