XCI. GLYCOGEN FORMATION IN THE FOWL.

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THE rate of glycogen formation in the liver of the rat after ingestion of various substances has been studied by many observers. It has been shown that, compared with other substances, the hexoses are more readily converted into glycogen, the order for the three commonest monosaccharides in this regard being glucose > fructose > galactose. It was considered of interest to carry out similar experiments in the fowl to ascertain whether the carbohydrate metabolism in birds was different from that in the mammal.

The research was begun by one of us (A. R. G. E.) in Toronto in 1931, the experiments taking the form of comparisons between the rate of deposition of liver-glycogen after ingestion of glucose and of several 3-carbon compounds. At Aberdeen the scope of the investigation was modified, so as to bring it into line with research being conducted there on the absorption and metabolism of carbohydrates in fowls. The object of the Aberdeen experiments was to determine the relationship between the rates of absorption and of glycogen formation after ingestion of various carbohydrates.

Toronto experiments (by A. R. G. E.).

The substances investigated were glucose, dihydroxyacetone, alanine, glycerol, sodium *dl*-lactate and sodium pyruvate. Barred Plymouth Rock chicks, weighing 100–200 g., and reared under the same conditions, were used¹. Chicks were selected for greater uniformity and because it was thought that, owing to more active metabolism, changes in the liver-glycogen would be more marked in the small birds. In addition, the livers of these chicks weighed about 5 g., a convenient amount for analysis. The procedure employed was similar to that of Cori and Cori [1926]. After a preliminary fast overnight, followed by access to the food hoppers for half an hour, the birds were fasted 24 hours and given, by stomach tube into the gizzard, 5 cc. of a 50 % aqueous solution of the substance to be tested, warmed to 38° and neutralised, if necessary, with NaOH. We found that if more than 5 cc. of fluid were given, there was danger of regurgitation into the crop. For comparison with the work of Cori and others on rats, all substances were administered in 50 % solution with the exception of alanine, which was given in 30 % solution. Glycogen was determined in the liver and pectoral muscles by Irving's method [1928], the Shaffer-Hartman method [1920] being used for estimating the sugar in the hydrolysate. The pectoral muscles were chosen, as they are easily accessible and quite large, even in a small chick. At the same time it was realised that a change in the percentage of glycogen in these muscles might not be comparable with Cori's

¹ These birds were kindly supplied by the Poultry Department, Guelph, Ontario.

results for the glycogen of "the rest of the body." Birds of both sexes were used because, as the following control experiment showed, there was no sex difference in the initial liver- or muscle-glycogen.

15 ♂ av. liver-glycogen, $0.126 \% \pm 0.029$; muscle glycogen, $0.186 \% \pm 0.114$
 15 ♀ " " $0.136 \% \pm 0.045$; " $0.192 \% \pm 0.100$

All chicks used in the investigation were fasted for 24 hours before the administration of the test substance; as the data given below indicate, the percentage of liver-glycogen of chicks fasted 48 hours was somewhat higher than that of those fasted 24 hours. Furthermore, the vitality of the birds was considerably lowered by a fast of 48 hours.

6 birds fasted 24 hrs., av. liver-glycogen, $0.149 \% \pm 0.059$; muscle-glycogen, $0.294 \% \pm 0.106$
 6 " 48 " " $0.229 \% \pm 0.033$; " $0.314 \% \pm 0.060$

Results.

Liver-glycogen. The average results are shown in Fig. 1. It can be seen that the glucose curve, so far as it goes, is of the same general type as that found by Cori and Cori [1926] in the rat. The curve for dihydroxyacetone, on the other hand, in its similarity to the smoothed glucose curve, differs from what might be expected from the figures of Cori and Cori [1928] for absorption and liver-glycogen deposition after ingestion of the triose in the rat, and suggests that the absorption of this sugar is more rapid in the chicken. Alanine gave a gradual increase in the liver-glycogen, the value reaching its maximum (1.5 %)

Fig. 1.

Fig. 2.

Fig. 1. Average liver-glycogen, Toronto experiments, after ingestion of: Glucose —; Dihydroxyacetone — — —; Alanine — · — ·; Glycerol — — — —; Na lactate — — — —; Na pyruvate · · · · ·.

Fig. 2. Average liver-glycogen, Aberdeen experiments, after ingestion of: Glucose —; Galactose — — —; Lactose — — —; Starch — · — ·; Egg-white — · — ·.

at 4.5 hours, a somewhat higher figure than that found by Wilson and Lewis [1929] in the rat. Glycerol, on the other hand, gave only a slight increase, the percentage obtained by Catron and Lewis [1929] in rats being almost three times as great. Neither sodium pyruvate nor sodium *dl*-lactate gave rise to

noticeable increases in liver-glycogen. Glycerol, and particularly sodium pyruvate, were found to be toxic, so that some birds died before the end of the experiment.

Muscle-glycogen. There was no increase in the percentage of glycogen in the pectoral muscles after feeding any of the trioses. Some of them, indeed, appeared to cause a fall in glycogen percentage. With glucose there was an increase, the maximum (0.8 %) occurring 2 hours after ingestion. It is of interest to note that dihydroxyacetone, which gave such large increases in liver-glycogen, caused no change in the glycogen of the pectoral muscles.

Aberdeen experiments (by A. R. G. E. and K. M. H.).

The substances tested were glucose, galactose, lactose, starch and egg-white. They were given in 50 % aqueous solution or suspension (starch), except egg-white which was dissolved in 0.9 % NaCl. The procedure was similar to that employed in the Toronto experiments, except that S.C. White Leghorn chicks were used, and glycogen determined by the method of Cori and Cori [1926] on "the rest of the body" after skinning and eviscerating the animals, and cutting off the heads, tips of wings and legs at the tibio-tarsometatarsal joint. The bodies were not frozen; preliminary experiments, cited below, showed that, under the experimental conditions, there was no marked difference in the initial levels of body-glycogen of birds which were not cooled, cooled in brine at -10° or frozen with CO_2 .

No cooling: av. body-glycogen	0.064 % ± 0.012
Brine, -10° "	0.091 % ± 0.013
CO_2 "	0.090 % ± 0.006

The average liver-glycogen of 8 control chicks was 0.049 %, considerably less than in the Toronto experiments.

The rates of absorption of glucose, galactose and lactose were determined, but owing to technical difficulties we were unable to do this with starch. Preliminary experiments showed that the whole digestive tract of chickens starved for 24 hours had no appreciable amounts of reducing substances, 1.7 mg. on an average in 12 birds. The recovery of glucose from 4 chickens killed immediately after feeding varied from 86 to 96 % with an average of 90 %; but, as will be shown later, the absorption of glucose was very rapid, and this might account, in part at least, for the poor percentage recovery. During the period of absorption the chicks were placed on filter-papers and at the conclusion of the experiment the washings from these were added to those of the digestive tract. In only a few instances, as after galactose feeding, was there any diarrhoea. Lactose was determined by adsorption on to yeast before and after hydrolysis. Blood-sugar determinations (Hagedorn-Jensen) were made in a few instances only, because of the rapidity with which the blood clotted.

Results.

Absorption. Table I shows the absorption as determined at different periods after ingestion. Comparing these results with Cori's [1925], it is seen that chickens differ from rats in that the rate of absorption of the sugars is not

Table I. *Absorption coefficients (g./100 g. live wt./hour).*

Substance	1.5 hrs.	3 hrs.	4.5 hrs.	6 hrs.
Glucose	0.655	0.422	0.341	0.245
Galactose	0.645	0.365	0.275	0.216
Lactose	0.355	0.175	0.145	0.115

constant between 1.5 and 6 hours, but falls considerably, especially during the later periods. Another difference is that galactose is absorbed somewhat more slowly than glucose. Lactose, as was expected, has a lower A.C. (absorption coefficient) than either glucose or galactose. At 1.5 hours the relative rates of absorption are: glucose 100, galactose 98, lactose 54. At the other periods, however, the order is glucose 100, galactose 85, lactose 43.

Table II. *Average blood-sugar (g./100 cc.).*

Substance fed	0 hrs.	1.5 hrs.	3 hrs.	4.5 hrs.	6 hrs.
Glucose	0.200	0.322	0.305	0.202	0.197
Galactose	0.200	0.308	0.284	0.295	—
Lactose	0.200	0.216	0.200	0.174	0.197
Starch	0.200	—	0.230	0.213	0.212
Egg-white	0.200	0.209	0.206	0.205	0.189

While the values given in Table II are of interest in indicating the more rapid return of the blood-sugar to normal after feeding glucose than after galactose, and that it was not affected by a protein such as egg-white, they should be considered with caution because they are averages of single determinations on different individuals, and as MacOwan and Magee [1931] and others have shown, there is considerable individual variation in adult fowls.

Liver-glycogen. The average results are shown in Fig. 2. Glucose caused a marked increase in liver-glycogen; this was, however, considerably less than in the Toronto experiments. In comparison with glucose, very little glycogen was deposited in the liver after galactose ingestion, still less after lactose or starch and none after egg-white. These results are somewhat surprising in view of the relatively rapid rates of absorption of the sugars. Harding and Moberley [1930] have shown that after ingestion of galactose by human beings the sugar is excreted in the urine, and Cori [1925] found that galactose was largely excreted in the urine of the rats in his experiments. Loss in the urine, however, could not account for the disposal of the galactose in our birds; for the average amount of galactose recovered from the mixed excreta of the birds killed at the different periods was as follows: 1.5 hrs., 0.439 g.; 3 hrs., 0.454 g.; 4.5 hrs., 0.481 g.; 6 hrs., 0.450 g. This indicates that although absorption continued, very little galactose was excreted after 1.5 hrs.

Body-glycogen. Glucose was the only substance which caused a marked increase in body-glycogen, the value rising from 0.064 % to a maximum of 0.42 % at 3 hrs. and falling to 0.33 % at 6 hrs. The muscle-glycogen of the chicks fed with the other substances was apparently not affected, the changes in the percentage of body-glycogen being insignificant.

Table III. *Percentage of sugar absorbed which was deposited as liver-glycogen.*

Sugar fed	Hours after ingestion			
	1.5	3	4.5	6
Glucose	4.71	2.92	11.84	9.03
Galactose	0.87	2.57	2.46	3.85
Lactose	0.83	0.39	0.10	0.16

Table III shows the percentage of sugar absorbed which was recovered as liver-glycogen (calculations being based on the figures obtained for the control chicks given previously).

DISCUSSION.

Wilson and Lewis [1929] point out that large experimental animals are unsuitable for studies of glycogen formation because they often have considerable reserves of glycogen even after fasts of several days. From the figures given for the percentage of glycogen in the livers and bodies of chickens starved for 24 hours, however, it appears that birds of this weight are as suitable in this respect as the rat; for in starvation the glycogen stores of the liver are rapidly depleted to a fairly constant level. It seems from a comparison of the data obtained in Toronto and in Aberdeen, that both liver- and body-glycogen may vary according to breed and environment.

In the Toronto experiments the liver-glycogen was slightly, but definitely, higher after 48 hours than after 24 hours of starvation. This fact may possibly be related to the observation made by MacOwan and Magee [1931] and by Henry, Macdonald and Magee [1933] that the blood-sugar of the fasting fowl shows unaccountable fluctuations, rising to a peak at about 72 or 96 hours after an initial fall. It is possible that this rise occurs earlier in the chicken and may be accompanied by a corresponding rise in the liver-glycogen.

From a study of the rates of absorption of glucose, galactose and lactose, birds seem to differ somewhat from the rat. While the question has not been studied sufficiently in detail, it appears that in young chickens, the absorption of galactose and more especially of glucose is not independent of the amount present in the digestive tract, as Cori [1925] has shown for the rat. Thus, in the Aberdeen experiments the A.C. for glucose fell from 0.655 g. at 1.5 hours when about 66 % of the sugar had been absorbed, to 0.422 g. at 3 hours when about 90 % of the sugar had been absorbed. Absorption is also considerably more rapid in the chicken than in the rat, as a comparison of our A.C. values with those of other workers will show. Another difference should also be noted, *viz.* that galactose is absorbed by the chick less rapidly than glucose, whereas Cori found the opposite in the rat.

From Fig. 2 it can be seen that the chick can rapidly deposit glycogen in its liver from absorbed glucose. Galactose and lactose, on the other hand, are not utilised in the same way, the liver- and muscle-glycogen during the absorption of these sugars showing only small increases. In view of the results published by MacOwan and Magee [1931] and Henry, Macdonald and Magee [1933] on the effect of feeding finely ground starch-rich foods on the blood-sugar level of adult birds, it is interesting to note that, with the birds in our experiments, the blood-sugar did not appear to increase, nor was there an appreciable deposition of glycogen in the liver or body as a result of starch feeding. This may be due to slower digestion of starch or more rapid utilisation of absorbed sugar by young chicks than by adult fowls.

It appears from the Toronto experiments that, if the rates of absorption of the two sugars are similar, dihydroxyacetone can be formed into liver-glycogen in the chick as readily as can glucose. It is curious, however, that there was no formation of glycogen in the pectoral muscles, and there is little reason to believe that there was in the other muscles. Mostkowski [1911], however, found an increase in the percentage of muscle-glycogen after feeding as much as 60 g. of the triose to adult hens previously starved for 4 or 5 days. The reason for these divergent results is not clear; they may be related to the differences in age of the birds used in the respective investigations.

Pyruvic acid was cited by Lusk [1928] as a possible intermediary in the conversion of alanine into glucose, but, in view of the definite increase in liver-

glycogen after the feeding of the amino-acid to our birds and the negative results obtained with sodium pyruvate, it is probable that pyruvic acid is not an intermediary in chickens. The experiments do not deny this possibility, however, for the pyruvic acid may have been metabolised in some other way. It is of interest that in the experiment where starch was given, there was little deposition of glycogen. Glycerol, also, while being formed into glycogen readily in the rat, was not utilised in this way in our experiments.

SUMMARY.

Experiments were conducted on chickens weighing 100–200 g. to find the rates of absorption of glucose, galactose and lactose and of glycogen formation after the ingestion of these sugars, of several 3-C compounds and of starch and egg-white. The relative rates of absorption were in the following order: glucose > galactose > lactose, and they fell gradually between 1·5 and 6 hours after ingestion. The rate of liver-glycogen formation was in the order glucose = dihydroxyacetone > alanine > glycerol > Na lactate > Na pyruvate in the Toronto experiments, and glucose > galactose > lactose = starch > egg-white in the Aberdeen experiments. Muscle-glycogen was increased by glucose ingestion but not by that of any of the other substances.

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