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The Mechanism of the Fructose Effect on the Ethanol Metabolism of the Human Liver *

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Ethanol is normally metabolized at a constant rate by the liver of man and animals. Attempts to change this rate have included treatment with metabolically active substances such as glucose, pyruvate, amino acids, dinitrophenol, insulin, and thyroid hormone. In some cases an increase has been observed, but usually only a very small one. The only exception so far discovered is the effect of fructose, first demonstrated by Stuhlfauth and Neumaier (1). This was confirmed by Pletscher, Bernstein, and Staub (2), who found an average increase in the elimination rate of ethanol of 80% in dogs receiving 1 to 2 g of fructose per kg per hour, and about 70% in human subjects who had fructose infused intravenously in comparable quantities (3). Oral ingestion of fructose in man gave the same result (4). Smaller or no effects have also been reported (5, 6), but in these cases the amount of fructose given was probably insufficient.

Several explanations of the phenomenon (which we shall refer to as the "fructose effect") have been offered, but since none of them were supported by direct experimental evidence, a detailed, quantitative investigation of the problem appeared desirable. Furthermore, it was of interest to study the circulatory and metabolic reactions of the liver during accelerated ethanol metabolism, as previous studies have shown that under normal conditions ethanol combustion accounts for practically all of the hepatic oxygen uptake (7).

In the present work the major metabolites of ethanol and fructose have been determined in arterial and hepatic venous blood, together with the splanchnic blood flow, in an attempt to account for the fate of ethanol and fructose in the liver of human subjects who received a continuous infusion of these substances together or one at a time.

Methods

Sixteen subjects with no known hepatic or metabolic disease were examined (Table I). They were kept fasting for 12 to 16 hours and premedicated with 200 mg of phenobarbitone. A double-lumen catheter was introduced via an antecubital vein, and the tip for the catheter, with the distal opening, was placed in one of the right hepatic veins. Through the proximal opening, situated in the upper part of the right atrium, ethanol or fructose or both were infused in the amounts stated in Table I.

TABLE I

Data of subjects examined and amounts of fructose and ethanol infused

Exp. no.	Age	Sex	Height	Weight	Infusion	
					Fructose	Ethanol
	years		*cm*	*kg*	*mmoles/min*	
1	55	M	181	49.5	10.61	
2	18	M	182	72.5	6.95	
3	26	M	181	81.9	9.33	
4	38	F	160	69.5	7.19	
5	22	M	177	70.0	6.16	
6	54	M	178	72.2	7.19	
7	36	M	176	106.5	9.48	I 2.72*
						II 4.64
8	29	M	177	71.8	8.06	I 2.93*
						II 4.89
9	20	M	170	60.1	9.96	3.08
10	21	M	177	65.0	9.19	3.13
11	23	F	163	55.6	8.08	3.04
12	31	F	166	57.5	7.21	2.98
13	45	M	160	58.0	7.16	2.93
14	40	F	165	58.5	7.38	2.33
15	18	F	175	53.7	10.86	2.89
16	19	M	183	68.3	7.02	4.34

* I, before fructose infusion; II, during fructose infusion.

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During the first 10 minutes of the infusion a priming dose was given (ethanol about 80 mmoles, fructose about 150 mmoles). Bromsulphalein (experiments no. 7 to 10, priming dose 150 mg, infusion 5 mg per minute) or indocyanine green (experiments no. 1 to 6 and no. 11 to 16, priming dose 15 mg, infusion 0.5 mg per minute) was given from the start of the experiments. Bromsulphalein, used in some experiments, was abandoned as a test substance, because in some experiments, not included here, the substances infused appeared to inhibit the hepatic elimination of Bromsulphalein so much that calculation of the hepatic blood flow was rendered unreliable. This phenomenon has been observed by others (8, 9). The removal of indocyanine green was not affected by the experimental procedures.

The material consisted of two series, one with infusion of fructose alone (experiments no. 1 to 6) and one with infusion of ethanol and fructose together (experiments no. 7 to 16). In the first series (fructose alone) the period with fructose infusion was preceded by a control period. In the second series (ethanol plus fructose) ethanol was given alone in the first period; this infusion was maintained (in two experiments with increased rate) during the second period in which fructose was infused. After the start of an infusion 30 minutes was allowed for equilibration; then three pairs of blood samples were taken simultaneously from the arterial and hepatic venous catheters with an interval of 10 minutes between each pair.

The blood was immediately transferred from the syringes, moistened by a heparin-fluoride solution, to iced test tubes. After cooling of the blood in an ice bath, cells and plasma were immediately separated by centrifugation for the determinations performed in plasma. The following determinations were performed by the methods indicated: ethanol (10), acetate (11), lactate (12), pyruvate (13),[1] glucose (14), Bromsulphalein (15) or indocyanine green (16), fructose (17), acetoacetate and β-hydroxybutyrate (18) (experiments no. 7, 8, 10, and 11 only), and glycerol (19) (experiments no. 5, 6, 15, and 16 only). In blood samples drawn during fructose infusion sorbitol was determined in Somogyi filtrates of whole blood after incubation for 1 hour at 37° C with Baker's yeast. Commercial yeast was washed six times with 5 vol of 10 mM KH_2PO_4. Each time the suspension was allowed to stand for 30 minutes before centrifugation. A 50% suspension was finally prepared in the same salt solution, and 0.1 vol of this was used for removal of the hexoses. In this way the blank value introduced in the subsequent analysis (20) was comparatively small and constant. Blood samples taken before the infusion with addition of known amounts were used as standards. The sorbitol analyses were not specific. Glycerol reacts with the periodic acid reagent and gives about the same color per molecule. The "sorbitol" figures therefore include glycerol. The formation of sorbitol was confirmed by examination of the urine from a subject receiving ethanol and fructose. The urine was evaporated to dryness, extracted with ethanol, and after removal of ethanol and extraction with water, hexoses were removed with yeast, and the solution was clarified by zinc hydroxide. The extract was subjected to column chromatography on Dowex-1 resin in borate buffer (21). The peak coincided with the peak in a control experiment with added sorbitol, whereas normal urine does not contain any material with this property. The amount of sorbitol found in the urine by chromatography agreed well with the quantity measured by direct periodate oxidation. For substances determined in plasma the concentrations in whole blood were calculated from the hematocrit value and their known distribution in red cell water.

In the middle of each sampling period arterial and hepatic venous blood was taken for determination of oxygen and carbon dioxide (van Slyke) and of pH and P_{CO_2} (22). Simultaneously, expiratory air was collected for a 5-minute period for determination of total body oxygen uptake and carbon dioxide production (Haldane). The cardiac output was determined in nine experiments (no. 1, 2, 6, 7, 8, 9, 12, 14, and 15) by the direct Fick method, using right atrial blood or, in one case (no. 9) in whom a small atrial septal defect was detected, the mean of superior and inferior caval blood, as a measure of mixed venous blood.

The infusion of ethanol caused slight dizziness in some of the patients, but none of them became visibly intoxicated. Six of the patients receiving ethanol and fructose together complained of sharp abdominal pains from about 5 to 15 minutes after the start of the fructose infusion; in some patients they disappeared spontaneously, in others after injection of 50 mg of Demerol. Similar complaints have been observed by other workers (6), even following infusion of fructose alone (23), but this was not noticed in the present series. No other ill effects of the procedures were observed.

Results

The experimental data of two typical experiments, one of the fructose series and one of the ethanol-fructose series, are given in Table II and Table III, respectively. Figure 1 shows the mean concentrations of some of the substances measured during the different experimental periods. Most of the arterial and hepatic venous concentrations are approximately constant within each period. In the series of experiments with fructose alone, however, the concentration of lactate rises continuously during the infusion of fructose, but the arteriohepatic venous difference is fairly constant. In the experiments with ethanol and fructose the hepatic venous concentration of ethanol is decreasing, and that of acetate is increasing, result-

[1] Control experiments showed that identical concentrations were obtained whether the samples were treated as mentioned or precipitated immediately by perchloric acid.

TABLE II

Data from an experiment with fructose infusion (exp. no. 6)

Time of sampling (minutes):		Control period 22.4	31.9	40.9	Fructose period starting at 41.3 70.3	81.2	91.9
Fructose mM in blood	Arterial				4.14	4.56	4.39
	Hepatic venous				2.42	2.79	2.57
	Difference				1.72	1.77	1.82
Sorbitol mM in blood	Arterial				0.44	0.23	0.25
	Hepatic venous				0.37	0.08	0.00
	Difference				0.07	0.15	0.25
Glucose mM in blood	Arterial	4.22	4.00	4.03	4.85	4.92	4.90
	Hepatic venous	4.19	4.17	4.33	5.44	5.30	5.18
	Difference	0.03	−0.17	−0.30	−0.59	−0.38	−0.28
Lactate mM in plasma	Arterial	0.74	0.74	0.76	3.88	4.63	4.99
	Hepatic venous	0.61	0.47	0.52	5.39	5.65	6.15
	Difference	0.13	0.27	0.24	−1.51	−1.02	−1.16
Pyruvate μM in plasma	Arterial	22	16	31	352	356	384
	Hepatic venous	21	20	8	593	609	662
	Difference	1	−4	23	−241	−253	−276
Glycerol mM in plasma	Arterial				0.09	0.07	0.08
	Hepatic venous				0.07	0.04	0.08
	Difference				0.02	0.03	0.00
Indocyanine green μg/L in plasma	Arterial	1,024	963	1,000	1,077	1,057	1,102
	Hepatic venous	522	486	437	494	526	420
	Difference	502	477	563	583	531	682
Oxygen mM in blood	Arterial		7.59			7.45	
	Hepatic venous		5.54			5.00	
	Difference		2.05			2.45	
Carbon diozide mM in blood	Arterial		20.29			17.78	
	Hepatic venous		22.61			20.81	
	Difference		−2.32			−3.03	
pH in blood	Arterial		7.39			7.37	
	Hepatic venous		7.35			7.28	
P_{CO_2} mm Hg in blood	Arterial		37			32	
	Hepatic venous		40			45	
				Fructose in urine 17 mmoles Sorbitol in urine 5 mmoles			

ing in increasing arteriohepatic venous differences of both substances. The splanchnic blood flow does not show consistent changes within the periods of measurements, and with the exceptions mentioned the experimental periods can reasonably be assumed to represent steady states.

Table IV shows some of the results obtained in the experiments with infusion of fructose alone. The splanchnic blood flow is not changed by the infusion of fructose. The splanchnic oxygen uptake increases by about 30%, but the increase is not statistically significant. The production of glucose and carbon dioxide does not change, in contrast to the output of lactate and pyruvate, which is greatly augmented. The mean glycerol uptake during fructose infusion in two experiments is 0.02 mmole per minute.

Table V shows similar data in the experiments with ethanol and fructose, together with the ethanol uptake and the acetate output. In these experiments there is a significant increase in the splanchnic blood flow, the oxygen uptake, and in the uptake of ethanol and pyruvate and the output of acetate, glucose, and lactate. The mean glycerol uptake during ethanol infusion alone in two experiments is 0.02 mmole per minute, and during ethanol-fructose infusion there is a mean glycerol output of 0.12 mmole per minute.

A comparison of the results in the two series of experiments shows the splanchnic uptake of

TABLE III

Data from an experiment with ethanol and ethanol-fructose infusion (exp. no. 15)

		Ethanol period			Ethanol-fructose period starting at 42.8		
Time of sampling (minutes):		23.6	31.1	41.7	69.8	81.5	91.2
Ethanol mM in plasma	Arterial		5.70	5.98	5.61	5.27	5.05
	Hepatic venous	4.02	4.45	4.90	4.41	3.81	3.57
	Difference		1.25	1.08	1.20	1.46	1.48
Acetate mM in plasma	Arterial	0.67	0.77	0.92	1.62	1.93	2.05
	Hepatic venous	1.45	1.59	1.57	3.00	3.41	3.50
	Difference	−0.78	−0.82	−0.65	−1.38	−1.48	−1.45
Fructose mM in blood	Arterial				7.29	7.20	7.46
	Hepatic venous				6.36	5.65	5.60
	Difference				0.93	1.55	1.81
Sorbitol mM in blood	Arterial				2.17	2.39	2.54
	Hepatic venous				2.61	2.70	2.93
	Difference				−0.44	−0.31	−0.39
Glucose mM in blood	Arterial	3.73	3.66	3.56	4.46	5.21	5.21
	Hepatic venous	4.00	4.05	3.69	5.41	6.09	5.92
	Difference	−0.27	−0.39	−0.13	−0.95	−0.88	−0.71
Lactate mM in plasma	Arterial	0.90	1.07	1.18	3.77	4.48	4.88
	Hepatic venous	1.02	1.14	1.32	4.49	5.36	5.66
	Difference	−0.12	−0.07	−0.14	−0.72	−0.84	−0.78
Pyruvate μM in plasma	Arterial	28	16	32	89	86	90
	Hepatic venous	12	9	12	36	37	31
	Difference	16	7	20	53	49	59
Glycerol mM in plasma	Arterial	0.079	0.114	0.086	0.48	0.45	0.50
	Hepatic venous	0.050	0.065	0.061	0.64	0.52	0.56
	Difference	0.03	0.05	0.03	−0.16	−0.07	−0.06
Indocyanine green μg/L in plasma	Arterial	930	857	816	616	685	673
	Hepatic venous	228	163	212	249	269	294
	Difference	702	694	604	367	416	379
Oxygen mM in blood	Arterial		6.85			6.47	
	Hepatic venous		5.33			4.64	
	Difference		1.52			1.83	
Carbon dioxide mM in blood	Arterial		19.10			15.81	
	Hepatic venous		19.99			15.90	
	Difference		−0.89			−0.09	
pH in blood	Arterial		7.42			7.27	
	Hepatic venous		7.38			7.21	
P_{CO_2} mm Hg in blood	Arterial		32			37	
	Hepatic venous		40			44	
				Fructose in urine 22 mmoles Sorbitol in urine 14 mmoles			

fructose to be 25% greater in ethanol-fructose experiments ($p < 0.05$). On the average 30 and 35% of the fructose administered is taken up in the splanchnic organs, and this percentage, as well as the extrasplanchnic removal, does not differ significantly in the two series. The same is the case with the urinary excretion of fructose, which is 5 and 10%, respectively, of the amount infused. In both series the excretion of sorbitol is about one-fourth of the excretion of fructose.

In Table VI the mean concentrations of lactate and pyruvate (with standard deviations) in arterial and hepatic venous plasma are given, together with the ratios between them. Due to very low concentrations of pyruvate in some experiments, the lactate/pyruvate ratio during the

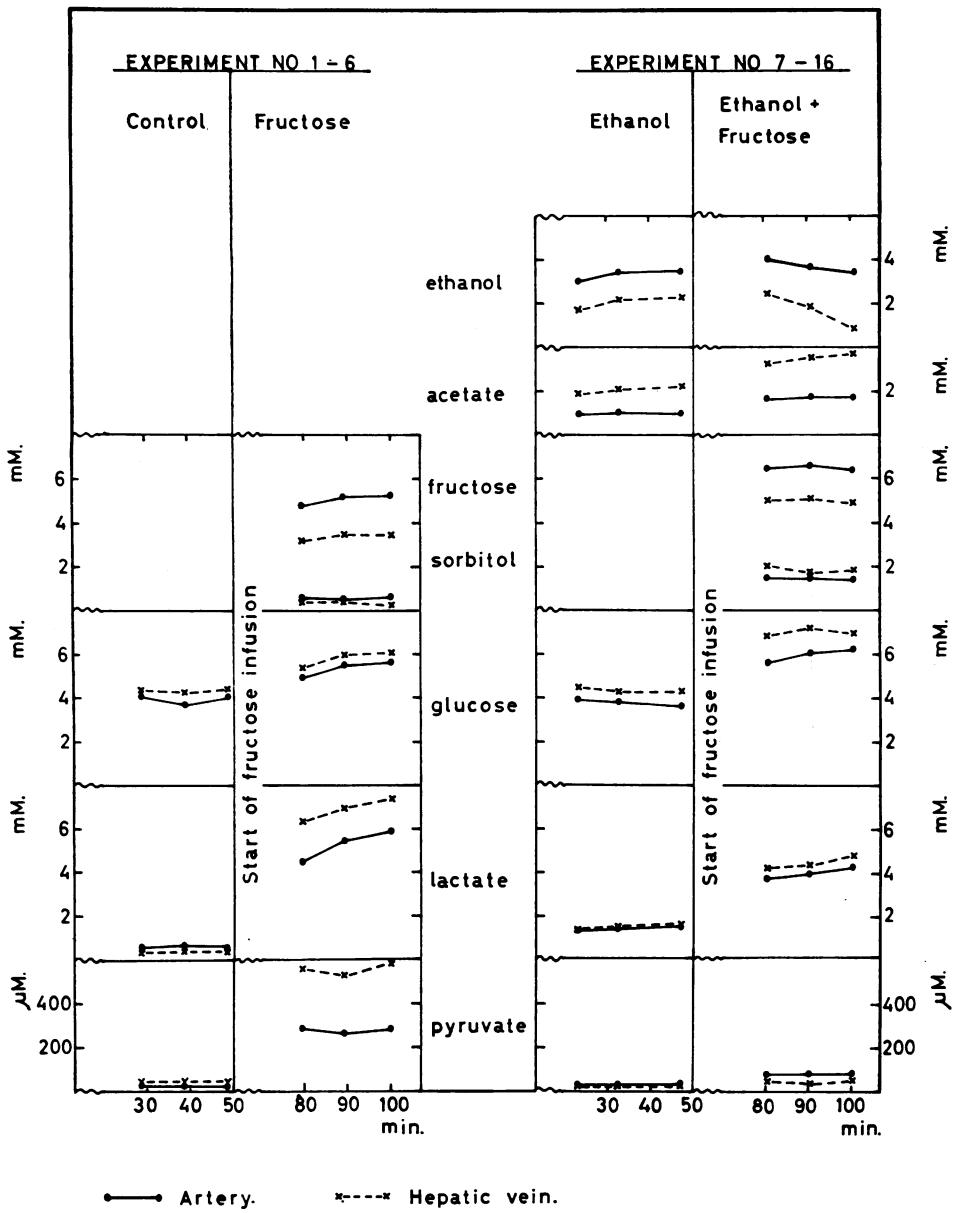

FIG. 1. THE MEAN CONCENTRATION CURVES IN ARTERIAL AND HEPATIC VENOUS BLOOD OF SOME OF THE METABOLITES MEASURED DURING INFUSION OF FRUCTOSE OR ETHANOL OR BOTH.

first period of fructose as well as of ethanol-fructose experiments is only approximative. Table VII shows the acetoacetate and β-hydroxybutyrate data from experiments in which these analyses were performed.

The total oxygen consumption and carbon dioxide production of the body (Table VIII) rise significantly when fructose is given in both series. When the two series are compared, the rise in carbon dioxide production is found to be significantly smaller when fructose is given together with ethanol than when fructose is given alone ($p < 0.05$).

Table IX shows the mean pH and Pco_2 in arterial and hepatic venous blood. All the measured pH values are significantly reduced by fructose

TABLE IV

Splanchnic blood flow and metabolism, before and during fructose infusion

Exp.	Period*	Hepatic blood flow	Splanchnic consumption						
			O_2	CO_2	Glucose	Lactate	Pyruvate	Fructose	Sorbitol
		L/min	*mmoles/min*			*mmoles/min*		*mmoles/min*	
1	I	0.99	2.56	−2.96	−0.49	0.26	0.000		
	II	1.03	3.21	−3.12	−0.87	−1.90	−0.273	2.38	0.21
2	I	2.29	3.21	−2.04	−0.41	0.50	0.027		
	II	2.26	3.44	−3.37	−0.75	−2.98	−0.208	2.89	0.41
3	I	1.42	2.15	−3.31	−1.36	−0.28	−0.048		
	II	2.00	4.02	−2.60	−1.22	−2.44	−0.350	2.06	0.34
4	I	0.93	1.70	−1.25	−0.04	0.17	0.007		
	II	1.12	2.84	−2.84	−0.18	−1.32	−0.342	2.49	0.03
5	I	1.29	1.56	−2.89	−0.48	0.06	−0.046		
	II	1.19	1.81	−2.40	−0.31	−1.36	−0.437	1.62	0.02
6	I	1.56	3.20	−3.62	−0.23	0.27	0.009		
	II	1.33	3.26	−4.03	−0.56	−1.35	−0.283	2.35	0.21
Mean	I	1.41	2.40	−2.68	−0.50	0.16	−0.009		
	II	1.49	3.10	−3.06	−0.65	−1.89	−0.316	2.30	0.20
p II-I		>0.5	>0.05	>0.3	>0.2	<0.005	<0.001		

* I = control period; II = fructose period.

TABLE V

Splanchnic blood flow and metabolism during infusion of ethanol alone and of ethanol plus fructose

Exp.	Period*	Hepatic blood flow	Splanchnic consumption								
			O_2	CO_2	Glucose	Lactate	Pyruvate	Ethanol	Acetate	Fructose	Sorbitol
		L/min	*mmoles/min*			*mmoles/min*		*mmoles/min*		*mmoles/min*	
7	I	1.36	4.12		−0.60	0.11	0.012	1.73	−1.74		
	II	1.21	5.45		−1.10	−0.42	0.051	2.76	−2.42	2.96	−0.16
8	I	1.39	2.72	−0.13	−0.86	0.06	0.000	1.49	−0.88		
	II	1.58	3.32	−0.77	−1.72	−0.47	0.057	2.67	−2.18	3.22	−1.23
9	I	1.50	3.02	−2.75	−0.69	−0.09	0.005	1.52	−1.31		
	II	2.06	3.50	0.00	−2.61	−0.36	0.024	2.81	−2.45	3.46	−0.18
10	I	1.54	2.36	−1.23	−0.69	0.09	0.008	1.31	−1.17		
	II	2.35	4.40	−0.73	−1.50	−0.87	0.005	2.49	−2.80	2.42	−1.20
11	I	1.16	2.46	−1.19	−0.51	−0.05	0.000	1.31	−1.04		
	II	1.48	5.71	−2.31	−0.88	−0.77	0.024	3.51	−2.19	3.02	−0.41
12	I	1.35	3.87	−3.73	−1.01	−0.09	0.004	1.76	−1.07		
	II	1.65	4.62	−0.83	−2.11	−0.87	0.063	2.34	−2.57	2.44	
13	I	1.19	2.68	−2.12	−0.35	−0.07	0.007	1.99	−1.77		
	II	1.62	6.54	−2.06	−1.78	−0.57	0.058	3.32	−2.82	3.69	−1.17
14	I	1.29	1.55	−3.11	−0.70	0.00	0.006	1.08	−1.21		
	II	1.74	2.95	−1.86	−4.80	−1.20	0.031	2.31	−2.37	3.10	−0.47
15	I	1.07	1.66	−0.97	−0.28	−0.10	0.013	1.10	−0.69		
	II	1.82	3.33	−0.16	−1.56	−1.22	0.084	2.18	−2.24	2.64	−0.69
16	I	0.95	2.46	−2.03	−0.67	−0.05	0.028	1.58	−0.90		
	II	1.33	3.79	−2.77	−1.38	−0.39	0.060	2.79	−2.14	2.07	−0.08
Mean	I	1.28	2.69	−1.92	−0.64	−0.02	0.008	1.49	−1.18		
	II	1.68	4.36	−1.28	−1.94	−0.71	0.046	2.72	−2.42	2.90	−0.62
p II-I		<0.005	<0.005	>0.2	<0.005	<0.001	<0.001	<0.001	<0.001		

* I = ethanol period; II = ethanol plus fructose period.

TABLE VI

Mean plasma concentrations and concentration ratios of lactate and pyruvate in arterial and hepatic venous blood

	Fructose experiments (No. 1–6)				Ethanol+fructose experiments (No. 7–16)			
	Concentration		Concentration ratio		Concentration		Concentration ratio	
	Artery	Hepatic vein	Artery	Hepatic vein	Artery	Hepatic vein	Artery	Hepatic vein
	mM				*mM*			
	Control period				Ethanol period			
Lactate (SD)	1.02 (0.30)	0.88 (0.45)	53.5	25.1	1.39 (0.25)	1.41 (0.25)	81.5	156.6
Pyruvate (SD)	0.019 (0.018)	0.035 (0.035)			0.017 (0.010)	0.009 (0.005)		
	Fructose period				Ethanol + fructose period			
Lactate (SD)	5.26 (1.21)	6.82 (1.53)	19.5	12.3	4.00 (0.58)	4.51 (0.69)	56.3	122.0
Pyruvate (SD)	0.270 (0.122)	0.554 (0.224)			0.071 (0.022)	0.037 (0.013)		

infusion, and there is no difference between the two series. In experiments with fructose alone the arterial Pco_2 falls and the hepatic venous Pco_2 rises ($p < 0.01$), whereas no change takes place when fructose is added to an infusion of ethanol. A comparison of the two series shows that the Pco_2 of hepatic venous blood is significantly lower during simultaneous ethanol and fructose infusion than during fructose infusion alone.

The cardiac output, determined in about half of the experiments of both series, increases on the average by 50% when fructose is infused. Thus the ratio splanchnic blood flow/cardiac output remains constant, i.e., one-third, in experiments with ethanol-fructose infusion. In the control period of experiments with fructose alone, this ratio is also one-third, and during the fructose infusion, one-fifth. The number of experiments is too small to permit a statistical evaluation.

TABLE VII

*Acetoacetate and β-hydroxybutyrate before infusions (control), during infusion of ethanol alone, and during infusion of ethanol plus fructose**

Exp. no.	Period	β-Hydroxybutyrate		Acetoacetate	Concentration ratio β-hydroxybutyrate acetoacetate	
		Arterial concentration	Splanchnic consumption	arterial concentration	Artery	Hepatic vein
		mM	*mmoles/min*	*mM*		
7	Control	0.41	−0.30	0.18	2.2	2.1
	Ethanol	0.49	−0.14	0.12	4.1	5.9
	Ethanol + fructose	1.10	0.19	0.20	5.5	4.3
8	Control	0.30	−0.31	0.04	7.5	4.0
	Ethanol	0.39	−0.18	0.09	4.3	5.2
	Ethanol + fructose	1.05	−0.03	0.15	7.0	7.6
10	Control	0.32	−0.14	0.07	4.6	
	Ethanol	0.38	−0.26	0.07	6.1	14.0
	Ethanol + fructose	1.16	−0.24	0.15	7.7	10.5
11	Control	0.21	−0.10	0.07	3.0	3.0
	Ethanol	0.25	−0.20	0.08	3.1	8.4
	Ethanol + fructose	0.83	−0.31	0.21	3.9	7.3

* The splanchnic consumption during the control period was calculated from the splanchnic blood flow values determined during the ethanol periods.

TABLE VIII

The influence of infusion of fructose or ethanol or both on the oxygen uptake and the carbon dioxide production of the body

	Body oxygen consumption	Body carbon dioxide production
	mmoles/min	*mmoles/min*
Exp. 1–6		
Control	10.27 (2.36)*	8.00 (1.73)
Fructose	12.89 (1.44)	12.62 (1.31)
Exp. 7–16		
Ethanol	9.87 (2.35)	7.46 (1.61)
Ethanol + fructose	13.55 (2.31)	10.26 (1.89)

* Standard deviation.

Discussion

Studying the fructose effect in the intact human organism has obvious advantages, but also entails certain limitations. The net splanchnic uptake or output of certain substances cannot give a complete picture of the metabolic changes in the liver cells, and this picture is, furthermore, obscured by independent changes in the metabolism of the extrahepatic splanchnic organs. There are also some practical difficulties. To spare the patients any unnecessary stress, the number of blood samples taken and the duration of the experiments were kept at a minimum. More extensive studies may reveal dynamic changes not clearly seen in the present experiments.

The hepatic elimination of ethanol. It is fairly well established that the first step, the oxidation of ethanol to acetaldehyde, is catalyzed exclusively by the nicotinamide adenine dinucleotide (NAD)-dependent enzyme, alcohol dehydrogenase (ADH), and that further dehydrogenation to free acetate is brought about by another NAD-dependent enzyme, aldehyde dehydrogenase. Theorell and Chance (24) have demonstrated that the dehydrogenation of ethanol to acetaldehyde by hepatic alcohol dehydrogenase consists of a number of partial processes of which the dissociation of the ADH-NADH complex is by far the slowest, thus limiting the rate of the process. The dissociation of the complex may be influenced by the concentration of NADH in the liver cells. Some observations indicate that the limitation of the ethanol dehydrogenation may depend on the capacity of the liver to reoxidize the NADH formed (25, 26). This view has been refuted by reference to the fact that the oxidative capacity of the liver greatly exceeds the requirements of ethanol dehydrogenation. The objection may not be relevant, however, since the oxidation of ethanol takes place outside the mitochondria, and other processes may be needed to carry the hydrogen from the extramitochondrial NADH to the respiratory chain because NADH cannot penetrate the mitochondrial membrane (27). If these transfer mechanisms have a limited capacity, then the NADH/NAD ratio will rise in the extramitochondrial cytoplasm.

This assumption finds some support in direct measurements of the concentrations of NADH and NAD in rat liver tissue during ethanol elimination, which show that the NADH/NAD ratio is two to three times higher than the control values (28, 29). Similar studies have not been performed in man, but an idea of the ratio may be obtained by determinations in the hepatic venous blood of certain metabolites that form parts of redox systems governed by NAD-dependent enzymes. Thus the concentration ratio of β-hydroxybutyrate/acetoacetate is found to rise during ethanol elimination in the present experiments

TABLE IX

The influence of infusion of fructose or ethanol or both on the pH and the carbon dioxide tension in arterial and hepatic venous blood

	pH (SD)		P_{CO_2} (SD)	
	Artery	Hepatic vein	Artery	Hepatic vein
			mm Hg	
Exp. 1–6				
Control	7.33 (0.04)	7.32 (0.04)	42.3 (3.8)	45.2 (4.2)
Fructose	7.30 (0.05)	7.23 (0.04)	37.5 (3.8)	49.8 (5.6)
Exp. 7–16				
Ethanol	7.34 (0.04)	7.30 (0.04)	40.0 (5.9)	44.0 (5.9)
Ethanol + fructose	7.27 (0.03)	7.22 (0.05)	39.9 (4.9)	42.6 (3.0)

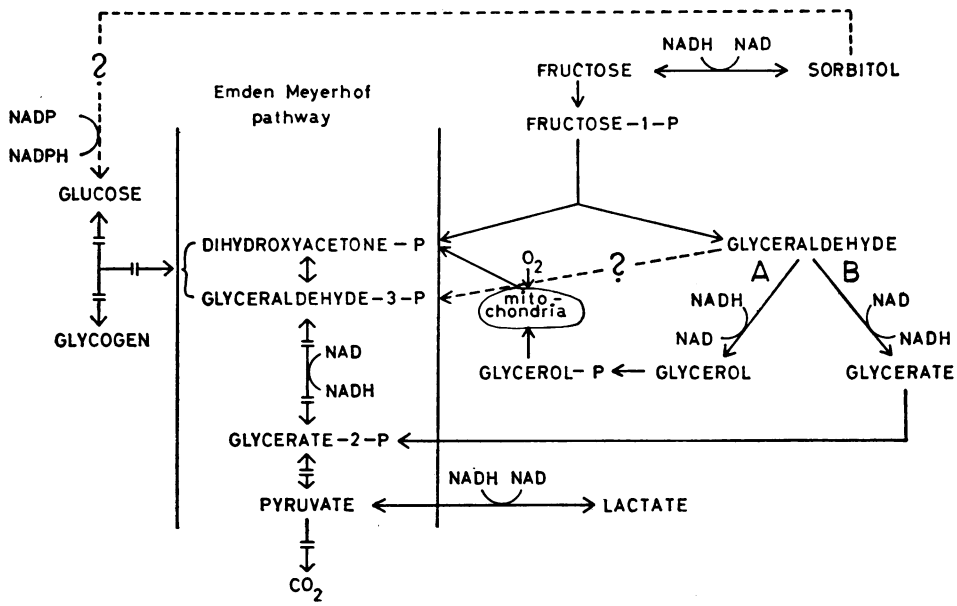

FIG. 2. OUTLINES OF THE PATHWAYS OF HEPATIC FRUCTOSE ELIMINATION. It is assumed that glyceraldehyde is metabolized mainly via pathway A during ethanol infusion and via pathway B under normal conditions. Hypothetical pathways are indicated by broken lines.

(Table VII) as well as in a previous study (7). The same is true of the ratio sorbitol/fructose as can be seen from Figure 1. The ratio lactate/pyruvate is assumed to be a particularly reliable index of the concentration ratio of free NADH/NAD in the liver cells (30), at least when the concentration of pyruvate is sufficiently high to be determined accurately. The results indicate (Table VI) that the NADH/NAD concentration ratio is 10 times higher during ethanol-fructose infusion than during fructose alone.

A rise in the concentration of NADH does not prove that the reoxidation of the coenzyme is maximal, but if it were not, one would expect the rise to increase the oxygen uptake of the tissue. Apparently ethanol does not by itself cause the oxygen consumption of the liver to rise, either *in vitro* (31) or *in vivo* (7).

Interrelations of ethanol and fructose metabolism. Figure 2 shows the generally accepted chart of the hepatic breakdown of fructose. The fructose-1-phosphate is split into equal parts of dihydroxyacetone phosphate and glyceraldehyde. The dihydroxyacetone phosphate is converted predominantly to glucose and glycogen, whereas glyceraldehyde appears to have the choice of at least three pathways: *1*) phosphorylation to glycerol phosphate by a triokinase (32), *2*) reduction to glycerol by alcohol dehydrogenase (33), and *3*) oxidation to glycerate by aldehyde dehydrogenase (34).

During infusion of fructose alone the following picture is obtained from the experimental data. About 2.3 mmoles of fructose is phosphorylated in the splanchnic area per minute. The release of about 2.2 mmoles per minute of lactate and pyruvate strongly indicates the predominance of pathway 3, leading directly to glycerate-2-phosphate, which is only one step removed from pyruvate, whereas pathways 1 and 2 lead to triose phosphates. Similar experiments with glucose loading give rise to only small quantities of lactate and pyruvate (35). Energy considerations support the suggestion that pathways 1 and 2 are of minor importance, as both require the participation of one extra molecule ATP to lead to the triose phosphate step, whereas the ATP used for phosphorylation of glycerate is recovered from phospho-enol-pyruvate.

When fructose is given in addition to ethanol, the picture is changed in the following ways. *a*) The quantity of fructose taken up is increased by about 0.6 mmole per minute. *b*) A corresponding amount of polyol (sorbitol and glycerol) is formed.

c) The output of lactate and pyruvate is decreased drastically (from 2.2 to 0.7 mmoles per minute). *d*) A corresponding increase is observed in the output of glucose (from 0.7 to 1.9 mmoles per minute). *e*) The ethanol oxidation to acetate is about twice that found in control experiments without fructose.

These changes are interpreted in the following way. The quantity of fructose phosphorylated per minute is approximately the same as in the experiments with fructose alone, and therefore the quantity of glyceraldehyde formed is also unchanged. The decrease in the output of lactate and pyruvate suggests that the pathway via glycerate is blocked under these conditions. It has been suggested that acetaldehyde competes efficiently for the aldehyde dehydrogenase responsible for glyceraldehyde oxidation to glycerate (36), as the K_m for acetaldehyde is several orders of magnitude smaller than for glyceraldehyde (37).

It therefore remains to decide which of the other two pathways is predominant and to bring this in relation to the increase in ethanol oxidation. Increased phosphorylation to triose phosphate may occur because the concentration of glyceraldehyde is increased, but this will give no possibility for explaining the increased ethanol oxidation. The reduction to glycerol, on the other hand, provides such an explanation, as pointed out by Holzer and Schneider (33). These authors found that glyceraldehyde could be reduced to glycerol by the ADH-NADH complex, thus providing an explanation for the fructose effect. By this process the limiting influence of the dissociation of the ADH-NADH complex could be circumvented, as NADH, formed by the dehydrogenation of ethanol to acetate, is reoxidized by glyceraldehyde without prior dissociation from the enzyme. This hypothesis was partly abandoned, when the same workers (36) showed that from an enzyme kinetic point of view it was more probable that glyceraldehyde is dehydrogenated to glycerate, a pathway that is supported by our experiments with fructose alone.

During ethanol metabolism, however, the inhibition of glyceraldehyde oxidation will cause the concentration of this substance to increase, and at the same time the increased concentration of NADH will facilitate the reduction to glycerol. Although the K_m value of ADH for glyceraldehyde (33) is much greater than for acetaldehyde (38), the concentration of the latter substance is probably extremely low (39). The concentrations of the aldehydes in the liver cells during these conditions are, however, not known.

If this mechanism is in fact active, what will happen to the glycerol formed? The liver is capable of metabolizing large amounts of glycerol, the first step being phosphorylation to glycerol phosphate. Some glycerol may escape phosphorylation, as is suggested by a slightly higher glycerol concentration in hepatic venous blood than in arterial blood, although changes in the lipid metabolism of extrahepatic splanchnic organs might contribute to this. The pathway via glycerol and glycerol phosphate is supported by the finding that the concentration of glycerol phosphate is increased more than 50% in the liver of rats receiving ethanol and fructose compared with rats receiving fructose alone (40). The glycerolphosphate is oxidized via the mitochondrial mechanism, and this may explain the increased splanchnic oxygen uptake observed during infusion of ethanol and fructose together.

The amount of glycerol maximally formed by this process can be assessed if it is assumed that glyceraldehyde is converted either to lactate and pyruvate or to glycerol. The formation of glyceraldehyde equals the phosphorylation of fructose, i.e., about 2.3 mmoles per minute. Since the splanchnic production of lactate and pyruvate amounts to 0.8 mmole per minute on the average, 1.5 mmoles per minute remains for formation of glycerol. Consequently conversion of glyceraldehyde to glycerol may account for reoxidation of 1.5 mEq of NADH per minute.

The increase in ethanol oxidation to acetate during fructose infusion is on the average 1.2 mmoles per minute, resulting in a rise of NADH produced of 2.4 mEq per minute, and therefore mechanisms other than glycerol formation may contribute to the fructose effect. The finding by Pletscher and associates (2) that ethanol prevented the rise in arterial pyruvate concentrations, seen after ingestion of fructose alone, made them suggest that a dismutation reaction with pyruvate might explain the fructose effect. Our data

show that this is quantitatively unimportant, as it can only account for an extra reoxidation of 0.04 mEq of NADH per minute. A dismutation reaction with fructose itself, being converted to sorbitol, was suggested by Holzer and Schneider (33). In our experiments the splanchnic production of 0.6 mmole per minute of polyol, mainly consisting of sorbitol, probably contributes significantly to the fructose effect.

During infusion of fructose alone the splanchnic production of glucose is not significantly higher than during the control period, and despite the high output of lactate and pyruvate an essential fraction of the fructose taken up in the splanchnic area may have been retained, probably as glycogen in the liver. During infusion of ethanol and fructose together, the splanchnic output of glucose and lactate is equal to the uptake of fructose (minus sorbitol production), indicating that no extra glycogenesis takes place under these circumstances. It is possible that ethanol inhibits the enzymatic conversion of glucose-6-P to glycogen, since the same effect has been demonstrated in animals and in perfusion studies (42, 43), but in the present experiments hormonal factors cannot be excluded. Ethanol intake has been shown to augment the urinary excretion of adrenalin (44), but it should be noted that during the infusion of ethanol alone the splanchnic output of glucose and lactate failed to reveal any clear adrenalin effect.

The hepatic blood flow and oxygen uptake. What effect ethanol has on the splanchnic blood flow is still not quite settled. The recent studies of Stein and co-workers (44) indicate that ethanol in sufficiently high concentrations (3 to 10 times higher than those used in the present work) results in a slight, parallel increase in the splanchnic and the systemic blood flow, probably by reducing the peripheral vascular resistance. At smaller concentrations, as used here, ethanol has a slight lowering effect on the splanchnic blood flow (7). In both studies the arterial concentration of ethanol is sufficiently high to ensure maximal hepatic elimination rate, according to the criteria of Larsen (45), i.e., incomplete hepatic extraction. The difference between the observations therefore cannot be explained by a greater metabolic load on the liver in the former studies, and the effect on blood flow and so on probably should be regarded as a pharmacological effect of ethanol on the blood vessels in general, which is unrelated to the hepatic ethanol metabolism. On the other hand, it is likely that the increased oxygen consumption in the liver during ethanol-fructose infusion is causally related to the augmentation in the splanchnic blood flow under these conditions, since the oxygen content in the hepatic venous blood is significantly lower during ethanol-fructose infusion than during infusion of ethanol alone. The experiments permit exclusion of carbon dioxide production as the determining factor in hepatic blood flow regulation, since the smallest production is seen in the experiments with the highest flow, i.e., during simultaneous ethanol and fructose infusion. The P_{CO_2} is also smallest in these experiments, despite a low pH value (Table IX).

The mean splanchnic oxygen consumption and carbon dioxide production under the experimental conditions studied are shown graphically in Figure 3. It illustrates how much ethanol elimination contributes to these values, assuming that the difference between ethanol uptake and acetate output represents breakdown of ethanol to carbon dioxide and water, presumably by way of acetate oxidation in the extrahepatic splanchnic organs. During ethanol infusion alone the splanchnic elimination of this substance requires 80% of the oxygen uptake, in close agreement with the 75% found previously (7).

The low "nonethanol" oxygen uptake during ethanol elimination means that the energy requirements of the liver cells are supplied almost exclusively by extramitochrondial NADH, the normal, intramitochondrial sources, notably the tricarboxylic acid cycle, presumably being inhibited. The metabolic consequences of this situation are difficult to evaluate, but it is tempting to believe that it provides the clue to the hepatic abnormalities frequently associated with chronic alcoholism.

Summary

Changes in the splanchnic metabolism and blood flow provoked by intravenous infusions of fructose and ethanol, given separately or in combination, have been studied by hepatic venous

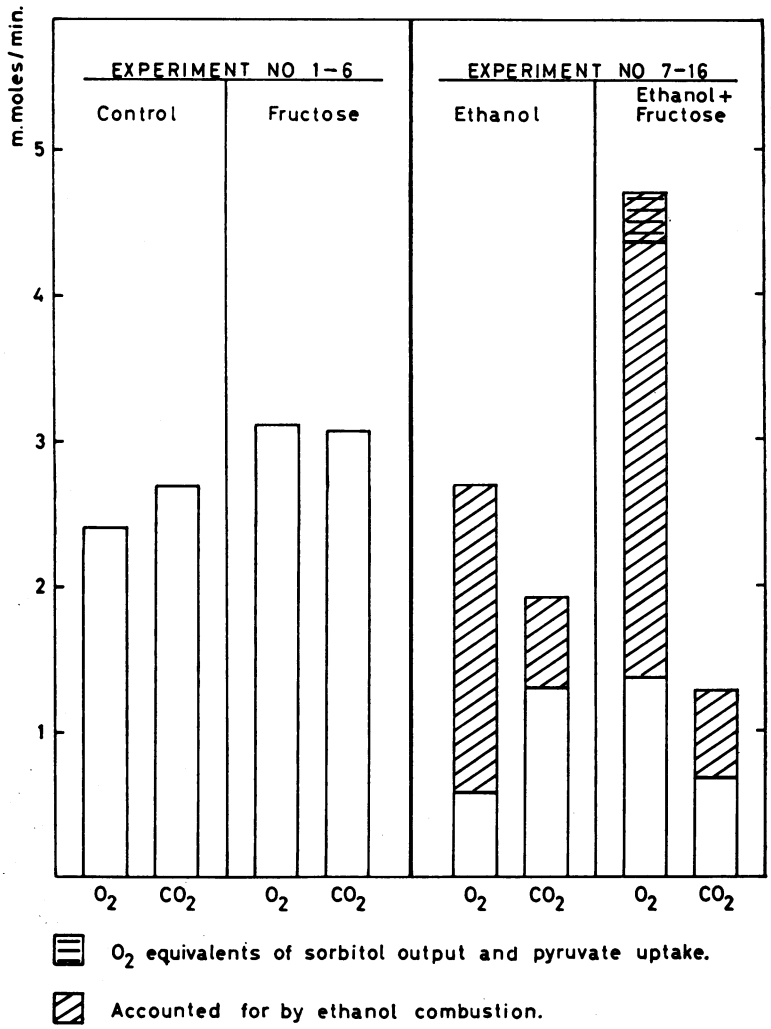

FIG. 3. THE INFLUENCE OF THE INFUSION OF FRUCTOSE OR ETHANOL OR BOTH ON THE SPLANCHNIC OXYGEN UPTAKE AND CARBON DIOXIDE OUTPUT. Calculation of the amounts accounted for by ethanol combustion is made on the assumption that ethanol is either converted to acetate, leaving the liver via the hepatic veins, or is oxygenated completely. The increase in sorbitol output and pyruvate uptake in this respect is equivalent to uptake of oxygen.

catheterization in human subjects with normal liver function. The following observations were made.

1) Fructose almost doubles the splanchnic ethanol uptake and acetate output.

2) When fructose or ethanol is given alone, it has no appreciable effect on the splanchnic blood flow and oxygen uptake, but when they are given together, the blood flow is increased by about 30% and the oxygen uptake by about 60%.

3) The lactate/pyruvate concentration ratio in hepatic venous blood is greatly increased during infusion of ethanol, whether fructose is given or not.

4) During infusion of ethanol the splanchnic uptake of fructose is on the average increased by 0.6 mmole per minute, compared with experiments with fructose infusion alone, and a corresponding amount of polyol (mainly sorbitol) is found in the hepatic veins.

5) The splanchnic output of lactate and pyruvate is reduced to one-third during ethanol-fructose infusion compared with fructose infusion alone.

6) The splanchnic output of glucose is three times greater during ethanol-fructose infusion than during fructose infusion alone.

The experimental findings support the view that the mechanism of the fructose effect on the metabolism of ethanol is complex. The quantitatively most important factor seems to be the formation of glyceraldehyde from fructose. Glyceraldehyde is normally dehydrogenated to glycerate, but in the presence of ethanol breakdown it is presumed to be reduced to glycerol by alcohol dehydrogenase. Thereby a limiting step in the dehydrogenation of ethanol to acetaldehyde, namely dissociation of the complex alcohol dehydrogenase and reduced nicotinamide adenine dinucleotide, is circumvented. The reactions can only be followed indirectly by the hepatic venous catheterization technique, but the data agree quantitatively with this concept. Dismutation reactions, notably involving the reduction of fructose to sorbitol, presumably contribute materially to the fructose effect.

The alterations in the splanchnic blood flow, oxygen uptake, and carbohydrate balance are discussed in relation to the other metabolic changes.

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