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GLYCOGEN SYNTHESIS IN THE SMALL INTESTINE.

By E. A. HORNE and H. E. MAGEE.

(*Rowett Research Institute, Aberdeen.*)

It has been claimed that the glycogen content of the portal blood increases during absorption of carbohydrates (see Verzar for references, 1932); but Charit [1926] was unable to obtain confirmatory evidence in angiostomized dogs. Lang [1928] criticized Charit's methods, and published findings which seemed to show that the glycogen in portal blood is increased to a variable extent in anæsthetized dogs during absorption of glucose and fructose. With glucose he found increases of 0·9–24 mg./100 c.c. of blood, with an average of 9·5 mg. in six experiments. He, therefore, concluded that the intestinal wall builds up glycogen and passes it into the portal blood during absorption of carbohydrates.

Verzar [1932] has made use of these results to support his argument that the phenomena of absorption can be interpreted in physico-chemical terms. He maintains that the fundamental basis of the selective absorption of glucose, as compared with foreign sugars such as xylose, is an unspecified transformation produced in the glucose as soon as it enters the epithelium. The glucose-diffusion gradient from intestinal lumen into cell is thereby kept at a high level, so that diffusion can proceed until no more sugar is left in the lumen. Verzar, assuming that Lang's conception is correct, makes no attempt to explain in terms of his physico-chemical theory of absorption why the mucosa either transforms only part of the absorbed glucose into glycogen; or, if it transforms all of it, why part of this newly-formed glycogen is again hydrolysed to sugar while the other part makes its way into the blood as glycogen. It appeared to us a more logical hypothesis that the absorbed glucose is entirely synthesized into a temporary labile form of glycogen and that it is rehydrolysed into sugar in order to pass through the attached border of the epithelium and the walls of the capillaries. This hypothesis is analogous with Loevenhart's hydrolysis-resynthesis theory of fat transport.

The object of the present research was to test this conception of carbohydrate absorption. It is clear, however, that Lang's results are incompatible with such a view, and a repetition of his experiments was obviously necessary at the outset.

Blood glycogen during glucose absorption. In dogs under urethane or ether the splenic artery was tied, blood massaged out of the spleen, the vein ligatured and a cannula fixed in the proximal end. One or two samples of blood (30 c.c.), depending on the size of dog, were then run into tared flasks containing 60 p.c. KOH and the weight of blood determined. Forty c.c. or more of 0·75 *M* glucose were then injected into the duodenum and as many blood samples (30 c.c.) as possible taken after 15 min. or more. Glycogen was estimated by Pflüger's method. Great difficulty was experienced in getting rid of the pigments, repeated precipitations and filtrations being necessary. In a preliminary test of the method, 3·63 mg./100 g. were found in the jugular blood of a dog, assuming that all the reducing substance found after hydrolysis of the alcohol precipitate was glucose. The assumption could not be confirmed experimentally because of the very small amount of reduction. It is noteworthy that the above value falls within the range found for blood (1·5–6·1 mg./100 c.c.) by Huppert [1894] and by Schöndorff [1903].

The glycogen content in the portal blood of four dogs was determined before and after injection of glucose into the duodenum. In two, no glycogen was found in any of the samples, and in one, none in the first sample and traces only 15 min. after giving the glucose. In the fourth dog the following data were obtained: before giving glucose, 3·55 mg.; 30 min. after, 2·67 mg.; 60 min. after, 2·33 mg./100 g. We formed the opinion from these results that glycogen is present in blood in such minute amounts that estimation of it with any degree of accuracy is wellnigh impossible. For this reason we think that very little significance can be attached to the small increases observed by Lang. Furthermore, it is very improbable that a colloid like glycogen could diffuse through cell membranes into the blood. As further research along the above lines appeared pointless, experiments were carried out to determine whether the glycogen content of the intestinal wall is increased as a result of the passage through it of glucose, either by diffusion in the case of surviving intestine, or by absorption in intact animals. In the earlier experiments the glycogen in the entire intestinal wall was estimated; but, later, the technique was modified so that estimations on the dry mucosa alone were possible.

Glycogen content of intestinal wall during glucose absorption; (1) *surviving intestine.* Intestinal loops were taken from rabbits fasted 15 hours, suspended in oxygenated Tyrode at 40° and 4–6 c.c. 0·75 *M* glucose or 0·9 p.c. NaCl put in adjacent segments. The order of the loops in which the solutions were inserted was varied, so as to allow for possible physiological variations in different levels of the intestine. At intervals after putting in the solutions adjacent loops were removed from the bath, slit open, dried between filter papers and transferred to warm KOH as rapidly as possible for glycogen estimation. The amounts of glycogen, expressed in g./100 g. of fresh tissue, were compared with those obtained for control segments which were digested in KOH immediately after excision.

As can be seen from the examples of results given below, the surviving intestine showed no indication of ability to build up, and temporarily store, glycogen. In the intestines of different animals large variations were found which were probably due, to some extent, to variations in the moisture content of the intestinal wall. In those segments with high initial values fairly rapid glycogenolysis was usual as Exp. II shows. To avoid this complication the remaining experiments were performed on living animals.

Glycogen in rabbit's surviving intestine.

	Solution in loop	Diffusion time (min.)	Glycogen mg./100 g.
Exp. I	Nil (control)	0	8·1
	"	0	8·4
	0·75 *M* glucose	30	6·8
	0·9 p.c. NaCl	30	5·9
	0·75 *M* glucose	60	2·4
	0·9 p.c. NaCl	60	1·1
Exp. II	Nil (control)	0	25·6
	"	0	30·0
	0·75 *M* glucose	15	17·0
	0·9 p.c. NaCl	15	13·3
	0·75 *M* glucose	45	13·5
	0·9 p.c. NaCl	45	15·0

(2) *Anæsthetized animals.* In rabbits under urethane the small intestine was divided into two portions of approximately equal length, by means of ligatures. Into one portion 20 c.c. of 0·75 *M* glucose were injected, and into the other 20 c.c. of iso-osmotic NaCl solution (2·66 p.c.). The abdomen was sewn up and, an hour later, the segments were taken out and the glycogen in the entire wall determined. Again, no sign of an increase in glycogen in the glucose-absorbing portion was found. In virtue of these results, the estimations in succeeding experiments were done on the mucosa alone. The procedure was to excise the loops at the

end of the absorption period, and then rapidly fill them with absolute alcohol. This precipitated the glycogen and fixed the mucosa, so that it could be readily removed by scraping with a blunt scalpel. The mucosa was dried thoroughly by pressing between filter papers, or by heat at 100°, and the glycogen estimated in 0·5 g. samples by Osterberg's [1929] modification of Pflüger's method. The results were expressed as p.c. of dried mucosa.

From the typical results shown below it can be seen that the evidence as to glycogen synthesis was contradictory. They suggested, however, that the mucosa of the lower half was initially richer in glycogen than that of the upper half.

Glycogen in dried mucosa after absorption for 1 hour.

		Glycogen mg./100 g.	Difference
Av. of 3 exps.	Upper half containing glucose	110	30
	Lower half containing saline	140	
Av. of 2 exps.	Upper half containing saline	115	38
	Lower half containing glucose	153	

It thus appeared possible that an increase in glycogen in the duodenal half might be completely masked by comparison with the ileal half, when this was used as a control.

This opinion proved to be correct; for, when the glycogen in the mucosa from different levels in the fasted rabbits was determined, it was found to be significantly lower in the duodenum than at other levels. The average values in four animals were:

	Glycogen mg./100 g.	Average
Duodenum	354	404
Upper jejunum	454	
Jejuno-ileum	505	468
Ileum	432	

The glycogen in the lower half was 64 mg. higher than in the upper half. In the foregoing data the same relationship existed; but the differences were similar, 30 and 38 mg., in spite of the order being reversed in the respective experiments. It is clear therefore that these data do not provide any evidence of glycogen formation during glucose absorption. It was thought that the failure to obtain such evidence might be due to rapid breakdown in the (hypothetical) glycogen. In the succeeding experiments, therefore, the mucosa was fixed *in situ* in the animal, while the circulation was still proceeding.

The next series of experiments was done on cats under urethane, because the relatively short small intestine of these animals is believed to possess correspondingly powerful absorbing capabilities. An hour after injecting glucose into the duodenum, absolute alcohol was forced through the intestine, and then retained in it under pressure for several minutes. The intestine became gradually paler, and circulation through it stopped. It was then excised and the mucosa was scraped off. The mean glycogen values for the mucosa of two (control) cats, which received no intestinal injection, and for two cats, which received glucose in the upper half and nothing in the lower half, are subscribed:

	Controls	Glucose injected into upper half	
Upper half	735	718	Glycogen mg./100 g. mucosa
Lower half	832	824	

As these findings threw no additional light on the problem, the next series of experiments was carried out on unanæsthetized animals.

(3) *Unanæsthetized animals.* Rats which had fasted 24 hours were given by stomach tube 5 c.c. 0·75 *M* or 3 c.c. 3 *M* glucose, and stunned after an hour. The abdomen was rapidly opened, and the mucosa of the whole small intestine devitalized with alcohol, as described above. The blood was always circulating when the intestine was excised immediately afterwards. The glycogen values obtained for the mucosa of animals given 5 c.c. 0·9 p.c. NaCl instead of glucose were used as standards for comparison.

Glycogen in mucosa of rats.

No. of animals	Solution fed	Glycogen mg./100 g.	Difference
10	Glucose	163	23
10	Saline	140	

The average results show that the glycogen content of the mucosa of the rats given glucose was higher than that of those given saline by 23 mg. This difference appeared too small to be significant, and, to settle the point, the experimental error in the glycogen estimation was determined by analysing mixed samples of dried mucosa. The mean of six samples was 580 mg., the maximum 593 mg., and the minimum 565 mg. The experimental error was therefore $\pm$ 14 mg. As the above difference falls within this range it must be regarded as insignificant.

Microscopical examinations were also made of sections of intestine fixed during the absorption of either saline or glucose and stained by iodine. No evidence of glycogen accumulations could be found in the epithelium of the glucose-absorbing intestine.

We do not consider the results of this study as being a complete answer to the question we set out to answer, for two reasons. In the first place our knowledge of the chemistry of glycogen is far from satisfactory, and it is very probable that present-day methods of estimation are too inaccurate for studies of this nature. Or again, a polysaccharide of smaller molecular weight and more soluble than the compound precipitated by 60–70 p.c. alcohol, may be formed in the epithelium so that it would escape detection altogether. In the second place, it is conceivable that glycogen is built up and broken down again with such rapidity inside the epithelium that the normal content is not exceeded. Obviously, the methods we have employed could not provide an answer to this question. Indeed, problems of this type rarely yield to direct attack, but rather to the accumulation of circumstantial evidence. It is not, however, clear how even evidence of this kind can be brought to bear on this particular problem. The prospect is, however, more encouraging in regard to a similar hypothesis on which experiments are now in progress in this laboratory. This is based on experiments which showed that absorption of glucose was increased by the presence of phosphate at *p*H 7 [Magee and Reid, 1931]. The theory is that glucose combines with phosphate inside the epithelium to form hexose phosphate, instead of glycogen as in the former hypothesis. This (hexose-phosphate) theory is rendered all the more probable by recent work from Verzar's laboratory [Wilbrandt and Laszt, 1932]. These workers found that injections of iodoacetic acid (which inhibits formation of hexose-phosphate) apparently stopped selective absorption of glucose in anæsthetized animals.

The only conclusion which appears justifiable from this study is that, as judged by present-day analytical methods, a building-up of glycogen in the intestinal mucosa during glucose absorption is extremely improbable.

Summary.

No evidence was obtained of an increase in the glycogen content of the portal blood of anæsthetized dogs during glucose absorption.

Glycogen determinations on the entire wall and the mucosa of the small intestine, either surviving or *in situ*, in anæsthetized rabbits and cats and in unanæsthetized rats, gave no evidence of formation of glycogen during absorption of glucose.

The glycogen content of the mucosa is lower in the duodenum than in other levels of the small intestine.

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