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Effect of Acetate on Glycogen Synthesis and Glucose Utilization in the Isolated Diaphragm of Rats

By I. PARNES AND E. WERTHEIMER

The Laboratory for Pathological Physiology, The Hebrew University, Jerusalem

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Central metabolic products are believed to be basic regulators of cellular metabolism, the influence of the hormones on metabolism being only secondary, or superimposed on these primary regulatory mechanism. It may be of interest therefore to investigate the influence of such products on certain metabolic processes *in vivo* and *in vitro*. Bloch (1947) has pointed out the central position of acetic acid in metabolism. In this paper we made a study of the influence of acetate on glycogen synthesis and glycogenolysis and glucose utilization by isolated rat diaphragms, using the technique of Gemmill (1940*a, b*).

EXPERIMENTAL

Male albino rats of laboratory stock weighing 80–100 g. were used. Preparation of diaphragm was carried out according to Gemmill (1940*a*), using diaphragms from rats fasted for 20 hr. The medium used for incubation was 'phosphate-buffered saline' containing 0.74, NaCl; 0.015, Na_2HPO_4 and 0.003% NaH_2PO_4 . All the added substrates were given as Na salts. The vessels containing the diaphragms in the medium were gassed for 2 min. with O_2 and then placed in a water bath at 37°. The flasks were shaken 120 times/min.

Glycogen synthesis. This was measured according to Tuerkischer & Wertheimer (1948).

Glucose utilization. For measurement of the glucose utilization by the diaphragm the methods of Krahll & Cori (1947) and Perlmutter & Greep (1948) were used.

Measurements of the glycogen synthesis by the isolated diaphragm of acetate-fed rats. The rats were given the following diet during 2 days: 58% carbohydrate, 12% Na acetate, 20% casein and 10% olive oil, with the usual addition of vitamins and salts. After fasting for 24 hr. the animals were killed, the diaphragms excised and the experiments carried out in the usual medium with 0.05% glucose.

Measurement of respiration. Warburg's direct method was used for the determination of the O_2 uptake (Dixon, 1943) in phosphate Ringer solution. The diaphragms were halved, one half was incubated in the medium containing 0.05% glucose and 0.1% acetate and the other half with glucose alone. Measurements were made over periods of 3 hr. and the average value for 1 hr. was recorded.

Chemical procedures. Determinations of glycogen in the

diaphragm were carried out by a micromodification of Pflüger's method (Good, Kramer & Somogyi, 1933). Glucose was estimated according to Somogyi (1937). Determination of acetic acid was carried out by steam distillation.

RESULTS

Glycogen synthesis by isolated rat diaphragm, with acetate in the medium

Incubation of rat diaphragm in the usual medium was followed by a marked glycogenolysis (Tuerkischer & Wertheimer, 1948). On addition of lactate (0.1%), citrate (0.1%) and succinate (0.1%) there was glycogenolysis of the same order of magnitude as in the medium without additional substrate. α -Ketoglutarate decreased, while acetoacetate markedly increased the glycogenolysis. Acetate in a concentration of 0.2% caused glycogenolysis of the same order of magnitude as was observed in the medium without substrate (-0.062 ± 0.009 in three experiments), but no glycogenolysis was observed in the presence of 0.05% acetate (-0.002 ± 0.010 in four experiments). Addition of 0.1% acetate gave rise to a small but significant net glycogen increase. Pyruvate caused net increase in glycogen, though not to the same extent as glucose (Table 1).

Table 1. *Glycogen synthesis by normal rat diaphragm in presence of acetate, pyruvate, lactate, etc.*

(The diaphragms were incubated at 37° for 3 hr. Glycogen synthesis is expressed as mg. glucose/100 mg. wet tissue.)

No. of experiments	Medium containing (0.1%)	Glycogen synthesis (mg./100 mg.)
14	Acetate	0.016 ± 0.008
11	Pyruvate	0.037 ± 0.013
7	Lactate	-0.062 ± 0.023
5	Citrate	-0.060 ± 0.026
5	Succinate	-0.069 ± 0.024
7	α -Ketoglutarate	-0.020 ± 0.019
4	Acetoacetate	-0.115 ± 0.024

Influence of acetate on glycogen synthesis from glucose

Glycogen synthesis by rat diaphragm from 0.05 % glucose was markedly increased by addition of 0.1 % acetate. This increase exceeded by far the sum of the values of synthesis caused by each of the substrates separately. This effect of acetate depended on the concentration of glucose in the medium. In presence of 0.1 % glucose, this increase was smaller but still significant as compared with 0.1 % glucose alone, while with 0.2 % glucose the values of glycogen synthesis were the same with and without acetate. With glucose concentration below 0.05 % the effect of acetate could again be demonstrated (Table 2).

not increase glycogen synthesis from glucose. With 0.2 % acetate and 0.05 % glucose the values found were 0.100 ± 0.029 (four experiments), while for 0.05 % acetate and 0.05 % glucose a value of 0.047 ± 0.039 (four experiments) was found. A 3 hr. incubation was chosen, since 45 min. values were far below the optimum, while after 5 hr. the amount of glycogen synthesis decreased. Lactate, succinate, citrate, propionate and acetoacetate in concentrations of 0.1 % added to a medium containing 0.05 % glucose did not increase the synthesis of glycogen; some of these substances—citrate and especially acetoacetate—inhibited glycogen synthesis from glucose (Fig. 1). A marked increase of glycogen synthesis from glucose could be demon-

Fig. 1. Effect of various substances on glycogen synthesis of rat diaphragm. ---, theoretical sum of the values for glucose and other substances acting separately.

Table 2. *Effect of acetate on glycogen synthesis from varying glucose concentrations*

(The diaphragms were incubated at 37° for 3 hr. Glycogen synthesis is expressed as mg. glucose/100 mg. wet tissue.)

No. of experiments	Medium containing		Glycogen synthesis (mg./100 mg.)
	Glucose (%)	Acetate (%)	
14	0.05	—	0.072 ± 0.007
28	0.05	0.1	0.166 ± 0.010
14	0.1	—	0.109 ± 0.020
6	0.1	0.1	0.161 ± 0.014
6	0.2	—	0.166 ± 0.027
6	0.2	1.0	0.163 ± 0.024

The acetate concentration of 0.1 % seemed to be optimal, as both higher and lower concentrations did

strated on addition of 0.1 % α -ketoglutarate only with diaphragms of fed rats. There was no increase in diaphragms from rats fasted for 24 hr. With diaphragms from fed rats the glycogen synthesis was 0.155 ± 0.026 (seven experiments); diaphragms from fasted rats gave values of 0.073 ± 0.011 (twelve experiments). Addition of pyruvate caused only a slight increase of glycogen synthesis, the value being not significantly higher than the sum of the values for pyruvate and glucose acting separately.

There was a further increase in the synthesis of glycogen from glucose in presence of acetate when insulin was added to the medium. The synthesis in the presence of insulin was 0.221 ± 0.024 (eleven experiments) as compared with 0.166 ± 0.010 (twenty-eight experiments) without insulin. The effect of

acetate on glycogen synthesis also could be demonstrated when serum was used instead of phosphate-buffered saline. When diaphragms were incubated in serum the difference value (Tuerkischer & Wertheimer, 1948) with and without acetate was 0.048 ± 0.015 (four experiments).

Influence of acetate on glucose utilization by rat diaphragm

The glucose uptake by rat diaphragm was markedly decreased in presence of 0.1% acetate. There was also a decrease in the oxidation of glucose as measured in the Warburg apparatus (Table 3). The

Table 3. *The effect of acetate on the oxygen uptake of the rat diaphragm*

(One half of the diaphragm was incubated in phosphate Ringer containing 0.05% glucose, the other half in the same medium with addition of 0.1% acetate.)

Exp. no.	Composition of medium	O ₂ uptake (μl.)
1	Without acetate	-3.85
	With acetate	-3.10
2	Without acetate	-4.05
	With acetate	-3.26
3	Without acetate	-4.11
	With acetate	-3.32

quotient, glycogen synthesis/glucose utilization rose from 0.12 for 0.05% glucose alone, to 0.47 for 0.05% glucose and 0.1% acetate. This effect of acetate was also observed when acetate was previously given *in vivo*. Rats were fed an acetate-containing diet, fasted for 24 hr. and their diaphragms incubated with 0.05% glucose without further addition of acetate *in vitro*. In these experiments a quotient of 0.32 was found.

This decrease in the glucose uptake could not be demonstrated in a medium with higher concentrations of glucose. The quotient, glycogen synthesis/glucose utilization, with 0.2% glucose alone was 0.25; when acetate was added this value remained unchanged. In a medium containing 0.05–0.1% glucose, the disappearance of acetate was 0.310 ± 0.042 mg./100 mg. of diaphragm. No definite relation was found between the amount of acetate

which disappeared from the medium and the amount of glucose utilized during the incubation of the diaphragm (Table 4).

DISCUSSION

Influence of acetate on glycogen synthesis. The absolute value for net glycogen increase from acetate by the muscle is small but significant. Considering that other substances such as lactate, citrate, succinate, ketoglutarate, propionate and acetoacetate do not show this effect, but on the contrary even cause marked net decrease of glycogen, the significance of the findings with acetate become enhanced. These experiments do not establish whether the acetate itself is incorporated into the glycogen molecule, or whether its participation in glycogen synthesis is indirect. This can be established only by experiments with labelled acetate. It should be stressed, however, that besides glucose only acetate and pyruvate gave rise to net glycogen increase, while all other substrates caused net glycogen decrease.

Gemmill (1940*a, b*) had found that the diaphragm cannot use lactic acid for synthesizing glycogen. This finding is in accord with the generally accepted belief that in mammals most of the lactic acid is transported by way of the blood to the liver, and only there resynthesized to glycogen (Cori cycle). Our findings demonstrate that muscle glycogen can be synthesized in the presence of some shorter carbon chains, as well as from glucose.

Influence of acetate on glycogen synthesis from glucose and on glucose utilization. Our main task was to investigate the influence of acetate on carbohydrate metabolism of the isolated diaphragm. Glucose utilization on the whole decreases, the glucose oxidation as measured in the Warburg apparatus is lowered, while glycogen synthesis increases. The quotient, glycogen synthesis/glucose utilization, is therefore markedly increased. Thus acetate influences carbohydrate metabolism by enhancing synthesis on the one hand, and lowering oxidation on the other. It should be noted, however, that this acetate effect can be demonstrated only when glucose concentrations are below the normal

Table 4. *Effect of 0.1% acetate on glycogen synthesis and glucose utilization by rat diaphragm*

(The diaphragms were incubated at 37° for 3 hr. Glycogen synthesis and glucose utilization are expressed as mg. glucose/100 mg. wet tissue.)

No. of experiments	Medium containing		Glycogen synthesis (mg./100 mg.)	Glucose utilization (mg./100 mg.)	Glycogen synthesis / Glucose utilization
	Glucose (%)	Acetate (%)			
10	0.05	—	0.066 ± 0.006	0.558 ± 0.031	0.12
14	0.05	0.1	0.147 ± 0.008	0.342 ± 0.013	0.47
12	0.05	<i>In vivo</i> *	0.126 ± 0.016	0.463 ± 0.033	0.32

* Rats were fed for 2 days a diet containing 12% acetate and fasted afterwards for 24 hr.; no acetate was added *in vitro*.

physiological concentration. With glucose concentration above 0.1 %, neither enhanced synthesis nor lowered utilization was observed. When looking for an explanation of this effect, it should be noted that when the acetate is given *in vivo*, and the diaphragm isolated 24 hr. after the last acetate feeding, the effect on glycogen synthesis and glucose utilization is the same as when the acetate is given *in vitro*. Acetate and glucose might be considered competitors in the tricarboxylic acid cycle. But since the level of glycogen synthesis *in vitro* remains high and that of general glucose utilization low, even 24 hr. after the acetate feeding was discontinued, this explanation is not acceptable.

According to these experiments, acetate in presence of low glucose concentrations causes changes of long duration in the muscle cell, presumably by sparing carbohydrate and increased metabolism of fat. The exact mechanism of this action is not known, but this appears to be the only explanation for the fact that the action of the acetate given *in vivo* can still be demonstrated 24 hr. later in the isolated diaphragm. It seems, therefore, that acetate can influence cell metabolism in a definite direction. No similar effect on the carbohydrate metabolism of the diaphragm was shown by any of the other substrates examined. Even pyruvate, which itself brings about net glycogen increase in the diaphragm, did not increase significantly glycogen synthesis from glucose.

Insulin markedly enhances glycogen synthesis in media containing acetate and glucose; this increase, however, can be explained by the enhancing action of insulin on the glycogen synthesis from glucose alone.

SUMMARY

1. Rat diaphragm in phosphate-buffered saline shows a significant net glycogen increase when incubated with 0.1 % acetate or pyruvate. When diaphragm is incubated in the medium without any addition, glycogenolysis takes place; this glycogenolysis is not affected by addition of lactate, citrate or succinate. Acetoacetate increases, whilst ketoglutarate decreases glycogenolysis.

2. Acetate (0.1 %) and 0.05 % or less glucose increase glycogen synthesis to a much greater extent than would be expected from the sum of the synthesis values for each substrate alone. Total glucose utilization and glucose oxidation are markedly lowered. The ratio of glycogen synthesis/glucose utilization therefore increases. No other substrate mentioned above has a similar effect. With 0.2 % glucose the synthesis of glycogen and the utilization of glucose are not affected by addition of acetate.

3. Acetate given *in vivo* causes, after an interval of 24 hr., an increase in glycogen synthesis from glucose and a decrease in glucose utilization of the isolated rat diaphragm *in vitro*.

4. Acetate is assumed to effect primary cellular regulation of metabolism.

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Effect of Acetate on the Glycogen Synthesis *in vivo*

BY I. PARNES AND E. WERTHEIMER

The Laboratory for Pathological Physiology, The Hebrew University, Jerusalem

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In experiments of the preceding paper it was shown that acetate at certain concentrations influences carbohydrate metabolism of the isolated rat diaphragm, providing that the glucose concentration does not exceed 0.1 %. The *in vitro* acetate effect, which consists of increased glycogen synthesis and decreased glucose utilization, was present even in diaphragms of rats which had previously been fed

a diet containing acetate and then killed after a 24 hr. fast. When these diaphragms were incubated with 0.05 % glucose, without further addition of acetate, there was increased glycogen synthesis and decreased glucose uptake.

In the following experiments the influence of acetate on carbohydrate metabolism *in vivo* was investigated. Our starting point was the following