LXVI. LACTIC ACID FORMATION IN MUSCLE EXTRACTS.

IV. A COMPARISON BETWEEN GLUCOSE AND GLYCOGEN IN RESPECT OF LACTIC ACID FORMATION AND PHOSPHORIC ESTER ACCUMULATION.

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(Received April 24, 1929.)

IN this paper are described eight experiments with individual extracts prepared in the same manner from eight healthy cats. In each case the reaction at the start of the incubation was between p_H 6.9 and 7.1; glucose and glycogen were used respectively in a concentration of 0.5 %. These eight experiments thus constitute a good series for the purpose of detecting any difference between glucose and glycogen with regard to the apparently related reactions of lactic acid formation and phosphoric ester accumulation.

EXPERIMENTAL.

The extracts were prepared from perfused muscle by the method described in a previous paper [Stiven, 1928]. The incubation was conducted in an atmosphere of nitrogen as described therein. The purity of the glycogen was estimated by hydrolysis to glucose and in weighing out the glycogen for making up the stock 10 % solution to be added to the extract, a correction was made for the purity as indicated by the yield of glucose. The lactic acid was estimated by the aeration method of Friedemann, Cotonio and Shaffer [1927]; the "inorganic" phosphorus by the colorimetric method of Fiske and Subbarow [1925]. Both lactic acid and "inorganic" phosphorus were estimated at intervals of 10 minutes up to the end of the first hour and thereafter at 90 minutes and 120 minutes from the start of the incubation. In this way the course of the reactions could be followed at short intervals and any resemblance or difference between the behaviour of these two carbohydrates could be detected.

The term "inorganic" phosphorus means in this paper the sum of the phosphagen phosphorus and the true inorganic phosphorus. In every experiment the lactic acid and the "inorganic" phosphorus changes were investigated in an aliquot sample of the extract to which no carbohydrate had been

added. In this "plain" extract the "inorganic" phosphorus increases rapidly during the first 40 minutes of the incubation but thereafter very slowly. In the case of the extracts to which carbohydrate was added this increase of "inorganic" phosphorus does not take place until the incubation has proceeded for 40 to 60 minutes if lactic acid is being formed from the added carbohydrate. In the early period of the incubation a decrease of the "inorganic" phosphorus may in some cases be observed. This decrease of "inorganic" phosphorus indicates that the formation of phosphoric ester is proceeding at a greater rate than the breakdown of the ester. The amount of this decrease between any two intervals is thus a measure of the excess of ester formed over that broken down in that interval and is called here ester accumulation.

Table I. *Lactic acid formation and phosphoric ester accumulation from glycogen and glucose both present to 0.5 %.*

Experiment	Lactic acid formed (mg. per 100 cc. extract) in				Maximum decrease of "inorganic" phosphorus (mg. per 100 cc. extract)	Time of maximum ester accumulation (mins.)
	20 mins.	40 mins.	60 mins.	120 mins.		
252 Glycogen	106	170	227	264	21	10
Glucose	118	213	300	427	3	10
254 Glycogen	72	98	114	130	25	20
Glucose	78	137	162	186	2	20
255 Glycogen	80	143	171	207	35	40
Glucose	84	162	224	295	0	—
256 Glycogen	107	157	195	284	29	10
Glucose	70	141	202	321	2	60
257 Glycogen	102	154	191	233	13	10
Glucose	64	104	113	119	0	—
260 Glycogen	127	183	229	260	38	20
Glucose	145	229	247	250	1.5	10
261 Glycogen	101	154	192	301	46	40
Glucose	79	125	192	332	1.5	50
262 Glycogen	90	167	216	252	6	10
Glucose	69	150	222	350	0	—

From the results in Table I it will be noticed that decidedly greater ester accumulation took place in every case with glycogen. With glucose it was very feeble and in some cases never took place at all. The striking difference between glucose and glycogen in this respect is not due to a less extensive formation of lactic acid from the hexose nor to a lower rate of formation of the acid. These results seem to indicate that at the start of the incubation, when the concentration of the glycogen is high, the formation of phosphoric ester generally proceeds at a greater rate than the subsequent removal of the phosphoric acid groups. With glucose, on the other hand, it would appear that these two opposing and concomitant reactions almost balance, and from an examination of a great many extracts it has been noticed that the greater the rate of glycolysis the more does the rate of ester formation tend to exceed that of the breakdown. If lactic acid originates only from carbohydrate that

has been through the intermediate stage of phosphoric ester, then it is difficult to understand why in the case of glycogen the ester should sometimes accumulate to the extent it does, particularly in those cases where the rate of lactic acid formation is greater than with glucose, indicating to all appearances a more rapid hydrolysis of the ester. It is conceivable, however, that the ester which accumulates in the glycogen samples does not have the same structure and hence does not yield the same end product as the intermediate ester formed from glucose. This point is under investigation and will be discussed in subsequent papers.

Another point deserves consideration. It will be noticed that the lactic acid formation from glucose varies greatly from one experiment to another. In some cases it is greater with glucose than with glycogen and in other cases it is not. It might be argued that in a series of experiments like the above where the conditions were kept as uniform as possible, this variation might be due rather to faulty technique than to a real difference produced by some unknown factor. The following experiment shown in Table II tests this point. 2 kg. of cat muscle were ground up and extracted in the usual manner, and the final volume of extract, 900 cc., was divided up into three portions of 180 cc. To each of these were added 9.5 cc. of 10 % glucose solution. The "inorganic" phosphorus and the lactic acid were estimated at the intervals indicated.

Table II, Exp. No. 265. *"Inorganic" phosphorus concentrations and lactic acid increases in three aliquot portions of the same extract, all containing 0.5 % glucose.*

Time in mins.	"Inorganic" phosphorus concentration (mg. per 100 cc.)			Increase in lactic acid (mg. per 100 cc.)		
	Sample A	Sample B	Sample C	Sample A	Sample B	Sample C
Start	71.3	71.0	71.0	—	—	—
10	70.5	70.5	70.5	36	36	38
20	71.0	71.0	71.0	72	75	72
40	72.3	72.3	72.1	166	167	166
60	75.7	76.0	76.0	219	222	228
90	82.3	82.9	82.9	291	308	311
120	89.5	89.5	89.5	313	325	327

The above results indicate the extent of the variation that is to be expected. The lactic acid values show greater fluctuations than the phosphorus values but these are not of sufficient magnitude to account for the erratic nature of the results in Table I. These must be due to some unknown factor connected with the preparation of the extract or even with the animals. In this connection it has been found that tame rabbits, as compared with wild rabbits or cats, give invariably most disappointing results as regards lactic acid formation from glucose.

SUMMARY.

Extracts from the muscles of eight cats have been examined for lactic acid formation and phosphoric ester accumulation from both glucose and glycogen. In the case of glucose the extent of the ester accumulation is very small compared with that from glycogen even when the rate of lactic acid formation from glucose is greater than from glycogen. Both the rate and extent of the lactic acid formation from glucose are, generally speaking, greater than from glycogen, but in this respect there is a great variation from one extract to another. It is shown that this variation is not due to faulty technique in the manner of incubation or in the analytical procedure, but is due to some factor as yet uncontrolled.

In concluding, the author wishes to express his thanks to Prof. E. Waymouth Reid, F.R.S., for his assistance in the perfusing of the cats.

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