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THE UTILIZATION OF GALACTOSE FOLLOWING COMPLETE REMOVAL OF THE LIVER

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One of the most important functions of the liver is the maintenance of the normal level of sugar in the blood. Except for the periods when glucose is added to the blood by alimentation, the liver is constantly adding glucose to the blood at the expense of its own glucose and glycogen. The liver is able to replenish its supply of glucose and glycogen by the conversion of other substances to glucose. The conversion of some of these substances appears to be specific for the liver since it is not accomplished by the other tissues in the absence of this organ. Amino acids (1) are not diaminized or converted to glucose in the absence of the liver. Lactic acid may be converted to muscle glycogen but is not utilized to increase the sugar content of the blood except by the liver. Fructose, however, may be converted to glucose in the absence of the liver. Bollman and Mann (2) found definite elevation of the glucose content of the blood of hepatectomized dogs and relief of symptoms of hypoglycemia following the administration of fructose to these animals. This conversion of fructose to glucose appeared to be accomplished by the intestine since no increase of blood glucose and no alteration of symptoms of hypoglycemia occurred when fructose was administered to animals from which both liver and intestines had been removed. Definite elevation of the blood glucose was also demonstrated following removal of the intestines of animals of which the liver had not been disturbed. Apparently both the liver and the intestines are able to convert fructose to glucose. The glycogen content of the muscles could be increased by administration of fructose in the absence of the liver but only under conditions that also increased the glucose of the blood. It could not be definitely established that the fructose was directly converted to muscle glycogen but this appeared unlikely since increases of glycogen were not obtained from administration of fructose to animals of which both liver and intestines had been removed.

The metabolism of galactose has been reviewed recently by Shay, Schloss and Bell (3). They concluded that galactose is an ideal sugar for liver function tests since it is readily absorbed from the intestine and is readily excreted by the kidneys even in the presence of severe renal damage. Cori (4) found that galactose increased the glycogen content of the liver much less than did equal amounts of glucose or fructose. Cori and Cori (5, 6) observed a greater utilization of galactose with decreased rates of absorption when the galactose was given enterally. The total amount utilized, however, increased with increased absorption so that as much as 52 mgm. could be utilized for each 100 grams rat per hour. Wierzuchowski and his collaborators (7, 8) used continuous intravenous injection of galactose, 2 grams for each kilogram of body weight each hour for three hour periods. Under these conditions their dogs excreted about 70 per cent of the injected galactose and galactose remained in the blood for nine hours after injection. The same animals excreted only 10 per cent of glucose or fructose when equal amounts of these sugars had been given in the same way. Injections of insulin did not greatly alter the excretion of glucose or fructose but slightly reduced the amount of galactose excreted in the urine. No significant changes were observed in the blood or urine glucose following the injection of fructose or galactose. Studies of respiratory metabolism indicated that the portion of retained galactose which was oxidized was much larger than was the portion of glucose or of fructose which underwent oxidation. Less storage or conversion of galactose to glycogen occurred than was the case with glucose or fructose.

METHODS. The rate of disappearance of galactose from the blood after injection of galactose, and the amount of this sugar excreted in the urine, were determined on dogs under three physiologic conditions: normality, following complete removal of the liver, and after the development of extreme degrees of experimental cirrhosis of the liver. All animals were accustomed to the laboratory procedures and variations in their blood sugar were not attributable to the handling incident to venipuncture or catheterization. All animals had been maintained on an adequate mixed diet for several weeks and were used each time after a fast of eighteen hours.

The method of administration of galactose which we found to be most suited for this type of experiment was the intravenous administration of 500 mgm. of galactose for each kilogram of body weight. Oral administration was found to give widely divergent results on normal animals. The dosage of 500 mgm. of galactose for each kilogram of body weight was adopted because smaller doses disappeared rapidly from the blood and the amount of galactose excreted in the urine was variable. Larger doses remained in the blood longer, and larger amounts of galactose were excreted in the urine so that the amount retained (and subjected to hepatic activity) was reduced in terms of percentage of the amount administered.

Galactose, 500 mgm. for each kilogram of body weight, showed the greatest difference in utilization of this sugar by the normal animal and by the animal totally deprived of hepatic tissue.

Specimens of blood were obtained at appropriate intervals by puncture of the jugular vein. Urine was obtained at timed intervals by catheterization.

Glucose and galactose in the blood were determined in the protein-free filtrates of unlaked blood prepared according to the method of Folin. For this purpose the freshly drawn blood, without an anticoagulant, was measured out immediately and delivered into a previously measured volume of cooled sulphate-tungstate reagent. The samples were stored in the refrigerator until the end of the experiment; then all were acidified, centrifuged, and filtered. Control experiments showed no changes to have occurred in the glucose or galactose content of the blood thus preserved in the refrigerator. The reducing power of the filtrates was determined by means of a Shaffer-Hartman alkaline copper solution similar in composition to the modified reagent devised by Somogyi (9), except that in accordance with the suggestion of DeLong (10), potassium iodide was omitted. This was added later, in each individual determination, just before the titration with 0.01 N thiosulphate. For the determination of galactose plus non-sugar reducing substances, the filtrates were subjected to fermentation with washed yeast according to Somogyi's (11) method as slightly modified by Spannuth and Power (12). Under the conditions employed glucose is removed quantitatively, while galactose, according to Cave (13), is unaffected. The difference in the reducing power before and after fermentation is therefore calculated as glucose, whereas the reduction after fermentation, corrected for the non-sugar reducing substances as determined on the control samples of blood before the administration of galactose, is calculated as galactose. In each case the titration values corresponding to glucose and galactose were converted to concentration values by means of curves previously constructed with the aid of pure glucose and galactose solutions respectively. In agreement with recent work it was found that the reducing power of galactose with the particular copper reagent used was about 80 per cent of that of glucose. Likewise, it was found that in mixtures of glucose and galactose, there was no detectable influence of either sugar on the reducing power of the other.

Urinary sugar was determined in a similar manner to that in the blood, by the Shaffer-Hartman method. The yeast treatment of the urine was omitted, however, in many instances because the high concentration of galactose and the absence of glucose from the urine allowed sufficient dilution so that the correction for the reducing substances in the urine became negligible.

Complete hepatectomy was performed by the method of Mann (14), in

which two preliminary operations are utilized. The first establishes an anastomosis of the portal vein and the vena cava; the vena cava is ligated above the stoma just below the liver. After several weeks an extensive collateral circulation of the portal and caval regions has developed. The second operation, ligation of the portal vein, serves to test the adequacy of the collateral circulation to return the blood to the heart. If this is inadequate death will follow in a few hours from portal stasis which would be attributed to the absence of the liver had that organ been removed at this time. After a week or two the liver may be removed. This is rapidly accomplished and the animal recovers from the ether anesthesia and appears normal. After several hours symptoms of hypoglycemia develop which may be entirely dispelled by the administration of glucose.

Partial impairment of hepatic function of a number of dogs was produced by repeated administration of carbon-tetrachloride (15). From 5 to 10 cc. of carbon-tetrachloride were administered by stomach tube three or four times each week. This produces a series of acute degenerative changes in the liver with subsequent adenomatous proliferation of hepatic cells and the formation of cicatricial tissue in the liver. Some of the animals used in this series had been under treatment for almost three years and all had moderate degrees of bilirubinemia and definitely retained bromsulphalein. Other animals were more acutely intoxicated with carbon-tetrachloride and the degree of bilirubinemia was used as an index of hepatic damage which was confirmed by histologic examination of the liver at the end of the experiment.

Results. Following the intravenous injection of 500 mgm. of galactose for each kilogram of body weight into normal dogs, the galactose content of the blood progressively diminished so that not more than traces remained in the blood after two hours. The urinary excretion of galactose was practically completed at this time. The amount of galactose which appeared in the urine of normal dogs varied from 50 to 150 mgm. per each 500 mgm. injected under these conditions. No significant changes were observed in the glucose content of the blood or urine. In the absence of renal excretion (nephrectomized dogs) the disappearance of the injected galactose from the blood was somewhat more prolonged, but the galactose usually was found to have completely disappeared within three hours.

After total removal of the liver, intravenously injected galactose, 500 mgm. for each kilogram of body weight, progressively disappeared from the blood so that only traces remained after three hours (fig. 1). The curve of the blood galactose was only slightly higher than that found when a normal dog was used. The complete urinary excretion of galactose ranged from 250 to 310 mgm. for each 500 mgm. injected in the different experiments. In the absence of renal excretion (nephrectomized-hepatectomized dogs) the galactose remained in the blood longer, so that four or

five hours after injection of galactose only traces were found in the blood (fig. 2). But it was apparent that the galactose was gradually being withdrawn from the blood.

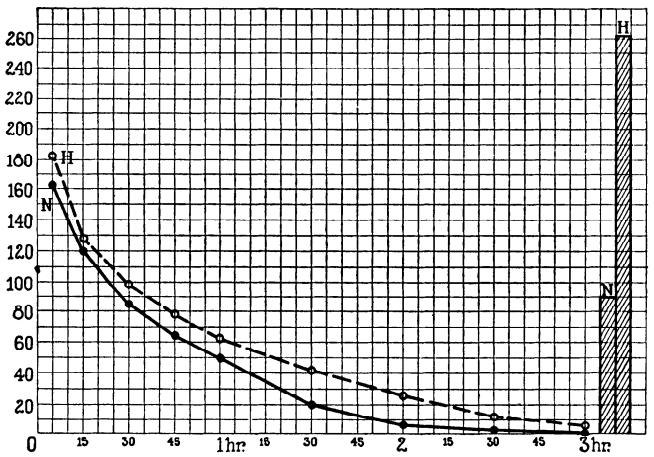

Fig. 1. Curves showing the clearance of galactose from the blood following the intravenous injection of 500 mgm. of galactose for each kilogram of body weight in the normal, *N*, and hepatectomized, *H*, dog. The curves are expressed in milligrams of galactose for each 100 cc. blood. The rectangles indicate the total amount of galactose excreted in the urine; shown as milligrams for each kilogram of body weight so that 500 would represent complete recovery of galactose.

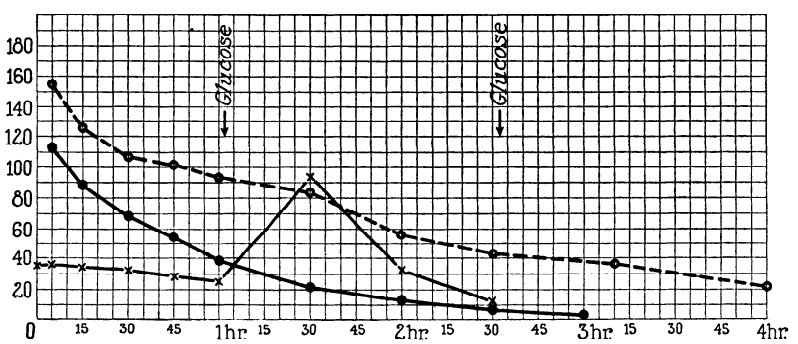

Fig. 2. Curves showing the clearance of galactose from the blood of nephrectomized dogs following the intravenous injection of 500 mgm. of galactose for each kilogram of body weight. The broken line is the blood galactose curve of a nephrectomized, hepatectomized dog; the solid line that of a nephrectomized dog in which the liver was not disturbed. The solid line marked *X* shows the glucose content of the blood of the hepatectomized nephrectomized dog.

The glucose content of the blood of hepatectomized dogs continued to decrease after administration of galactose, and symptoms of hypoglycemia developed in relationship to the glucose content of the blood and were independent of the galactose content. In most experiments it could not be shown that there was any change in the usual rate of decrease of the glucose content of the blood. In a few experiments some delay appeared and minor increases of 1 or 2 mgm. of glucose for each 100 cc. of blood were noted. This will be discussed later. No significant changes were found in the glycogen content of the muscles of the hepatectomized dog which had received galactose. The decrease in glycogen content of the muscles that follows hepatectomy was present after administration of galactose. However, most of the animals would not have survived the hypoglycemia unless glucose had been given; so the survival time without glucose was too short to allow significant changes in the glycogen of the muscles to be demonstrated, even if muscle glycogen could be formed from galactose. Changes are seldom demonstrated within the first six hours after hepatectomy, when large amounts of glucose are given.

The disappearance of injected galactose from the blood of animals which had marked experimental cirrhosis was similar to that of normal animals. The urinary excretion of galactose was usually found to be from 100 mgm. to 200 mgm. for each 500 mgm. injected. If acute degenerative changes were present in the liver, as occurs for several days following administration of carbon-tetrachloride, more galactose appeared in the urine; 150 mgm. to 250 mgm. for each 500 mgm. injected were recovered. Similar results were obtained with hepatic injury from carbon-tetrachloride of animals that previously had been normal. Histologic examination of sections of liver removed immediately following the experiment indicated that the amount of galactose excreted was roughly proportional to the degree of acute hepatic injury and bore no relation to the amount of cicatricial tissue present in the liver.

Galactose was injected into a few animals from which part of the liver had been removed (fig. 3). From 50 to 70 per cent of the liver was removed after an Eck fistula had been made. Regeneration of liver such as occurs in the normal dog was not present. These animals excreted essentially the same amount of galactose after operation as they did before. The liver that remained, however, appeared approximately normal on histologic examination.

Discussion. The normal dog utilizes galactose much more slowly than it utilizes glucose or fructose. In the absence of the liver, utilization is still further retarded and a much larger portion of the galactose which has been administered is excreted in the urine. It should be noted that not all of the galactose administered is recovered in the urine. The retained galactose disappears completely from the blood and it is extremely unlikely that

it is stored elsewhere in the body as galactose. No appreciable galactose depots have been demonstrated, and after repeated administrations of galactose to liverless animals, there is similar disappearance of galactose, with no evidence of storage of galactose as such.

In our experiments we have been unable to determine the fate of the galactose which is apparently utilized without the intervention of the liver. Three possibilities present themselves. The galactose may be oxidized

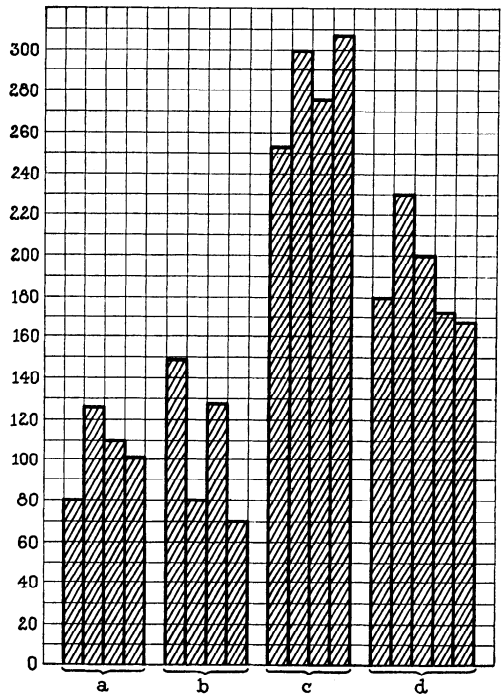

Fig. 3. Rectangles representing the excretion of galactose following administration of 500 mgm. of galactose for each kilogram of body weight. The height of the rectangle indicates the number of milligrams of galactose in the urine for each kilogram of body weight. Five hundred would represent complete recovery of the galactose. *a*, normal dogs, *b*, dogs with more than 50 per cent of the liver removed, *c*, dogs with the liver completely removed, and *d*, dogs with acute hepatic degeneration following carbon-tetrachloride.

directly, converted to glucose, or converted to glycogen. Direct oxidation of galactose might be of a non-specific nature; its fate might be that of a foreign substance introduced into the body, and it might be destroyed in a manner comparable to the destruction of alcohol, with little direct effect on the normal carbohydrate metabolism of the tissues. If the galactose retained by the liverless animal were oxidized as glucose, or exerted the equivalent sparing action on metabolism of glucose a definite alteration in

the curve of the decreasing blood glucose should be obtained. Injection of comparable amounts of glucose produces definite alteration in the blood glucose curve following hepatectomy. We have obtained only questionable changes following administration of galactose. The amount of galactose that is apparently utilized after hepatectomy does not quantitatively alter the metabolism of glucose. The foregoing considerations also indicate that the retained galactose is not quantitatively converted to glucose, since such conversion should alter the blood glucose curve, as is the case when fructose is administered. That galactose may form glycogen in the muscles without the intervention of the liver is a possibility that we are unable to rule out by our experiments. The amount of glycogen formed in the muscles from the galactose retained would be too small to be recognized as definite in this type of experiment. Administration of equivalent amounts of glucose would not be recognizable in the changes found in the glycogen content of the muscle. Any change in the glycogen content of the muscle of the hepatectomized dog would not be reflected in the glucose content of the blood.

Since essentially normal amounts of galactose were excreted by animals of which the liver was the site of extensive cirrhosis (with no acute degenerative changes in the liver) and by animals from which 50 to 70 per cent of the liver had been removed, it is obvious that no direct relationship exists between the amount of liver present and the utilization of galactose. These findings are quite in keeping with similar observations on other functions of the liver such as the regulation of the glucose content of the blood, deaminization and formation of urea, excretion of bile, and so forth. Within rather wide physiologic limits a small portion of normal liver is capable of maintaining all of the known functions of the entire organ with an efficiency that appears to be well within the observed normal variations. Degenerative changes of the hepatic cells, however, may greatly alter this picture, so that mild changes which cannot be detected by functional tests of the entire organ may cause complete cessation of certain functions when only a small portion of the liver is present.

The presence of acute changes in the liver, such as are produced by the administration of carbon-tetrachloride, chloroform, phosphorus or toluylenediamine definitely impairs the utilization of galactose. More galactose was excreted in the urine of these animals in the presence of such changes than when the same amount of galactose was injected before injury to the liver or after the animals had recovered from the effects of the hepatic poison. At the time of decreased retention of galactose, definite histologic evidence of degenerative changes was present in the liver and other functions were also impaired. The animals retained bilirubin in the blood, and the elimination of bromsulphalein was impaired. The amount of galactose excreted was roughly proportional to the extent of the hepatic injury but did not approach that returned by the hepatectomized dog unless

the animal was in the premortal stage from extensive hepatic destruction. In this respect utilization of galactose differs from utilization of glucose in the presence of necrosis of the liver. There is no gradual change in the maximal tolerance of glucose. Not until lethal injury to the liver has been produced does the maximal tolerance of approximately 2 grams of glucose for each kilogram of body weight per hour of the normal dog fall abruptly to the 0.75 gram tolerance of the hepatectomized dog.

SUMMARY

Galactose injected intravenously into normal dogs (500 mgm. for each kilogram of body weight) disappears from the blood in about two hours, and 10 to 30 per cent of the amount given appears in the urine. In the absence of the liver similar injections are followed by similar disappearance of galactose from the blood, but 50 to 60 per cent is recovered in the urine. There is greater delay in the clearance of galactose from the blood of nephrectomized animals, and still greater delay when the liver is also removed. Utilization of galactose is definitely impaired by hepatectomy but appreciable amounts appear to be utilized in the absence of the liver. This galactose is probably not converted to glucose, for it is without effect on the hypoglycemia of hepatectomized animals, and there is little sparing action on the blood glucose.

Removal of 50 to 70 per cent of the liver is without effect on the amount of galactose excreted in the urine following intravenous administration. The presence of acute degenerative lesions of the liver, such as are produced by carbon-tetrachloride, chloroform, phosphorus or toluylenediamine increases the amount of galactose recovered in the urine proportionally to the histologic changes present in the liver. Other physiologic evidence of impairment of hepatic function was present in the animals that gave evidence of decreased tolerance for galactose.

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