THE GLYCOGEN CONTENT IN THE LIVER AND MUSCLES OF RATS IN RELATION TO THE TIME ELAPSED AFTER ADMINISTRATION OF CHLOROPROPAMIDE AND TO THE DURATION OF FASTING

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The antidiabetic sulfonamides decrease the liberation of glucose from the liver into the blood. This frequently leads to an increase in the glycogen content of the liver [1]. In conjunction to considerable increase in the glycogen content of the liver in some cases only slight enlargement of the liver [12] can be observed and sometimes no changes of that organ are found at all [9,10,11,14]. No changes in the glycogen content of the liver were found after 5-14 days of administration of one of the strongest antidiabetic preparations: metahexamide [8]. And what is more: under certain circumstances carbutamide even causes a decrease in the glycogen content of the liver [13]. The results of investigations concerning the glycogen content in the skeletal muscles are also of contradictory character. Administration of carbutamide either failed to change the glycogen content of muscles in dogs or even caused a slight decrease [13].

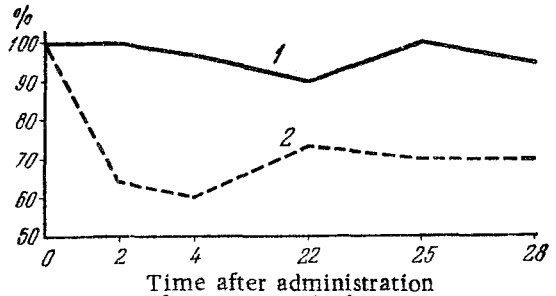
The contradictory data in the literature induced us to study the causes responsible for the different influence exerted by antidiabetic sulfonamides upon the glycogen content of the liver and the muscles.

In previous investigations [2-5] we had shown that butamide, cyclamide and chlorocyclamide exerted, when injected into rats for 5,10 or 20 days, no influence upon the glycogen content of the liver within 15-17 hours after consumption of food and administration of sulfonamides. After five days of administration of cyclamide the glycogen content of the skeletal muscles decreased whereas administration lasting 10 or 20 days caused no changes in the glycogen content. Butamide and chlorocyclamide increased the glycogen content of the skeletal muscles whatever the time of administration. Butamide, chlorocyclamide and chloropropamide caused a marked increase in the glycogen content of the liver four hours after consumption of food and administration of sulfonamides without exerting any influence upon the glycogen content of the muscles.

In the present paper we studied the influence of chloropropamide (synthetized in the Ukrainian Institute of Experimental Endocrinology by T. F. Sysoeva and N. I. Makhnenko) upon the glycogen content of the liver and muscles, following up the pattern of changes over various periods, administering the drugs for one day or for many days and in relation to the time elapsed since the last consumption of food and the last intake of the antidiabetic substance.

METHOD

Male rats weighing 150-200 g served as control and experimental animals. They were given the standard diet consisting of roots (carrots and beets - 3 g), concentrated foods (oats, barley, bran, maize, millet, and sunflower seed-15 g), bread (15 g) and soup (50 g); the latter included meat (5 g), milk (8 g), semolina (5 g), salt and water. At 9-10 a.m. the animals were given the grain and the roots and at 1-2 p.m. the other ingredients of their ration. Chloropropamide was administered perorally in a dose of 100 mg per 1 kg body weight (in milk; with addition of a small quantity of gum arabic). Within a certain time after the last consumption of food and administration of chloropropamide the animals were decapitated; pieces of the liver and the m. gastrocnemius were excised to estimate their glycogen content by the method of Pflüger in a modification developed by us [6]. The blood sugar level was estimated by the method of Hagedorn-Jensen. The control animals were different from the experimental animals in the respect that they were not given chloropropamide. After each period of investigation groups of 5-10 experimental rats and 5-15 control rats were killed. The findings were evaluated statistically by the method of Fisher and



Changes in the blood sugar level under the influence of a single dose of chloropropamide against a background of 22-50 hours fasting (in per cent of the original value defined as 100). 1) Control animals, 2) experimental animals.

Student. The statistical significance of the difference P was calculated according to the table of Fisher [7].

RESULTS

The influence of a single administration of chloropropamide against a background of 22-50 hours' starvation.

Rats which had been fasting for 22 hours were given a single dose of chloropropamide and 2,4,22,25 and 28 hours later the blood sugar level and the content of glycogen in the liver and the skeletal muscles were estimated. Throughout the period in question the animals were not given any food and were kept in special cages provided with a double bottom securing the elimination of faeces.

The curve (figure) shows that the blood sugar level was in the control animals more or less stable whereas in the experimental animals the blood sugar level decreased considerably two and four hours after administration of chloropropamide respectively. Although subsequently the blood sugar level showed a slight increase it was still even after 28 hours far from reaching the original level.

The glycogen content of the liver showed a marked increase two and four hours after administration of chloropropamide respectively (Table 1). The difference between the findings obtained in the control and the experimental rats respectively proved to be statistically significant even after 22 hours, as P was < 0.02. At later periods of the experiments the glycogen content in the liver of the experimental animals was the same as that in the control animals. The glycogen content in the skeletal muscles remained unchanged at all periods of investigation.

TABLE 1. The Influence of a Single Administration of Chloropropamide Against a Background of 22-50 Hr Fasting upon the Glycogen Content in the Liver

Duration of fasting (in hr)	Time since chloropropamide administration (in hr)	Glycogen content of liver ($\bar{x} \pm S\bar{x}$)				
		Control animals	n	Experimental animals	n	P
22	—	223±9.7	6	—	—	—
24	2	214±5	6	305±20	6	<0.001
26	4	200±10.9	15	280±11.9	10	<0.001
44	22	200±9.6	12	242±10.6	6	<0.02
47	25	118±15.6	12	92±20.2	6	0.3
50	28	100±16	12	97±16	6	—

Remark: $\bar{x} \pm S\bar{x}$ represents the mean arithmetical ± deviation of the means; n-the number of experiments; P-the criterion for the statistical significance of the difference.

Administration of chloropropamide for ten days and the content of glycogen in the tissues 4-45 hours after the last consumption of food and administration of the preparation.

The rats were given chloropropamide daily for ten days. On the last day the animals were kept in the fasting cage and were given the preparation. The blood sugar level and also the concentration of glycogen in the liver and the muscles of these animals were investigated 4,6,8,25 and 28 hours (first series of experiments) and also 17,19,21,42 and 45 hours (second series of experiments) after the last feeding and the last administration of chloropropamide. Within 4-28 hours the blood sugar level showed a marked decrease which averaged 33%; the fall was much less in rats of the second series of experiments after 17-19 hours.

The data set forth in Table 2 show that the glycogen content in the liver increased considerably 4,6 and 8 hours after the last consumption of food and the last administration of chloropropamide. At the other intervals the glycogen content was the same as in the control animals. The glycogen content in the skeletal muscles of the experimental animals was somewhat higher at all periods of investigation. This difference, however, was significant only 8,17 and 19 hours after the last consumption of food and last intake of chloropropamide. Later the glycogen content of the

TABLE 2. The Influence of Administration of Chloropropamide Continued for Ten Days upon the Glycogen Content in the Liver and Muscles 4-45 Hours after the Last Consumption of Food and Last Administration of the Preparation (Average Findings Obtained on Six Animals; the Data Referring to 45 Hours Represent the Average Findings Obtained on Five Animals)

Time since intake of food and chloropropamide (in hr)	Glycogen ($\bar{x} \pm S\bar{x}$)					
	Liver			Muscles		
	Control animals	Experimental animals	P	Control animals	Experimental animals	P
4	1085±35.5	2330±62.3	<0.001	453±32.5	510±20	<0.3
6	1046±43.6	1906±24.1	<0.001	483±24.3	527±14.4	<0.1
8	945±43.5	1390±126	<0.001	403±10	729±32.4	<0.001
17	475±24	490±11	<0.5	545±22.9	663±25.6	<0.01
19	465±36	487±19	—	540±20.7	600±30.7	<0.001
21	250±22.5	250±23.4	—	455±14.8	477±20.4	—
25	226±13	224±13.5	—	400±12	410±8.7	—
28	210±10	222±13	—	420±12.8	500±32	—
42	190±8.3	170±20	—	450±14	440±12.8	—
45	133±9.2	127±9	—	427±14.3	417±14.3	—

Remark: $\bar{x} \pm S\bar{x}$ represents the mean arithmetical ± deviation of the means; P represents the criterion for the statistical significance of the difference.

muscles approached that of the control animals.

The data quoted above show that the glycogen content in the liver and the muscles depends not only on the action of chloropropamide but also to a considerable degree on the time through which the experimental animals were fasting. After administration of chloropropamide for ten days the glycogen content of the liver increases; this increase is particularly marked in the first eight hours after the administration of the preparation whereas the glycogen content shows no changes in the period between 17 and 45 hours after the last consumption of food and the last administration of the preparation.

It thus appears that the increase in the glycogen content of the liver observed after administration of chloropropamide for several days is the more intensive the shorter the time of fasting and the shorter the time elapsed after the last administration of the preparation. The influence of chloropropamide upon the glycogen content of the liver is the weaker the longer the time elapsed after the last administration of the preparation and the last consumption of food.

If only a single dose of chloropropamide is administered the glycogen content of the liver increases also after fasting lasting 24 and 26 hours respectively and two and four hours after the administration of the preparation. It thus appears that given fasting of equal duration the glycogen content of the liver depends on the time elapsed after the last administration of chloropropamide.

Chloropropamide also exerted a varying influence upon the glycogen content of the muscles depending on the duration of fasting. After fasting lasting four hours the glycogen content of the muscles showed a slight rise at almost all periods of investigation (first series) although the difference was statistically significant only after eight hours; it proved nevertheless to be increased under the influence of chloropropamide also after fasting lasting 17 and 19 hours respectively (second series). If the fasting lasted longer, chloropropamide exerted no influence upon the glycogen content of the skeletal muscles.

If one compares the effect of chloropropamide upon the blood sugar level and the glycogen content in the liver and muscles the absence of any regularity deserves attention. Chloropropamide decreases the blood sugar level independently of the time of fasting. At the same time the glycogen content of the liver and muscles may be increased or may be unchanged. For example, after a single administration of chloropropamide into rats which had been fasting for 22 hours the glycogen content in the liver increased 2-4 hours after the last administration of the preparation and remained subsequently unchanged notwithstanding the considerable decrease in the blood sugar level.

The glycogen content in the skeletal muscles did not change throughout the period of investigation.

The discrepancy between the decrease of the blood sugar level under the influence of chloropropamide and the glycogen content of the liver and muscles became particularly manifest if the preparation was administered into rats which had been fasting for four hours. In these animals the glycogen content showed a marked increase in the first 4-8 hours of action of the sulfonamide but later, notwithstanding the continued decrease in the blood sugar level, the glycogen content in the liver showed a marked decrease just as in the control animals. In the muscles on the other hand the glycogen content increases within 8-19 hours after the administration of the preparation.

The results obtained by us explain the discrepancy found in the literature concerning the influence of antidiabetic sulfonamides upon the glycogen content in the liver and muscles.

SUMMARY

A study was made of the effect of chloropropamide(100 mg per kg) on the blood sugar level and the content of glycogen in the liver and skeletal muscles of rats in 4,6,8,17,19,21,25,28,42 and 45 hours after the food intake was stopped and the preparation was administered. Marked reduction of the blood sugar level was noted for a period of 28 hours, whereas the glycogen content in the liver increased considerably only in 4,6 and 8 hours; in the muscles it increased in 8,17 and 19 hours. The content of glycogen in the liver and muscles depends not only on the chloropropamide action but also on the duration of the previous starvation.

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