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The Metabolism of Glucose in Diaphragm Muscle from Normal Rats, from Streptozotocin-Treated Diabetic Rats and from Rats Treated with Anti-Insulin Serum

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1. The metabolism of [U-^{14}C]glucose by the isolated diaphragm muscle of normal rats, rats rendered diabetic with streptozotocin and rats with transitory insulin deficiency after an injection of anti-insulin serum was studied. 2. The incorporation of [^{14}C]glucose into glycogen and oligosaccharides was significantly decreased in the diabetic diaphragm muscle and in the muscle from rats treated with anti-insulin serum. 3. Neither diabetes nor transitory insulin deficiency influenced the oxidation of glucose, or the formation of lactate and hexose phosphate esters from glucose. 4. Insulin fully restored the incorporation of glucose into glycogen and maltotetraose in the diabetic muscle, but the incorporation into oligosaccharides, although increased in the presence of insulin, was significantly lower than the values obtained with normal diaphragm in the presence of insulin.

The only diabetogenic agent available for experimental work until recent years was alloxan. However, the toxic effects of this substance, which manifest themselves in addition to its effect on the pancreas, have always introduced considerable complications in the study of experimental diabetes. The introduction of streptozotocin as a diabetogenic agent, with a specific and rapid action on the β-cells of the pancreas, resulting in total degranulation without cytological disintegration (Rakieten, Rakieten & Nadkarni, 1963; Arison, Ciaccio, Glitzer, Cassaro & Pruss, 1967), has now considerably facilitated such studies. It was therefore decided to study the glucose metabolism in diaphragm muscle from streptozotocin-diabetic rats. It was also decided to carry out a similar study of glucose metabolism in diaphragm muscle from rats treated with anti-insulin serum and thus rendered temporarily insulin-deficient as manifested by hyperglycaemia (Wright, 1961). The results of these experiments are reported in the present work.

EXPERIMENTAL

Animals. Fed male Sprague–Dawley rats (280–320g.) were used. Streptozotocin (65mg./kg.) was given intravenously 7–10 days before the animals were killed. The animals were fed *ad libitum* during the week after the injection and had free access to water; in most cases they lost about 50g. in weight during this period. The rats treated with anti-insulin serum were given intravenously 1·0ml. of guinea-pig anti-insulin serum 1hr. before removal of the diaphragm. The potency of the anti-insulin serum used was such that 0·5ml. injected intravenously produced an increase of blood glucose concentration of 150$\pm$20mg./100ml. 1hr. after the injection in 120–140g. rats.

Blood glucose concentration was determined, by the glucose oxidase method, both with the streptozotocin-treated and the anti-insulin-serum-treated animals, on deproteinized whole blood obtained by venepuncture at the time of removal of the diaphragm.

Materials. [U-^{14}C]Glucose was obtained from The Radiochemical Centre, Amersham, Bucks. Guinea-pig anti-insulin serum was prepared in this Department as described by Mansford (1967). Streptozotocin was kindly given by Upjohn Ltd., Kalamazoo, Mich., U.S.A. Crystalline glucagon-free insulin was obtained from Burroughs Wellcome, Beckenham, Kent.

Incubation of diaphragm muscle. In all experiments the animals were anaesthetized with ether and the diaphragm muscle was rapidly removed. In experiments in which the diaphragm muscle of normal and streptozotocin-treated animals or normal and anti-insulin-serum-treated animals were compared, portions, each weighing 80–120mg., of the hemidiaphragms of each animal were incubated separately. In those experiments in which the influence of insulin was studied, the hemidiaphragms were divided into two parts, two parts being incubated without insulin and two parts with insulin. In all experiments incubation was carried out in Warburg vessels in 2ml. of Krebs–Henseleit Ringer phosphate buffer (Krebs & Henseleit, 1932) containing 11mM-glucose and approx. 10μc of [U-^{14}C]glucose. Insulin concentration was 0·01 unit/ml. Incubation was carried out for 90min. at 37° in O_2.

Preparation of samples and radioactive measurements. The method of preparation of tissue extracts after incubation and the chromatography methods were identical with those described by Beloff-Chain *et al.* (1955).

The radiochromatograms were scanned by an automatic scanning device developed in this Department (E. B. Chain,

A. E. Lowe & K. R. L. Mansford, unpublished work). The scanning device is a computerized modification of that developed by Frank, Chain, Pocchiari & Rossi (1959) made approximately twice as sensitive by replacing the end window Geiger counters with gas-flow counters. The counting efficiency is 11% and the reproducibility of the scanning results is ±7%. The computer programme corrects for counting efficiency and variations between scanners and the output expresses the radioactivity of the intermediates separated on the chromatograms in μμc.

CO_2 was collected during incubation in the centre well of the vessel in 0·15 ml. of Hyamine and transferred at the end of the incubation period to 10 ml. of scintillation fluid (Bray, 1960). At the beginning of each experiment samples of the incubation medium were also transferred to 10 ml. of scintillation fluid. The ^{14}C contents of the CO_2 produced during incubation and of the [U-^{14}C]glucose initially present in the medium were counted in a liquid-scintillation counter. The counting efficiency was determined by the channels-ratio method (Baillie, 1960), so that the results could be expressed as μμc. The specific radioactivity of the glucose present in the incubation medium was thus determined and the results are expressed in all Tables as mμmoles of substrate converted into the intermediates measured by radiochromatography and into CO_2.

Table 1. *Metabolism of glucose in diaphragm muscle from normal and streptozotocin-diabetic rats*

Results are expressed as mμmoles of substrate converted and of unchanged substrate/100 mg. of tissue after 90 min. incubation at 37° in O_2 in 2 ml. of Krebs–Ringer phosphate-buffered medium, pH 7·4, containing 11 mM-glucose (approx. 10 μc of [U-^{14}C]glucose/vessel). Mean values ±S.E.M. for 11 normal and 11 diabetic rats are given, with the numbers of pieces of diaphragm muscle in parentheses.

	Normal	Diabetic
CO_2	344±24·3 (19)	347±10·1 (21)
Lactate	604±67·3 (20)	465±19·8 (22)
Glycogen	79±5·8 (20)	48±3·4* (20)
Maltotetraose	32±3·8 (20)	16±3·0* (19)
Maltotriose	68±6·5 (21)	47±2·9* (21)
Maltose	66±7·2 (21)	61±4·5 (21)
Glucose in tissue	370±19·7 (21)	435±2·8 (21)
Phosphate esters	63±7·1 (19)	50±6·9 (21)

* Difference between normal and diabetic significant ($P \leqslant 0·01$).

RESULTS

Blood glucose concentration. The mean values of the blood glucose concentration of the 16 streptozotocin-diabetic rats used in the following experiments (Tables 1 and 2) was 371±21 mg./100 ml. That of the six anti-insulin-serum-treated rats (Table 3) was 267±10 mg./100 ml. The mean blood glucose concentration in the normal rats was 109±6 mg./100 ml.

Metabolism of glucose in diaphragm muscle from normal, diabetic and anti-insulin-serum-treated rats. The results given in Table 1 show that there was a significant decrease in glucose incorporation into glycogen and oligosaccharides in the diaphragm muscle from diabetic rats. There was no significant effect on the oxidation of glucose to carbon dioxide; lactate formation from glucose appeared to be somewhat decreased, but there was a wide variation in the [^{14}C]lactate produced and there was no statistically significant difference between results obtained with two groups of animals. The concentrations of neither the free glucose in the tissue nor the phosphorylated hexose esters appeared to be decreased in the diabetic muscle in these experiments.

The results given in Table 2 show that there was a very marked stimulatory effect of insulin on the incorporation of glucose into glycogen and oligosaccharides in the diabetic diaphragm muscle. (In

Table 2. *Influence of insulin on the metabolism of glucose in diaphragm muscle from normal and streptozotocin-diabetic rats*

Results are expressed and experimental conditions are as given in Table 1. The insulin concentration was 0·01 unit/ml. Mean values ±S.E.M. for six normal rats and nine diabetic rats are given, with the numbers of pieces of diaphragm in parentheses.

	Normal		Diabetic	
	No insulin	With insulin	No insulin	With insulin
CO_2	127±4·6 (12)	143±5·8 (12)	326±47·3† (14)	448±40·6 (14)
Lactate	1013±60·7 (12)	1129±56·1 (12)	920±52·9 (17)	1603±242* (16)
Glycogen + maltotetraose	110±9·9 (11)	224±15·8* (10)	67±8·6† (16)	367±49·4*† (15)
Maltotriose	125±10·2 (11)	269±17·4* (10)	34±2·5† (16)	175±23·3*† (15)
Maltose	136±13·3 (11)	321±14·6* (10)	31±3·0† (16)	147±21·4*† (15)
Glucose in tissue	319±23·5 (11)	381±19·3 (10)	344±22·8 (16)	424±29·7 (15)
Phosphate esters	50±9·0 (11)	58±8·2 (10)	31±2·3 (16)	65±7·2* (15)

* Influence of insulin significant ($P \leqslant 0·01$).
† Difference between normal and diabetic significant ($P \leqslant 0·01$).

Table 3. *Metabolism of glucose in diaphragm muscle from normal and anti-insulin-serum-treated rats*

Results are expressed and experimental conditions are as given in Table 1. Mean values ± S.E.M. for six normal rats and six anti-insulin-serum-treated rats are given, with the numbers of pieces of diaphragm in parentheses.

	Normal	Anti-insulin-serum-treated
CO_2	474±45·4 (12)	409±34·1 (12)
Lactate	918±85·5 (12)	971±102 (12)
Glycogen	71±7·0 (10)	34±4·8* (11)
Maltotetraose	28±6·3 (10)	11±2·5† (10)
Maltotriose	65±7·6 (10)	28±4·9* (12)
Maltose	61±4·4 (10)	29±6·0* (11)
Glucose in tissue	412±18·0 (10)	377±13·0 (12)

* Difference between normal and anti-insulin-serum-treated rats significant ($P \leqslant 0{\cdot}01$).

† Difference probably significant ($P = 0{\cdot}02$), but a very high biological variation.

these experiments the maltotetraose results have been incorporated into those of glycogen, as there was not a satisfactory separation in some of the chromatograms.) As the basal values for the incorporation of glucose into glycogen and oligosaccharides were low in the diabetic muscle the insulin effect was much more striking than that observed in normal animals. The incorporation into glycogen and maltotetraose was significantly higher in the diabetic muscle treated with insulin than in the normal muscle so treated, whereas the incorporation into maltotriose and maltose did not reach that obtained with normal muscle in the presence of insulin. In this group of experiments the phosphorylated esters formed from [^{14}C]glucose appeared to be lower in the diabetic animals than in the normal ones, and insulin increased these values to the normal level.

The decreased glucose metabolism in diaphragm from anti-insulin-serum-treated rats (Table 3) was very similar to that found in the streptozotocin-diabetic animals.

The results reported in Table 2 for the normal rats differ from those in Tables 1 and 3 in that in this group of experiments $^{14}CO_2$ values were lower, whereas the maltotriose and maltose values were considerably higher. Thus the differences in the values obtained for the ^{14}C content of the latter intermediates in the normal and in the diabetic animals was more striking in this group of experiments than in those reported in Table 1. On the other hand the apparent increase in $^{14}CO_2$ values in the diabetic as compared with the normal diaphragm muscle is probably due to the unusually low values of $^{14}CO_2$ observed with this group of normal rats.

DISCUSSION

The present results show that diaphragm muscle from rats rendered diabetic by a single injection of streptozotocin has an abnormal glucose metabolism, manifesting itself in the decreased incorporation of glucose into glycogen and oligosaccharides. The results are in marked contrast with previous findings with isolated diaphragm muscle from alloxan-diabetic rats, where no abnormality in the glucose metabolism was observed in animals that had been treated with alloxan 18–26 days before an experiment and with starvation blood glucose concentrations exceeding 200mg./100ml. at the time of experiment (Beloff-Chain *et al.* 1955). Mansford & Opie (1968) also have reported marked contrasts between the metabolic patterns observed in the blood and in the perfused heart tissue of rats with streptozotocin-diabetes and alloxan-diabetes. Several authors have reported differences between the glucose uptake in the diaphragm muscle from normal rats and that from alloxan-diabetic rats (Krahl & Cori, 1947; Villee & Hastings, 1949; Manchester & Young, 1960). However, in considering the discrepancy between these results and those reported by Beloff-Chain *et al.* (1955) it should be emphasized that, as discussed by Manchester & Young (1960), the lowered glucose uptake is much more marked in the 'short-term' diabetes, i.e. 48hr. after alloxan injection, than in the 'long-term' diabetes, i.e. at least 2 weeks after alloxan injection. At 48hr. after alloxan injection the toxic effects of this compound are probably at a maximum and therefore it is more difficult to distinguish between the diabetogenic effect and the toxic effects.

It has been shown that, whereas insulin added *in vitro* to the diabetic diaphragm muscle fully restored the incorporation of glucose into glycogen (in fact the incorporation was significantly higher in the presence of insulin in the diabetic muscle than in the normal muscle), the ^{14}C content of the oligosaccharides, although increased by insulin, was not restored to the value obtained in the normal muscle in the presence of insulin. This suggests that a primary site of insulin action in muscle is on glycogen synthesis.

Attention should perhaps be drawn to the differences observed in the three sets of experiments carried out with normal rats (i.e. those reported in Tables 1, 2 and 3 respectively), although at present these findings cannot be interpreted. It would appear that in some animals (see Table 2) there was a biological factor that decreased the complete oxidation of glucose to carbon dioxide, whereas the formation of lactate and oligosaccharides from glucose was increased. The values for the incorporation of glucose into glycogen and maltotetraose did not appear to vary greatly in the

different groups of experiments, nor did the total glucose metabolized.

In diaphragms from anti-insulin-serum-treated rats there was a significant decrease in the incorporation of glucose into glycogen and oligosaccharides, whereas the lactate formation and carbon dioxide production from glucose were not significantly affected. These results provide strong evidence that the abnormalities encountered in glucose metabolism in the streptozotocin-diabetic muscle are due to insulin deficiency. The results with the diaphragm muscle differ from those reported by Mansford (1967) in experiments with the perfused hearts of rats treated with anti-insulin serum, in which he found, not only a decrease in the incorporation of glucose into glycogen, but also a significant decrease in the carbon dioxide and lactate formation from glucose.

Gregor, Martin, Williamson, Lacy & Kipnis (1963) found that in diaphragm muscle removed from rats 90min. after an injection of anti-insulin serum there was a diminished rate of phosphorylation of 2-deoxyglucose to 2-deoxyglucose 6-phosphate.

In the present study no significant difference was found between normal and insulin-deficient diaphragms in the ^{14}C content of the hexose phosphates. It has been found that glucosamine is phosphorylated in diaphragm muscle to form glucosamine 1-phosphate and glucosamine 6-phosphate, without being further metabolized to any great extent, and that insulin stimulates this phosphorylation (A. Beloff-Chain, P. Betto, R. Catanzaro, E. B. Chain, L. Longinotti & F. Pocchiari, unpublished work). Further work is required to find out whether the phosphorylation of glucosamine, known to be insulin-sensitive, is diminished in the diaphragm of streptozotocin-diabetic rats.

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