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Determination of Fermentable Sugar in Muscle.*

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It was noted[1] that short tetanic stimulation of rat muscle resulted in an increase in fermentable sugar, suggesting that some of the split-products of glycogen escaped phosphorylation and remained as fermentable sugar in muscle. Since several intermediates might be formed between glycogen and the first phosphorylized product, it seemed of interest to determine the nature of the fermentable sugar formed as a result of contraction. It was shown previously that heart muscle contained a larger amount of fermentable sugar than resting skeletal muscle. This was attributed to the continuous activity of the former organ and made it desirable to determine the nature of the fermentable sugar in heart muscle as well.

In view of the low concentration of fermentable sugar in muscle, large amounts of tissue had to be used for each experiment. Cats were anesthetized with nembutal and the gastrocnemii dissected free of surrounding tissue for rapid removal. One muscle was stimulated tetanically for four 10-second periods through the sciatic nerve, while the other muscle served for the determination of the resting value. Generally the muscles of 2 cats were combined; these yielded from 40 to 60 gm. of resting muscle and a similar quantity of stimulated muscle.

In some preliminary experiments the muscles were frozen in a CO_2 snow-ether mixture prior to extraction. It was found that the contraction elicited by the freezing resulted in an increase in the fermentable sugar. Hence, this method of fixing the muscles was replaced by one in which the muscles were immersed immediately after removal in N sulfuric acid cooled to about —4°.

It seemed doubtful at first that a muscle of the size of a cat gastrocnemius could be fixed in this manner. In control experiments it was found that the fermentable sugar content of thin cat muscles (weighing 1 to 2 gm.) fixed in cold acid was the same as that of gastrocnemii. In both cases the values were lower than those found in previously frozen muscle. Freezing apparently produces a measurable breakdown of glycogen, because in addition to

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1 Cori, G. T., Closs, J. O., and Cori, C. F., *J. Biol. Chem.*, 1933, **103**, 13.

the increase in fermentable sugar there occurs also an increase in hexosemonophosphate.

After half an hour the weighed muscles were removed from the acid and ground in a meat chopper. The muscle pulp and acid were transferred to a mortar and after grinding with sand the mixture was allowed to extract over night. "Iron and mercury" reagent† was added equal to two-tenths of the final volume and enough water to make the dilution 1:8, assuming the water content of the muscle to be 80 %. The mass was then neutralized with $BaCO_3$, filtered through a Büchner funnel, Fe+Hg removed as sulphides and H_2S and Ba removed. A second precipitation was usually carried out by addition of Fe+Hg reagent equal to two-tenths of the filtrate volume. After treatment as above, the pH of the filtrate was adjusted to between litmus and congo and evaporated to dryness *in vacuo*. The solids were taken up in 2.5 cc. of water and 50 cc. of 95 % alcohol was added to remove salts. After remaining over night in the refrigerator, the salts were filtered off. Control experiments showed that the salts did not include any fermentable reducing substance before or after hydrolysis with acid.

The alcohol extract was evaporated to a small volume at a temperature of 35° while the pH was kept at about 6. Last traces of alcohol and water were removed *in vacuo*. The solids were dissolved in water and made up to 25 cc. After filtration one portion was hydrolyzed in N H_2SO_4 for 4 hours at 100° and the SO_4 removed with Ba. The remainder was analyzed for maltose and glucose as outlined below. The filtrates were free of glycogen and hexosemonophosphate, since these are removed by the treatment with heavy metal and Ba.

A high alkalinity copper-iodometric reagent and a heating period of 20 minutes was used in order to insure complete oxidation of such sugars as maltose. The reducing power of maltose with this copper reagent was found to be 54 % of that of glucose and hence a factor of 1.85 was used for conversion.

At an alkaline pH glucose but not maltose is fermented, while at an acid pH both sugars are removed by fermentation with (Fleischmann's) yeast. After trying out this method, which was kindly communicated to us by Dr. Somogyi, on known mixtures of glucose and maltose, it was applied to the muscle extracts.

In order to gain further information on the nature of the fer-

† 28 gm. $Fe_2(SO_4)_3$ + aq. dissolved in 100 cc. of 1.5 N H_2SO_4. After cooling below 10°, 34 gm. $HgSO_4$ are dissolved. (Private communication from Dr. E. S. West.)

mentable sugar in muscle, an aldose determination was carried out by means of the Willstätter-Schudel titration. An extract in which stimulated and unstimulated muscle were combined, showed 18.4 mg. % of fermentable aldose by means of the hypoiodide titration and 18.5 mg. % fermentable sugar by copper reduction. The close agreement between these 2 values indicates that fructose was not present and since mannose is only slowly fermented at an alkaline pH, the fermentable hexose present was most likely glucose.

The accompanying table shows the values obtained in mg. sugar per 100 gm. muscle. In spite of the vigorous treatment with heavy metal precipitants the extracts were free of non-fermentable reducing substances only in 2 instances. Experiment 1 shows that the combined treatment with Fe and Hg is more effective in removing non-fermentable reducing substances than treatment with Hg alone. The first precipitation with Fe+Hg removes the bulk of the non-fermentable reducing substances, while the second precipitation removes only small amounts.

TABLE I.

	Unhydrolyzed				Hydrolyzed		
Tissue	Total Reduction	Non-Fermentable	Maltose	Glucose	Total Reduction	Non-Fermentable	Plasma Sugar
Gastrocnemius*	22	8	5	11	34	5	96
Gastrocnemius†	51	19‡	6	29	82	29	
Gastrocnemius*	20	4	2	15	15	0	90
Gastrocnemius†	37	4	1	33	37	0	
Gastrocnemius*	12	0	0	12	9	0	112
Gastrocnemius†	25	0	0	25	29	0	
Gastrocnemius*	18	7§	2	10			102
Gastrocnemius†	29	6§	0	23			
Heart Ventricle	34	0		34	45	0	
Heart Ventricle	43	13§	0	30			

* Resting muscle.
† Stimulated muscle.
‡ $HgSO_4$ used instead of $Fe_2(SO_4)_3$ + $HgSO_4$.
§ One precipitation with Hg + Fe.

The average glucose content of the gastrocnemius at rest was 12 mg. % at an average plasma sugar level of 100 mg. %. This corresponds closely to the values obtained previously on rat muscle[1] at comparable plasma sugar levels. Significant amounts of a sugar behaving like maltose were found only in the first experiment and in this case acid hydrolysis caused an increase in fermentable sugar. In the other cases only traces of maltose were present and the fermentable sugar content after acid hydrolysis was practically the same as

that before hydrolysis. After short tetanic stimulation the glucose content of the gastrocnemius more than doubled, the average being 28 mg. %. Stimulation was, however, without effect on the maltose content of muscle or on the fermentable sugar content after acid hydrolysis. Apparently, intermediates between glycogen and glucose such as disaccharides do not accumulate in muscle as a result of stimulation. Heart muscle contained about as much glucose as stimulated skeletal muscle and did not contain an appreciable quantity of maltose.

Summary.—The increase in fermentable sugar content of muscle following tetanic stimulation is due to an accumulation of glucose. Maltose does not accumulate as a result of contraction.

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Cataract in Rats Fed on Galactose.

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A previous publication[1] reported the occurrence of cataractous changes in the eyes of all rats fed on rations containing lactose as the chief source of carbohydrate. Negative results with other carbohydrates tested led to an investigation of galactose as the next logical step. This sugar was fed to young rats at 35% and 25% levels corresponding to the galactose available from the 70% and 50% lactose rations fed in previous experiments.

Four rats on the 35% galactose ration developed mature bilateral cataract in 12, 14, 14, and 37 days respectively (average 19 days), whereas those on the 25% galactose ration were somewhat more delayed. The average time for the development of mature bilateral cataract in 49 rats fed the 70% lactose ration was 10 weeks, approximately 4 times as long. Controls fed on the 70% starch ration showed no eye changes.

The rations used in the experiment are shown in Table I.

Growth was subnormal on both galactose and lactose rations but galactose caused no diarrhea, a consistent result from lactose feeding. Galactosuria was more severe on galactose than on lactose rations. The calcium content of cataractous eyes was of the same

[1] Mitchell, H. A., and Dodge, W. M., *J. Nut.*, 1935, **37**, 37.