Fructose Absorption and Metabolism by the Growing Chick (35079)

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Virtually all species studied can absorb and utilize fructose. In some species such as the guinea pig (1, 2) and hamster (3), fructose is largely converted to glucose during transfer across the intestinal wall; whereas in other species fructose is absorbed largely unchanged. Man (4, 5) and the laboratory rat (2) represent species in which the latter is true. The conversion of fructose to glucose in the intestinal wall involves phosphorylation to fructose-1-phosphate, conversion to trioses via fructose-1-phosphate aldolase, condensation of trioses to fructose-1,6-diphosphate and conversion to glucose-6-phosphate and glucose (6, 7). Species which absorb fructose without conversion can utilize the hexose by similar reactions in liver (8).

The chicken has been shown to absorb fructose (9) and good rates of growth are observed in chicks fed sucrose-containing diets, indicating that the fructose moiety is effectively utilized. However, it is not known whether fructose is absorbed intact in these species or whether it is converted to glucose during absorption. The studies herein reported were conducted to establish whether fructose is converted to glucose during absorption.

Materials and Methods. Male crossbred chicks (Columbian ♀ × New Hampshire ♂) were used for all experiments. Except for one experiment, the chicks were fed a stock diet which provided 23% protein, 3.7% fat, and all essential minerals and vitamins. In one experiment a purified diet was fed which contained (g/100 g): isolated soybean protein, 29.4; DL-methionine, 0.3; glycine, 0.4; mineral mix,[1] 5.31; vitamin mix,[2] 0.4; choline Cl, 0.2; corn oil, 5; nonnutritive fiber, 4; and glucose, 55 or glucose 27.5 and fructose, 27.5. Food and water were provided *ad libitum* and the chicks were housed in cages having raised wire floors.

Fructose and glucose absorption were studied by administering 10 ml of a 30% solution of fructose or glucose into the crop of chicks weighing 350–400 g which had been fasted for 24 hr. The chicks were killed at varying intervals after intubation. The digestive tract was removed and the contents were analyzed for glucose or fructose. The difference between the amount of hexose administered and that recovered was used as an estimate of the amount absorbed. For the *in vitro* study of fructose transport, chicks weighing about 1100 g were killed and the small intestine was removed. Everted intestinal sacs were prepared as described by Wilson and Wiseman (12). The mucosal solution was Krebs–Ringer bicarbonate buffer (pH. 7.4) containing 5 mM fructose, the serosal solution was fructose-free buffer. The sacs were incubated for 1 hr at 38° in a shaking water bath (90 strokes/min). Following incubation the serosal solution was removed and analyzed for fructose and glucose. Glucose was determined by a glucose oxidase procedure[3] and fructose by the method of Van Handel(13).

Two experiments using fructose-U-^{14}C were conducted. In one, an 800 g chick received, intraperitoneally, 1.5 ml of saline containing 5 μCi of fructose-U-^{14}C; 30 min later a blood sample was obtained by heart puncture. In a second experiment, a chick of similar size was employed; the body cavity was exposed and a segment of ileum, approximately 3 cm

[1] For composition of mineral mix see Leveille *et al.* (10).

[2] For composition of vitamin mix see Yeh and Leveille (11).

[3] Glucostat, a prepared enzymatic glucose reagent, Worthington Biochemical Corporation, Freehold, New Jersey.

TABLE I. Intestinal Absorption of Glucose and Fructose by the Chick.[a]

Hexose administered	Time elapsed after administration of hexose (hr)	Percentage of administered hexose absorbed
Glucose	1	84.5 ± 5.5 (5)
	2	99.2 ± 0.3 (5)
Fructose	0.5	57.2 ± 6.7 (5)
	1.0	95.1 ± 1.7 (3)
	1.5	98.6 ± 0.4 (3)

[a] Ten ml of a 30% solution of glucose or fructose were placed into the crop of 350–400 g chicks which had been fasted for 24 hr. After the times indicated, the chicks were killed and the hexose remaining in the digestive tract was determined. The values represent the percentage disappearance of administered hexose ± SEM for the number of chicks shown in parentheses.

long and drained by a prominent mesenteric vein, was tied-off. Into this segment was injected 1.5 ml of saline containing 5 μCi of fructose-1-^{14}C and after 10 min the mesenteric vein was cannulated and blood was collected. The blood was deproteinized by the method of Somogyi (14) and the protein free filtrate was passed over a mixed resin bed containing IR-120 (H^+ form) and IR-45 (OH^- form). The eluates were concentrated under vacuum and glucose and fructose were separated by descending paper chromatography on Whatman No. 1 filter paper in a solvent system consisting of *n*-butanol:acetic acid:water (4:1:5). The chromatographs were developed for 24 hr and air-dried, and guide spots were identified by spraying with a 0.5% solution of $NaIO_4$ followed by 0.5% benzidine. The sugar spots appear as white areas in a blue field. The strips containing the radioactive sugars were cut into narrow strips 0.5 cm wide, placed in scintillation vials and 10 ml of toluene scintillation fluid were added. Radioactivity was determined in a Packard Tricarb spectrometer.The radioactivity associated with glucose and fructose could be ascertained by these measurements.

Fructokinase activity was determined by the method of Parkes *et al.* (15). The chicks were killed by cervical dislocation; about 1 g of liver was homogenized in 10 ml of 0.15 *M* KCl. The homogenates were centrifuged at 100,000*g* at 5° for 1 hr and the resultant supernatant fraction was used for enzyme assay. Protein content of the high-speed supernatant fraction was assayed by the method of Lowry *et al.* (16).

Results. The rate of glucose and fructose absorption in chicks was determined in the experiment summarized in Table I. The hexoses were very rapidly absorbed and absorption was nearly complete within 1 hr after administration of glucose or fructose. In order to determine whether fructose was converted to glucose during absorption, an experiment was conducted with two chicks in which a fructose solution was administered into the crop as in the experiment summarized in Table I. Thirty min later, while the chicks were actively absorbing the hexose, the body cavity was exposed, a mesenteric vein was cannulated and blood was collected. The plasma from these samples contained 16.5 and 19.5 mg of fructose/100 ml, suggesting that some fructose was absorbed unchanged.

An experiment was conducted with intestinal sacs to determine the degree of conversion of fructose to glucose during transport across the chick intestine. The results, shown in Table II, indicate that, of the hexose appearing on the serosal side, only 17% was in the form of glucose, while 83% appeared as fructose. The experiments summarized in Table III were conducted to evaluate the form in which fructose is absorbed and its metabolism in the intact chick. When fructose-U-^{14}C was injected into an isolated seg-

TABLE II. *In Vitro* "Transport" of Fructose by Chick Intestine.[a]

	Glucose and fructose in serosal fluid	
	(m*M*)	(%)
Fructose	0.988 ± 0.165[b]	83.4 ± 1.2
Glucose	0.190 ± 0.017	16.6 ± 1.2

[a] Everted intestinal sacs were incubated in Krebs–Ringer bicarbonate buffer for 1 hr. The mucosal solution initially contained 5 m*M* fructose.

[b] Mean ± SEM for 4 chicks.

TABLE III. Conversion of Fructose to Glucose in the Chick.

Treatment	Radioactivity in: Glucose (cpm)	Radioactivity in: Fructose (cpm)	Percentage of total radioactivity in: Glucose (cpm)	Percentage of total radioactivity in: Fructose (cpm)
Mesenteric blood[a]	7.5	54.5	12	88
Peripheral blood[b]	289.3	8.4	97	3

[a] Blood was obtained from a mesenteric vein draining a segment of the small intestine into which 1.5 ml of saline containing 5 μCi of fructose-U-^{14}C was injected.

[b] Blood was obtained by heart puncture from a chick given, ip, 1.5 ml of saline containing 5 μCi of fructose-U-^{14}C.

ment of the ileum, the mesenteric blood draining this segment contained radioactive hexose, of which 12% was present as glucose and 88% as fructose. However, if fructose-U-^{14}C was given intraperitoneally and blood was withdrawn 10 min later only 3% of the hexose radioactivity was present as fructose and 97% as glucose. These data suggest that some organ was able to effectively utilize fructose and convert it to glucose. We therefore assayed for fructokinase activity in the liver of chicks fed diets high in carbohydrate derived exclusively from glucose or from glucose and fructose. The results, shown in Table IV, demonstrate that hepatic fructokinase activity is high in the chick and is not increased further by fructose ingestion.

Discussion. The results presented support the concept that the chicken absorbs fructose with little conversion to glucose. The results of both the *in vitro* and *in vivo* experiments are in excellent agreement and indicate that the transformation of fructose to glucose during absorption occurs to the extent of only about 15%. These observations are similar to findings for the rat (2), and man (5), which have been reported to convert 10%, and 18% of fructose to glucose during absorption, respectively. These species are in contrast to the guinea pig (1, 2) and hamster (3) in which the conversion of the fructose to glucose during absorption is virtually complete.

TABLE IV. Hepatic Fructokinase Activity in Chicks Fed Glucose or Fructose Supplemented Diets.

Dietary carbohydrate	Final body wt (g)	Hepatic fructokinase (units/mg of protein)
Glucose	568 ± 12[a]	21 ± 3[b]
Glucose and fructose	519 ± 7	27 ± 5
p	<0.01	ns

[a] Final body weight is mean ± SEM for 10 chicks. Initial body weight was 48–60 g.

[b] Fructokinase activity is the mean ± SEM for 5 chicks. A unit is defined as the phosphorylation of one nmole of fructose/min at 37°.

Like other species studied, the chicken has a very active hepatic fructokinase. In fact, the activities observed in the present study (21 and 27 mμmole units/mg of protein) are similar to those reported for rat liver (17, 18)and for human liver (17, 19). Also, as observed in the present studies for the chick, hepatic fructokinase specific activity in the rat is not increased by fructose ingestion (18, 20). Thus, the chicken is similar to the rat, dog, and man with regard to its relative inability to convert fructose to glucose during absorption, and as in these other species the liver appears to be the major organ responsible for fructose metabolism. That fructose is metabolized very rapidly is indicated by the observation that 10 min after intraperitoneal administration of fructose-^{14}C only 4% of the circulating hexose radioactivity was found as fructose.

Summary. Experiments were conducted to evaluate whether fructose was converted to glucose during absorption in the chick. Chicks were found to absorb glucose and fructose rapidly and absorption of an orally

administered dose of either hexose was essentially complete within 1 hr. Intestinal sacs incubated *in vitro* transported fructose from the mucosal to the serosal surface and only 17% of the hexose in the serosal fluid was glucose. Experiments with intact chicks using fructose-U-^{14}C demonstrated that only 12% of the radioactive hexose entering the portal blood was glucose. Thus, it was concluded that only about 15% of absorbed fructose is converted to glucose during the process of absorption. However, 10 min following the intraperitoneal administration of fructose-U-^{14}C, 97% of the hexose radioactivity in blood was present as glucose. The activity of hepatic fructokinase was observed to be high and was not influenced by fructose ingestion. It is proposed that fructose is rapidly utilized by the chick and that the liver is a major site of fructose metabolism.

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