CARBOHYDRATE METABOLISM IN LOCAL AND GENERALIZED TETANUS

by

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The majority of experimental data supports the hypothesis that muscular rigidity resulting from intramuscular injection of tetanus toxin is not of central origin but induced by functional disturbances residing at the myoneural junction or in muscle metabolism. The symptoms of a generalized tetanus are readily explained on the basis of an impaired function of the muscle, the central nervous system and possibly the liver. No systematic investigation into the character of metabolic disturbances in local tetanus has been carried out. Information has so far been limited to incidental data on glycogen and phosphate content and the activity of a few enzymes like ATP-ase and cholinesterase in tetanized muscles. In the present paper experiments are described which form part of an investigation into the metabolism of animals treated with tetanus toxin, which involves a study of the glycogen synthesis and glycolysis in that condition.

Glycogen synthesis

Male albino rats, weighing 100–150 g and starved for 48 hours, were used. After the starvation period, glucose was administered by stomach tube or intraperitoneal injection, while in other experiments laboratory stock diet was fed *ad libitum*. Local tetanus was induced by intramuscular injection, generalized tetanus by intraperitoneal injection of toxin. Glycogen was isolated by the method of GOOD *et al.*[1]; results are expressed as glucose (NELSON's method[2]). Glycogen synthesis *in vitro* was evaluated according to WALAAS AND WALAAS[3].

In contrast to the marked increase in glycogen content of normal muscle following the ingestion of food by starved animals, glycogen in tetanized muscle remains practically unchanged (Table I).

The administration of glucose by stomach tube to animals in the pretetanic period results in glycogen figures in muscle (pretetanic period of local tetanus) and liver (pretetanic period of generalized tetanus), which are significantly lower than those observed in controls. Impairment of glycogen synthesis, therefore, seems to precede symptoms of tetanus intoxication (Table II). Further investigation showed that even in animals with a severe general intoxication synthesis of liver glycogen

TABLE I

GLYCOGEN CONTENT OF NORMAL AND TETANIZED GASTROCNEMII OF PREVIOUSLY STARVED RATS DURING A 96-HOUR FEEDING PERIOD

Feeding period (hours)	*Glycogen content (%)*	
	Normal	*Tetanus*
0 (3)*	0.31	0.18
24 (8)	1.17	0.32
48 (5)	0.57	0.14
96 (2)	0.52	0.24

TABLE II

GLYCOGEN CONTENT OF NORMAL AND TETANIZED MUSCLE (PRETETANIC PERIOD OF LOCAL TETANUS) AND LIVER (PRETETANIC PERIOD OF GENERALIZED TETANUS) 3 HOURS AFTER ADMINISTRATION OF GLUCOSE (1.5 g) BY STOMACH TUBE TO 48-HOUR STARVED ANIMALS

		Glycogen (%)
muscle	normal (15)*:	0.70 ± 0.033
	tetanus (15):	0.51 ± 0.031
liver	normal (12):	2.40 ± 0.194
	tetanus (12):	0.69 ± 0.120

TABLE III

ANAEROBIC GLYCOLYSIS OF DIAPHRAGMS FROM NORMAL AND TETANIZED RATS (GENERALIZED TETANUS)

Q_{CO_2} = micro l CO_2/mg dryweight/60 min. Glucose concentration 0.2%.

	Q_{CO_2}
normal (6)*:	3.65 ± 0.38
tetanus (6):	2.03 ± 0.12

* Figures between brackets give number of experiments.

occurs at a normal rate during the first hours after intraperitoneal glucose administration; after 4 hours, however, nearly all glycogen has disappeared. *In vitro* experiments showed that glycogen synthesis in diaphragms from rats with a generalized tetanus proceeds normally during a 35 min period; the insulin stimulation of glycogen synthesis and the breakdown of glycogen by adrenalin are not altered.

Glycolysis

Glycolysis in diaphragms was measured by the conventional WARBURG technique. Glucose was added in a final concentration of 0.2%. Muscle extracts were prepared by extraction of minced muscle with *M*/15 phosphate buffer pH 7.4. Glycogen, glucose-1-phosphate, and hexose diphosphate or 3-phosphoglyceric acid were used as alternate substrates. Samples were deproteinized with trichloroacetic acid and analysed for lactic acid[4], pyruvic acid[5], fructose[6] and phosphate[7] before and after incubation (60 min, 37° C). A separation of intermediates was accomplished by Ba-fractionation[8].

Anaerobic glycolysis in intact diaphragm (generalized tetanus) and in muscle extracts (local tetanus) is inhibited (Tables III and IV). It is concluded from the results of Table IV that differences in lactic acid formation are due to the accumulation of hexosediphosphate in tetanus extracts, which could be isolated in the Ba-insoluble fraction. At first sight this seems suggestive of a decreased aldolase activity in tetanized muscle. We found, however, that the addition of coenzyme I to extracts of such muscles completely restored glycolytic activity. This activation by coenzyme suggests that a blockade at the triosephosphatedehydrogenase stage should also be kept in mind.

TABLE IV

GLYCOLYTIC ACTIVITY OF EXTRACTS FROM NORMAL AND TETANIZED MUSCLES (LOCAL TETANUS)

Substrates: glucose-1-phosphate, hexosediphosphate, 3-phosphoglyceric acid. Figures between brackets give initial amounts of substrates. Results are expressed as mg found after incubation (60 min, 37° C).

	Normal	*Tetanus*
Glucose-1-phosphate (8.5 mg)		
Glucose-1-phosphate	< 1.0	< 1.0
Hexosediphosphate (as fructose)	< 0.10	1.39 ± 0.062
Pyruvic acid	0.36 ± 0.072	< 0.10
Lactic acid	3.18 ± 0.077	1.64 ± 0.148
Hexosediphosphate (3.8 mg as fructose)		
Hexosediphosphate (as fructose)	0.40 ± 0.080	1.90 ± 0.220
3-*Phosphoglyceric acid* (5 mg)		
Pyruvic acid	1.47 ± 0.025	1.42 ± 0.023

DISCUSSION

It is found that the glycogen synthesis by skeletal muscles *in vivo* (local tetanus) and the glycolysis in diaphragms from rats with a generalized tetanus are severely impaired; the glycolytic activity of muscle extracts, also, is diminished. The site of the blockade can be located at the aldolase or the triosephosphatedehydrogenase stage. As it can be established, moreover, that the synthesis of liver-glycogen is considerably diminished in generalized tetanus, the conclusion seems justified that tetanus intoxication is accompanied or caused by disturbances in carbohydrate metabolism.

In contrast to rigor mortis, the amount of energy-rich phosphate compounds is only slightly diminished in local tetanus. No evidence was obtained for the occurrence of gross changes in aerobic metabolism (cyclophorase activity, respiration in diaphragm and liver slices). Full details and discussion of results will be published in due time.

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