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*

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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

experiment. Rats fed a diet containing 12 % acetate show after a 24 hr. fast more glycogen in the liver and muscle than controls fed a diet containing an equivalent amount of carbohydrate.

EXPERIMENTAL

Animals and diet. Male albino rats of our stock, weighing 80–120 g., were used. For at least 2 days before the experiment they received the following diet: 70 % carbohydrate, 20 % casein, and 10 % fat, with the usual addition of salts and vitamins. After this the experimental animals were given for at least 2 days a diet consisting of 58 % carbohydrate, 12 % acetate, 20 % casein, and 10 % fat, and the usual additions. The controls continued to get the 70 % carbohydrate diet. In some of the experiments paired feeding was used. This was, however, discontinued when it was found that acetate had no influence on the amount of food eaten.

The protein-rich diet contained 58 % casein, 12 % acetate, 20 % carbohydrate and 10 % fat, salts and vitamins, while the corresponding control diet contained 70 % casein, 20 % carbohydrate and 10 % fat.

In the phlorrhizin experiments the rats were fasted for 24 hr. and 30 mg. phlorrhizin were injected subcutaneously during the fasting period. In a number of the experiments the animals were killed 3 hr. after the phlorrhizin administration, while in other experiments the rats were killed 17 hr. after the administration of phlorrhizin, i.e. following a recovery period. The chemical procedures used were the same as described in the previous paper. Alloxan diabetes was induced by intravenous injection of 6–8 mg. of alloxan/100 g. rat.

RESULTS

After fasting for 24 hr., acetate-fed rats showed more glycogen in liver and muscle than controls. If the acetate diet was given for 1 day instead of the usual 2 days the differences were less marked. Acetate (6 or 3 %) gave nearly the same results as 12 % acetate. With a 1 % acetate concentration the effect was abolished (Table 1).

The influence of the duration of the fasting period. The glycogen contents of liver and muscle were similar in both acetate-fed and control rats, if the animals were not previously fasted. After an 8 hr. fast the glycogen content decreased in both, but no differences were observed between the acetate-fed rats and the controls. After 18 hr. fasting there was even more glycogen in the livers of the control, than in those of the acetate-fed animals. Fasting periods of 32 and 48 hr. gave the same results as the initial experiment. With a 72 hr. fast again no significant differences could be observed between the acetate-fed and the control rats.

Other substances. Addition of 6–12 % propionate, butyrate or stearate to the diet had no influence on the liver and muscle glycogen. With similar amounts of lactate or citrate, the glycogen content, after a 24 hr. fast, was even lower in the experimental rats than in the controls (Table 2).

The glycogen content, 3 hr. after the administration of phlorrhizin, reaches very low values in the

Table 1. *Glycogen after a 24 hr. fast, as influenced by the acetate concentration in the diet*

No. of experiments	Acetate added to diet (%)	Glycogen of liver (as mg. glucose/g. wet tissue)		Glycogen of muscle (as mg. glucose/g. wet tissue)	
		With acetate	Without	With acetate	Without
12	12	0.41 ± 0.081	0.12 ± 0.030	0.27 ± 0.018	0.21 ± 0.018
7	6	0.42 ± 0.079	0.11 ± 0.013	0.22 ± 0.014	0.19 ± 0.011
6	3	0.44 ± 0.16	0.13 ± 0.028	0.26 ± 0.042	0.19 ± 0.011
5	1	0.16 ± 0.028	0.17 ± 0.048	0.18 ± 0.039	0.18 ± 0.011

Table 2. *Liver and muscle glycogen, following a 24 hr. fast after a diet containing lactate, citrate, propionate, butyrate or stearate*

No. of experiments	Addition to diet (%)	Glycogen (as mg. glucose/g. wet weight)			
		Liver		Muscle	
		With acetate	Without*	With acetate	Without
5	Lactate 12	0.22 ± 0.067	0.49 ± 0.020	0.28 ± 0.053	0.28 ± 0.040
5	Citrate 12	0.15 ± 0.044	0.31 ± 0.061	0.30 ± 0.010	0.26 ± 0.030
8	Propionate 6–12	0.077 ± 0.021	0.14 ± 0.035	0.16 ± 0.028	0.14 ± 0.017
6	Butyrate 6–12	0.22 ± 0.075	0.18 ± 0.032	0.16 ± 0.017	0.17 ± 0.017
5	Stearate 12	0.26 ± 0.058	0.24 ± 0.028	0.22 ± 0.021	0.20 ± 0.013
12	Acetate 12	0.41 ± 0.081	0.12 ± 0.030	0.27 ± 0.018	0.21 ± 0.018

* The 24 hr. fasting values of liver glycogen of normal rats usually vary greatly in different seasons. This work was carried out during summer, fall and winter.

Table 3. *The acetate effect on liver and muscle glycogen as influenced by the duration of the fast*

No. of experiments	Duration of fast (hr.)	Glycogen (as mg. glucose/g. wet weight)			
		Liver		Muscle	
		With acetate	Without	With acetate	Without
5	0	1.54 ± 0.18	1.69 ± 0.20	0.41 ± 0.037	0.39 ± 0.036
5	8	0.58 ± 0.13	0.51 ± 0.22	0.30 ± 0.022	0.24 ± 0.017
5	18	0.27 ± 0.072	0.52 ± 0.20	0.32 ± 0.046	0.34 ± 0.019
12	24	0.41 ± 0.081	0.12 ± 0.30	0.27 ± 0.018	0.21 ± 0.018
5	32	0.30 ± 0.055	0.08 ± 0.026	0.19 ± 0.017	0.23 ± 0.023
6	48	0.49 ± 0.10	0.14 ± 0.036	0.20 ± 0.040	0.14 ± 0.029
5	72	0.35 ± 0.11	0.28 ± 0.12	0.11 ± 0.031	0.12 ± 0.022
5	24	0.04 ± 0.009	0.06 ± 0.018	0.13 ± 0.029	0.14 ± 0.021
5	(3 hr. after phlorrhizin)				
	24	0.38 ± 0.076	0.12 ± 0.049	0.26 ± 0.010	0.20 ± 0.035
5	(17 hr. after phlorrhizin)				
	24				

acetate-fed animals as well as in their controls. After a recovery period of 17 hr. the differences in the amount of glycogen of experimental rats and controls were about the same as in the 24 hr. fast experiment (Table 3).

The influence of acetate on the glycogen of animals fed a protein-rich diet. Acetate caused an additional increase of the already high glycogen values (the 'protein effect' described by Mirski, Rosenbaum, Stein & Wertheimer, 1938); the liver glycogen, after a 24 hr. fast, was 1.18 ± 0.12 in the acetate-fed rat as compared with 0.77 ± 0.17 in the protein-fed controls; the muscle glycogen was 0.29 ± 0.032 as compared with 0.23 ± 0.021 (five experiments).

The influence of acetate on the glycogen content of alloxan diabetic rats. The effect of acetate on liver and muscle glycogen could be demonstrated in alloxan diabetic animals (three experiments).

DISCUSSION

In the preceding *in vitro* experiments, 0.1 % acetate was found to increase the glycogen synthesis and decrease the glucose utilization by the isolated diaphragm in presence of 0.1 % or less glucose. We might assume that acetate in the presence of a normal or lowered blood glucose concentration causes a decrease in carbohydrate utilization and therefore, in spite of the fast, the glycogen content remains high, a shift in metabolism from carbohydrate to fat utilization having taken place. This could explain the *in vivo* as well as the *in vitro* experiments. However, other factors which might be responsible for the high glycogen content of the acetate-fed animals must be taken into consideration.

An alkalosis effect could be excluded as, according to McKay, Wick, Carne & Barnum (1941), alkali administration depresses the liver glycogen and enhances the ketonaemia. Furthermore, if alkalosis were responsible for the high glycogen values, citrate

and lactate should have the same effect as acetate. We found, however, that citrate and lactate depressed the glycogen values.

Acetate itself could be an excellent glycogen precursor, or, being an available energy source, might have a carbohydrate-sparing action, and thus be responsible for an enhanced glycogen synthesis. If this were the case, differences in the glycogen content would be expected after short fasting period. Differences were observed, however, only after longer fasts.

Acetate might cause an inhibition of glycogenolysis. Here again the absence of differences in glycogen content after short fasting periods does not fit in with this assumption. Moreover, after phlorrhizin administration, the glycogen drops to about the same level in both the acetate-fed animals and the controls, and only after a long recovery period do the differences reappear.

Acetate may cause enhanced gluconeogenesis. This assumption is supported by the above-mentioned finding that after phlorrhizin the liver-glycogen values drop to minimal traces, in the acetate-fed rat as well as in the controls. Only after a recovery of 17 hr., i.e. time enough for neogenesis, do the differences reappear. In further support of this assumption is the fact that after short fasting periods the glycogen content in the livers of acetate-fed animals is even lower than in the livers of the controls, and only after a fasting period of 24–48 hr. do the glycogen values in the acetate-fed rats become much higher than in the controls. After a fasting period of 72 hr. the differences disappear, as by then gluconeogenesis has been initiated in the controls as well. There was, however, no consistent increase in nitrogen excretion during the fasting period in the acetate-fed animals. This point therefore still remains to be cleared up. After taking into consideration all these points, it appears that the explanation offered in the *in vitro* experiments is suitable in the case of the *in vivo* experiments as well. In the latter there may be gluconeogenesis as

well as lowered glucose utilization and enhanced glycogen synthesis. The fact that acetate administered 24 hr. beforehand, *in vivo*, causes enhanced glycogen synthesis, and a drop in the glucose utilization in the isolated diaphragm confirms the explanation offered at the beginning of the discussion.

According to Todd, Barnes & Cunningham (1947), addition of glycine to the normal diet is followed by a higher fasting liver glycogen as compared with the corresponding controls. When insulin is administered to these animals the drop in the liver and muscle glycogen and in the blood sugar is markedly smaller than in rats kept on a normal diet (Cunningham, Barnes & Todd, 1948). This phenomenon is in accordance with the so-called 'protein effect' described by Mirski *et al.* (1938). It remains to be seen whether a connexion exists between the acetate effect and the glycine action.

Acetate and cellular regulation. In this connexion it should be mentioned that when rats were fed a protein-rich diet (Mirski *et al.* 1938) or a diet containing mostly fat (Stein, Tuerkischer & Wertheimer, 1939) the carbohydrate utilization was decreased and gluconeogenesis increased. This was also demonstrated by Samuels, Reinecke & Ball (1942) on hypophysectomized rats and even on eviscerated preparations (Roberts, Samuels & Reinecke, 1944; Reinecke & Roberts, 1944). Most important are the experiments on isolated diaphragms of rats, reported by Lundbaek & Stevenson (1948) and by Gilmore & Samuels (1948) which indicate that this muscle in a carbohydrate-fed rat utilizes glucose at twice the rate of that of the fat-fed animal when both are exposed to the same environment. It would appear, therefore, that the local cellular metabolic system is affected. A cellular adaptation to certain nutritional conditions must

have occurred in this case by depressing carbohydrate metabolism. This shifting in cell metabolism from carbohydrate to fat utilization persists even in tissues isolated from the organism. The findings in these experiments cannot be due primarily to hormonal regulation, but seem to be brought about by some metabolic products in the cell itself, acting as cellular regulators whose effects are of long duration. Acetate and glycine are important metabolic products of the cell possibly responsible for this cellular regulation. This brings us to the assumption that the utilization of carbohydrates on the one side and of fats and proteins on the other side, is regulated primarily by the cell itself, certain metabolic products being responsible for this regulatory action. The action of hormones would then be secondary.

SUMMARY

1. More glycogen in liver and muscle is found after a 24 hr. fast in rats, which received 3–12 % acetate with their diet, than in their controls, where equivalent amounts of carbohydrate replaced the acetate. This was not the case after shorter fasting periods or after a lengthy fast of 72 hr.

2. Acetate-fed rats which received phlorrhizin during the 24 hr. fast showed the same low glycogen values as the controls 3 hr. after the phlorrhizin administration, but had much more liver glycogen than the controls 17 hr. after the phlorrhizin injection.

3. A similar effect on the fasting glycogen was not evinced by propionate, butyrate, stearate, lactate or citrate.

4. This *in vivo* action of acetate is discussed in connexion with the results found with acetate *in vitro*.

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