of thiamin is succeeded by a much slower rate of absorption, presumably after the diffusion equilibrium between the lumen of the gut and the intestinal mucosa has become established. Secondly, absorption of thiamin is roughly proportional to its concentration in the intestine, as was seen when the dose of thiamin was increased from 100 μg to 1 mg. On the other hand, if the absorption of a substance is aided by phosphorylation, as, for instance, is the case with dextrose, the rate of absorption remains constant and is within wide limits independent of the concentration of the substance which is being absorbed.

12002

Relationship Between Sugar Concentration and Glycogenetic Action of Insulin on Rat Diaphragm *in vitro*.

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Previous work has shown that the rate of glucose utilization by the peripheral tissues of both normal and depancreatized dogs depends on the blood sugar level.[1, 2] Insulin appears to act as a catalyst in this process, accelerating a reaction which proceeds at a slower rate in its absence. However, if the blood sugar be sufficiently raised the absence of insulin makes little difference.

The present work is a study of the relationship between the action of insulin and glucose concentration in respect to the storage of muscle glycogen. Using the technic of Gemmill[3] we have observed the relationship of sugar concentration to glycogen formation in rat diaphragm *in vitro*, with and without added insulin.

Methods. Normal fed rats weighing between 80 and 100 g were used for all experiments. The animals were killed by a blow on the head, the diaphragms were quickly removed, trimmed, divided into approximately equal halves, weighed while moist, and introduced into Warburg vessels. The medium used was Hastings'[4] Ringer

* Aided by the Max Pam Fund for Metabolic Research.

1 Soskin, S., and Levine, R., *Am. J. Physiol.*, 1937, **120**, 761.

2 Dye, J. A., and Chidsey, J. L., *Am. J. Physiol.*, 1939, **127**, 745.

3 Gemmill, C. H., *Bull. Johns Hopkins Hosp.*, 1940, **66**, 232.

4 Hastings, A. B., Muus, J., and Bessey, O., *J. Biol. Chem.*, 1939, **129**, 295.

buffered with phosphate or bicarbonate at pH 7.4. The gas phase was 100% O_2, or 95% O_2, 5% CO_2, depending upon the buffer. The insulin employed was an amorphous preparation low in zinc, containing 22 units per mg.* One to 3 units were added per vessel. The total volume in all cases was 3 ml. The incubation period was 3 hours at 38°C. At the conclusion of the experimental period, KOH was pipetted into the main chamber to stop the reaction. The contents of the vessels were quantitatively removed and glycogen was determined by the method of Good, Kramer, and Somogyi.[5]

Inasmuch as Gemmill had already shown that the insulin effect on glycogen deposition was specific, we omitted controls with inactivated insulin preparations or non-specific proteins.

Results. The results are detailed in Table I and graphically illustrated in Fig. 1. The 2 values compared in the horizontal lines

TABLE I.

Glycogen Deposition in Rat Diaphragm *in vitro*, at Different Glucose Concentrations, with and without Insulin.

Rat No.	Initial	100 mg % glucose	Rat No.	100 mg % glucose	100 mg % glucose + insulin
1	133	147	6	285	490
2	90	60	7	110	350
3	210	100	8	130	300
4	130	110	9	60	145
5	127	125	10	100	460
			11	125	285
			12	137	591
			13	158	450
Avg	138	90		138	384
Avg % increase due to sugar				0	
Avg % increase due to insulin					178

Rat No.	200 mg % glucose	200 mg % glucose + insulin	Rat No.	400 mg % glucose	400 mg % glucose + insulin
14	219	282	20	480	450
15	257	461	21	370	540
16	123	366	22	520	320
17	253	294	23	132	160
18	90	400	24	271	323
19	100	150	25	200	450
			26	350	220
			27	570	720
Avg	174	326		362	398
Avg % increase due to sugar	26			162	
Avg % increase due to insulin		110			26

* We are indebted to the Eli Lilly Company for supplies of this insulin.

5 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

of each section of Table I, represent the 2 halves of the diaphragm of a single rat. It will be seen that no glycogen was deposited at a glucose concentration of 100 mg % in the absence of added insulin. As the glucose concentration was raised glycogen was deposited in increasing amounts. At a glucose concentration of 400 mg % the final glycogen content reached 3 times the initial value.

The catalytic activity of insulin was very great at a glucose concentration of 100 mg %, still significant at 200 mg %, and negligible at 400 mg %.

It may also be of interest to record that our results resembled Gemmill's, in that insulin did not increase the O_2 consumption of the diaphragms. The average QO_2 with insulin was 7% less than the control.

Discussion. Our results show that in the absence of added insulin the rate of glycogen formation in the diaphragmatic muscle of rats *in vitro* depends upon the concentration of sugar in the medium. Cori and Cori[6] have shown a similar relationship between liver glycogen deposition and blood sugar concentration *in vivo*. Our results further indicate that, in the presence of added insulin, glycogen formation in the diaphragm *in vitro* occurs at a high rate even at comparatively low sugar levels. As the sugar concentration is increased the catalytic effect of insulin becomes less and less evident.

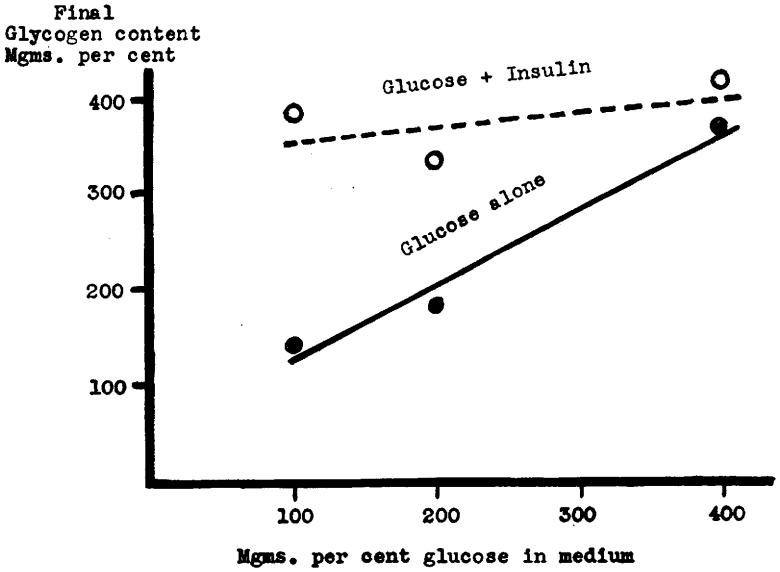

FIG. 1.

[6] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1929, **85**, 275.

The dispensability of insulin at high sugar concentrations is in agreement with the well established evidence that completely diabetic animals can store some glycogen if high blood sugar levels are maintained.[7, 8]

The relationship between the action of insulin and sugar concentration demonstrated for glycogen deposition in the present work, is similar to the relationship previously demonstrated for glucose utilization *in vivo*.[1] The presence of this same relationship in both instances is in agreement with our conception that insulin acts by catalyzing the conversion of glucose into some intermediary metabolite which precedes and is necessary for both glycogen formation and sugar utilization.[9] Thus insulin influences the rate at which sugar enters into the metabolic processes of the cell, but the fate of the sugar which has entered depends on other factors, and insulin cannot be regarded as being a *specific* accelerator of either the storage or the oxidation of carbohydrate.

Summary. Glycogen deposition in rat diaphragm *in vitro* varies directly with the concentration of glucose in the medium. Insulin catalyzes this process greatly at low sugar concentrations but very little at high concentrations. The bearing of these results on the mechanism of insulin action is briefly discussed.

12003 P

Effect of Shearing Forces on the Cortex of the Fertilized Egg of *Arbacia punctulata.*

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In previous communications[1, 2] the theory was advanced that, after monaster formation, the cortex of the fertilized egg of *Arbacia punctulata* consists in part of a bound calcium compound. Data were presented showing that this labile combination can be broken down in the living cell by various chemical agents, with the subsequent release

[7] Major, S. B., and Mann, F. C., *Am. J. Physiol.*, 1932, **102**, 409.

[8] Barker, S. B., and Sweet, J. E., *Science*, N. S., 1937, **86**, 270.

[9] Soskin, S., and Levine, R., *Am. J. Physiol.*, 1940, **129**, 782.

[1] Churney, L., *Anat. Rec. Suppl.*, 1938, **72**, 65.

[2] Churney, L., and Moser, F., *Physiol. Zool.*, 1940, **13**, 212.