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Metabolism of Fructose by the Liver of Diabetic and Non-Diabetic Subjects.* (19186)

JAMES W. CRAIG,† WILLIAM R. DRUCKER, MAX MILLER, J. EVAN OWENS, HIRAM WOODWARD, JR., BERNARD BROFMAN, AND WALTER H. PRITCHARD.

From the Department of Medicine, Western Reserve School of Medicine, Cleveland, O.

The metabolic effects of fructose administered intravenously to man have been studied in this laboratory recently(1). During the period of infusion, fructose was removed at the same rapid rate from the peripheral venous blood of both normal subjects and diabetic patients deprived of insulin. The concentration of blood pyruvic acid rose markedly in both groups of subjects. There was an increase in the blood glucose level of most of the normal subjects and of all of the diabetic patients investigated; in the latter group the glucose rise was of greater magnitude than in the normal group. Other investigators have demonstrated the participation of the liver in fructose metabolism in experiments with tissue slices or homogenates and with intact animals(2-8). Accordingly, hepatic vein catheterization studies were performed to evaluate the role of the liver in fructose metabolism in man.

Experimental procedure and methods. The subjects were 3 diabetic and 3 non-diabetic patients. In 2 of the cases, the diabetes mellitus was mild and easily regulated. Subject M.M. had had severe, labile diabetes mellitus for 23 years; she was in diabetic ketosis during the test and her CO_2 combining power was 30 volumes per 100 ml at the end of the test. None of the patients had evidence of hepatic disease. For 3 days prior to the test each patient received B complex capsules which provided at least 20 mg of thiamine daily; these were given to eliminate any possible effect of a sub-clinical thiamine deficiency on pyruvate metabolism. The diabetic patients were given only regular insulin for 3 days before the test and none on the morning of the test; therefore, no insulin effect was present at the time of the procedure. The tests were performed after an overnight fast. Pre-medication consisted of 0.032 g of codeine phosphate and either 0.2 g of phenobarbital or 0.1 g of seconal. A radiopaque catheter was inserted into a superficial arm vein and its tip was guided under fluoroscopic control into a right hepatic vein. Following a control period, 1.0 g of fructose‡ per kg was administered intravenously in a 10% solution in approximately one hour. Specimens of blood were obtained simultaneously from the hepatic vein and a peripheral artery during the control period; at approximately 30, 45, and 60 minutes after the beginning of the fructose infusion; and 30 minutes after the end of the infusion. These specimens were analyzed in duplicate for the following substances: Fructose(9); total hexose (Somogyi's

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† Stober Fellow in Medicine.

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TABLE I. Effects of Intravenous Fructose on Blood Concentrations and Net Splanchnic Assimilations of Fructose and Pyruvic Acid.

Date	Subject, age, wt, S.A.*	Time, min	Fructose: Artery, mg/100 ml	Fructose: Hepatic vein, mg/100 ml	Fructose: Net splanchnic assimilation, mg/min/m²	Pyruvic acid: Artery, mg/100 ml	Pyruvic acid: Hepatic vein, mg/100 ml	Pyruvic acid: Net splanchnic assimilation, mg/min/m²
				A. Non-diabetic subjects				
5/15	P.	0	2	2	0	.7	.6	1
	39 yr	41†	90	49	250	2.9	4.4	—9.2
	72 kg	66†	82	48	320	3.3	4.6	—12
	1.85 m²	97	23	19	49	2	2.6	—7.4
5/29	M.	0	2	3	—6	.9	.7	1.2
	59 yr	31†	170	132	300	2.4	3	—4.7
	57 kg	41†	130	87	330			
	1.66 m²	46†	108	64	400	2.6	3.9	—12
		81	26	20	43	2	2.6	—4.3
9/17	F.	0	1	1	0	.8	1.3	—4
	41 yr	35†	94	46	380	3.2	6	—22
	73 kg	48†	99	54	360	3.1	5.3	—18
	1.71 m²	60†	110	59	370	3.4	5.8	—18
		92	21	15	30	2.5	3.1	—3
				B. Diabetic subjects				
5/22	R.	0	2	2	0	1.2	.8	1
	52 yr	31†	128	71	160	3.5	5.7	—6.2
	68 kg	55†	150	76	240	3.9	6.9	—9.6
	1.66 m²	91	34	28	17	3.4	4.5	—3.1
6/5	B.	0	2	1	8	.9	.8	.8
	33 yr	30†	85	32	390	2.8	4.4	—12
	70 kg	45†	86	42	360			
	1.82 m²	60†	72	42	250	4	6	—17
		90	20	16	36	2.7	2.7	0
9/24	M.M.	0	2	2	0	1	.8	2
	28 yr	30†	96	54	390	1.3	1.1	1.8
	61 kg	46†	84	52	320	1.7	.9	8
	1.60 m²	54†	84	52	310	1.4	1.1	2.9
		85	24	21	39	1.7	.7	13

* Surface area. † During fructose infusion.

iodometric method for glucose)(10); pyruvic acid(11); citric acid(12,13); and, in some cases, malic acid(14) and lactic acid(15). Glucose was computed as the difference between total hexose and fructose. There was no interference with the fructose determination by blood glucose levels up to 300 mg per 100 ml; corrections were made for concentrations above 300 mg per 100 ml. The hepatic blood flow was estimated at appropriate intervals by the BSP technic(16,17). The estimated net splanchnic assimilation of a substance was calculated by multiplying its arterial-hepatic venous concentration difference by the estimated hepatic blood flow; the results were expressed in milligrams per minute per square meter of body surface. A positive value indicated retention of the substance by the splanchnic system, and a negative value indicated that the substance was being released from the system. It was assumed that the metabolic changes which took place in the splanchnic system occurred chiefly in the liver.

Results. Results of these studies are recorded in Tables I and II. Large amounts of fructose were retained by the liver in both diabetic patients deprived of insulin and in non-diabetic subjects. It was estimated that the liver removed approximately one-third of the administered fructose during the period of the infusion; during the 30 minutes after the infusion, approximately another one-sixth of the infused fructose was retained by the liver. During the *control period* the liver removed small amounts of pyruvic acid from the blood in 5 out of 6 cases; in the sixth case a small amount of pyruvic acid was released. When fructose was administered, there was a large output of pyruvic acid from

TABLE II. Effects of Intravenous Fructose on Blood Concentrations and Net Splanchnic Assimilation of Glucose.

Subject	Time, min	Glucose		
		Artery, mg/100 ml	Hepatic vein, mg/100 ml	Net splanchnic assimilation, mg/min/m²
		A. Non-diabetic subjects		
P.	0	115	124	—86
	41*	127	135	—49
	66*	143	145	—19
	97	130	130	0
M.	0	87	91	—24
	31*	103	113	—78
	41*	111	132	—160
	46*	130	128	18
	81	85	90	—36
F.	0	85	94	—72
	35*	85	88	—24
	48*	91	89	16
	60*	83	85	—15
	92	94	115	—100
		B. Diabetic subjects		
R.	0	171	174	—8
	31*	198	204	—17
	55*	205	216	—35
	91	201	204	—8
B.	0	117	130	—100
	30*	144	164	—150
	45*	153	166	—110
	60*	156	168	—100
	90	150	146	36
M.M.	0	386	430	—440
	30*	471	519	—440
	46*	513	545	—320
	54*	516	560	—430
	85	542	567	—320

* During fructose infusion.

the liver in all cases except patient M.M., who had severe diabetes mellitus and was in ketosis during the test. In the latter case the liver continued to remove pyruvic acid from the blood during the fructose infusion and removed an increased amount in the post-infusion period; in spite of the continued uptake of pyruvic acid by the liver, the arterial level of pyruvic acid increased. *Lactic acid* levels were determined in 4 cases. The changes in lactic acid usually paralleled those in pyruvic acid, but the magnitude of the lactic acid changes was greater. In one diabetic and 2 non-diabetic subjects the liver released lactic acid during the fructose infusion. In the fourth case (M.M., a diabetic patient in ketosis), lactic acid was retained by the liver in the control period, neither retained nor released at the mid-point of the infusion, and retained in increasing amounts in the subsequent periods; the arterial level of lactic acid showed a marked increase. In the 3 cases in which pyruvic and lactic acid were released from the liver, approximately one-fourth of the fructose uptake could be accounted for by the hepatic output of these two acids. In all 6 cases the liver released glucose during the control period. During fructose administration there was an increase in the hepatic glucose output in 3 cases (A.M., a non-diabetic subject, and E.R. and W.B., diabetic patients) and a decrease in the other 3 cases (non-diabetic subjects B.P. and L.F. and diabetic patient M.M.). There was an increase in the arterial level of glucose during the fructose infusion in all 6 cases.

Small quantities of citric acid were retained by the liver during the control period, and the administration of fructose did not produce any significant change in the splanchnic balance of

this acid. In the 3 cases in which blood malic acid levels were determined, the arterial-hepatic venous concentration differences were too small to be significant both in the control period and after fructose infusion.

Discussion. Other investigators have studied the metabolism of fructose in liver slices or homogenates and in the liver of intact animals. Their results indicate that fructose may be (a) phosphorylated under the influence of a hexokinase separate from that for glucose (2,3); (b) converted to glycogen(4); (c) transformed to glucose(3,5); (d) metabolized to lactic acid(6,7); (e) converted to fatty acids(8); or (f) oxidized completely to CO_2 (8). Our studies furnish evidence of the occurrence of (d) in the human liver *in vivo*; it is probable that hepatic production of lactic and pyruvic acid plays an important role in the rise in the concentration of these substances in the peripheral blood after fructose administration. Transformation of fructose to glucose may also have occurred in some of the cases; but in only 3 instances could increased hepatic glucose production have been responsible for an elevation in the peripheral blood level of glucose; other mechanisms must be invoked to account for the glucose rise in the other cases. Our technics of investigation do not permit an evaluation of the relative importance of the other possible routes of fructose disposal. Slein, Cori, and Cori(2) concluded from tissue studies that fructose utilization by the liver was not inhibited by glucose; this conclusion is apparently applicable also to the human liver *in vivo*. The depletion of liver glycogen which occurs in diabetic acidosis(18) may explain the divergent results obtained in case M.M. In the absence of normal hepatic glycogen stores, fructose may be converted to glycogen or glucose rather than to pyruvic and lactic acid. The rise in pyruvic and lactic acid concentrations in the arterial blood in this case must have been of extra-hepatic origin. Although the estimation of hepatic blood flow is admittedly inexact, the determination was used in this study to obtain an approximate value for the net splanchnic assimilation of certain substances. The conclusion that a substance was being retained by the liver or released from that organ was based only on the relationship of its concentrations in arterial and hepatic venous blood, and did not depend upon the magnitude of the hepatic blood flow. While errors in the estimation of hepatic blood flow would result in an erroneous calculation of the quantities of substances involved, the possible range of error does not appear to be great enough to invalidate any of our conclusions.

Summary and conclusions. The metabolism of fructose in the liver was investigated by hepatic vein catheterization studies in 3 diabetic patients deprived of insulin and in 3 non-diabetic subjects. (1) There was a large hepatic uptake of fructose during the period of intravenous administration in both diabetic and non-diabetic subjects. (2) In all but one case there was a large hepatic output of pyruvic and lactic acid during the fructose infusion. The liver of one diabetic patient in ketosis continued to remove pyruvic and lactic acid from the blood; hepatic glycogen depletion may have accounted for this divergent result. (3) In half the cases (2 diabetic patients and 1 non-diabetic subject) the output of glucose by the liver was increased during fructose administration. (4) In the absence of ketosis, the liver of the diabetic subject without insulin, therefore, metabolized fructose in a manner similar to that of the liver of the non-diabetic individual. The presence of ketosis, however, was accompanied by a decreased output of pyruvic and lactic acid, despite a normal uptake of fructose.

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Effect of Rabies Street Virus in the Cotton Rat and the Swiss Albino Mouse. (19187)

REGINALD L. REAGAN AND A. L. BRUECKNER.

From the Live Stock Sanitary Service Laboratory, Maryland State Board of Agriculture, University of Maryland, College Park.

The rabies virus strain V 308 used in the present study was isolated in Swiss albino mice (21-day-old Webster Carworth strain) from a dog brain sent to this laboratory for a routine examination for rabies. The history of this strain is given in a previous paper which dealt with electron microscope studies of Negri bodies(1). The strain was pathogenic for 2 species of bats(2). Furious rabies was produced in the Syrian hamster by various routes of inoculation with this strain (3).

Methods. Each of four 3-week-old cotton rats (*Sigmodon hispidus*) was inoculated intracerebrally with 0.03 cc of a 10% virus-bearing brain suspension of the 2nd mouse passage which titrated 10^{-4} in mice. Five days after injection the cotton rats showed tremors and paralysis, but no evidence of furious rabies was noted. The brains of 2 paralyzed cotton rats were removed aseptically. Touch preparations from the Ammon's horns of these brains were stained with Seller's stain(4) and upon examination under an optical microscope were found to contain Negri bodies. A 10% suspension in physiological saline was made from the 2 brains. This material was used to initiate the present study in cotton rats and Swiss albino mice. By intracerebral inoculation it titrated $10^{-4.5}$ in cotton rats and $10^{-4.2}$ in mice. Diagnosis of rabies was made on the following factors: presence of symptoms of rabies in the rat or mouse and demonstration of Negri bodies in the Ammon's horn of the brain of the animal showing symptoms. The presence of Negri bodies was determined by staining touch preparations of the Ammon's horn with Sellers' stain and examining the slides with the optical microscope. Twenty-seven healthy cotton rats (21 days old) were divided into 9 groups of 3 animals each. The 3 rats of each group were administered the virus by one of the following methods: intracerebral, intraperitoneal, intradermal, intramuscular, intralingual, and intracardial injection and by oral, rectal, and intranasal instillation. In all routes of exposure the inoculum consisted of 0.1 cc of the above described suspension, except in the intracerebral group where the inoculum was 0.03 cc. Twenty-seven healthy Swiss mice of the same age were divided into 9 groups of 3 each and were treated in a like manner. After inoculation all groups were observed daily for symptoms of rabies. After symptoms of rabies appeared and the animal became paralyzed or moribund, it was sacrificed and the brain removed and examined for the presence of Negri bodies. Some animals died during the night and the brains of these