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Oxidation of Free Fatty Acids by Dog Liver.* (29782)

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It has been shown in several laboratories (1-5) that the liver takes up large quantities of free fatty acids (FFA) and that hepatic uptake is dependent on plasma FFA concentration(6). The perfused rat liver has also been found to remove FFA and to release esterified fatty acids(7-9). In the present study the oxidation of FFA to CO_2 was investigated in dogs under *in vivo* conditions employing isotopically labeled substrate.

Materials and methods. Seven dogs of either sex, weighing about 20 kg, were used after 16 hours of fasting. Simultaneous blood samples (1-6 per dog) were collected from the femoral artery, portal vein and hepatic vein. The latter 2 vessels were catheterized after laparotomy under manual guidance. Palmitate-1-C^{14} or oleate-1-C^{14} was infused as described previously(10).

Plasma FFA concentrations were determined by the method of Dole and Meinertz (11). After completion of the FFA determination, the bottom phase of the titrated mixture was maintained in an alkaline state by addition of NaOH. The top phase (designated as neutral lipids) was removed, the bottom phase washed 3 times with ligroine or hexane and the washings added to the withdrawn top phase. The remaining bottom phase (containing FFA) was acidified, washed 3 times with hexane and the washings were pooled. Both FFA and neutral lipids were counted in a Packard liquid scintillation detector using 0.5% 2,5-diphenyloxazole and 0.05% 2-p-phenylenebis-4-methyl, 5-phenyloxazolone in toluene as scintillation fluid.

Blood was collected for $C^{14}O_2$ and CO_2 determinations under mineral oil into a mixture of saponin and oxalate. Analysis of $C^{14}O_2$ was performed according to the method of Passman, Radin, and Cooper(12). Concentration of CO_2 was determined by the Technicon AutoAnalyzer.

Results and discussion. The results are summarized in Table I. The portal supply of blood to the liver was asumed to be 2 parts portal vein to 1 part hepatic artery (13). The mean portal supply of FFA was .229 mEq/l. The liver removed 39% of this amount. A consistent production of $C^{14}O_2$ by the liver was also evident. The oxidation index indicated that on the average .02 mEq/l of FFA, or about ¼ of the removed amount was oxidized. Thus, about 10% of the arterial FFA content is oxidized by the liver through one passage. A portion of the labeled fatty acid was recirculated in the form of neutral lipids, as judged by the frequent release of net counts in the neutral lipid fraction. Assuming that the average FFA has 17 carbon atoms, the oxidation of FFA would account for approximately 38% of the CO_2 produced by the liver.

The present data obtained in dogs under *in vivo* conditions are not unlike those described for the perfused rat liver, where 6.27% and 18.00% of the C^{14} labeled palmitic acid was oxidized to CO_2 in 4 hours by the fed and starved livers respectively(8).

Summary. The hepatic oxidation of FFA to CO_2 was investigated in dogs. Thirty-nine per cent of the mean portal supply of FFA was removed by the liver and about ¼ of this amount was oxidized to CO_2. This amount of CO_2 could account for approxi-

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TABLE I. Hepatic Removal and Oxidation of FFA.*

Dog No.	Sample No.	Portal supply of FFA $\left[\frac{2P+1A}{3}\right]$ mEq/l	Extraction ratio, % $\left[\frac{\frac{2P+1A}{3}-H}{\frac{2P+1A}{3}}\right]\times 100$	Portal supply of radio FFA $\left[\frac{2P+1A}{3}\right]$ cpm/ml	Hepatic removal of radio FFA $\left[\frac{2P+1A}{3}-H\right]$ cpm/ml
7	4	.177	25.4	809	297
	5	.202	47.0	851	515
10	1	.221	38.0	N.A.	N.A.
	2	.219	31.1	N.A.	N.A.
	3	.240	38.8	N.A.	N.A.
	4	.210	33.8	N.A.	N.A.
	5	.203	41.9	N.A.	N.A.
11	1	.156	39.8	158	65
	2	.160	70.0	159	94
	3	.211	54.5	165	107
	4	.245	61.2	176	110
	5	.251	56.6	191	132
	6	.253	66.0	201	127
12	1	.190	20.0	681	237
	2	.226	33.6	718	270
	3	.199		N.A.	N.A.
	4	.209	8.1	N.A.	N.A.
16	4	.399	37.8	603	191
17	4	.255	17.3	504	110
18	4	.349	22.9	797	105
Mean		.229	39	463	182
S.E.		.013	3.9	81	34
N		20	19	13	13

Dog No.	Sample No.	Hepatic CO_2 production $\left[H-\frac{2P+1A}{3}\right]$ mEq/l	Hepatic $C^{14}O_2$ production $\left[H-\frac{2P+1A}{3}\right]$ cpm/ml	Oxidation index $\left[\frac{H-\frac{2P+1A}{3}\,C^{14}O_2}{\text{A FFA spec. act.}}\right]$ mEq/l	$\left[\frac{\text{Counts removed as FFA}}{\text{Counts produced as } CO_2}\right]\times 100$
7	4	.8	54	N.A.	25.8
	5	.5	29	.007	9.2
10	1	.8	74	N.A.	N.A.
	2	2.5	94	N.A.	N.A.
	3	1.1	64	N.A.	N.A.
	4	1.9	89	N.A.	N.A.
	5	3.4	112	N.A.	N.A.
11	1	.3	5	.005	10.4
	2	.3	42	.042	62.7
	3	− .1	21	.027	37.5
	4	−2.7	12	.017	15.2
	5	−1.5	16	.021	16.7
	6	−2.6	10	.013	10.5
12	1	1.5	6	.002	4.0
	2	.7	21	.007	12.1
	3	1.4	42	.012	N.A.
	4	1.1	63	.015	N.A.
16	4	.6	65	.043	56.5
17	4	.8	54	.027	74.0
18	4	1.2	84	.037	112.0
Mean		.6	48	.020	34.2
S.E.		.33	7	.004	9.1
N		20	20	14	13

* Abbreviations: A = artery, P = portal vein, H = hepatic vein; N = No. of samples; S.E. = standard error of mean; N.A. = not available.

mately 38% of the total CO_2 produced by the liver.

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Simian Enterovirus SV2 Hemagglutination Studies. I. Relationship of Infectivity to Hemagglutination.* (29783)

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While studying the characteristics of a number of simian enteroviruses it was noted that SV2 agglutinated rhesus monkey red blood cells (RBC) at 4°C and room temperature but not at 37°C(1). To determine the nature of the hemagglutinin produced by this virus, a study was made of the relationship of the infectious virus particle to the hemagglutinating activity of the virus. The results have shown that the two are very closely associated and that the hemagglutinating activity probably resides in the viral capsid.

Materials and methods. Virus. The virus was obtained from the American Type Culture Collection, Viral and Rickettsial Registry and plaque purified in LLCMK2 cells. This virus served as a source for passages in primary rhesus monkey kidney cultures. These passages were used during this study. To increase the hemagglutination (HA) titer of the infected culture fluids (usually 32 to 128) the latter were concentrated on several occasions by dialysis against polyvinyl pyrrolidone after homogenization with genetron 113 (2 parts virus : 1 part genetron).

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Tissue culture. Primary rhesus kidney cell cultures were grown from suspensions of trypsinized kidney cells in Melnick's lactalbumin hydrolysate-Hanks' saline medium(2) containing 2% calf serum and 0.2% anti-SV5 rabbit serum (Microbiological Associates, Inc.). Bottle cultures were maintained on lactalbumin hydrolysate-Earle's saline medium(2) containing 2% calf serum. Tube cultures were maintained on 50% 199 medium-50% bovine amniotic fluid.

Virus assay. Infectivity titrations were carried out in tube cultures of primary rhesus kidney cells by inoculating 0.1 ml of 10-fold dilutions of the virus into each of 4 tubes per dilution. The cultures were incubated at 37°C and observed daily for 1 week. Infectivity endpoints ($TCID_{50}$) were determined by the method of Reed and Muench(3).

HA test. Rhesus monkey RBC were collected in Alsever's solution. These cells were washed 3 times with phosphate buffered saline (PBS) and used as a 0.5% suspension in PBS. Cells not used immediately were stored in Alsever's solution at 4°C for no longer than 1 week. A mixture of 0.5 ml RBC and 0.5 ml of 2-fold dilutions of virus in 13 × 100 mm glass tubes was used in the