Relationship of Hepatic Uptake of Free Fatty Acids to Plasma Concentration.* (25712)

WILLIAM T. MCELROY, JR., WILLIAM L. SIEFERT AND JOHN J. SPITZER
(Introduced by M. A. Spirtes)
Dept. of Physiology, Hahnemann Medical College and Hospital, Philadelphia, Pa.

The metabolism of free fatty acids (FFA) presents an intricate picture to investigator who seeks to relate plasma FFA concentration to cellular handling of this material. Net FFA plasma concentration at any instant must equal the algebraic sum of production, destruction, storage, or conversion. These parameters in turn may be regulated by local mechanisms at cellular level, or, vary each in a particular direction under the influence of endocrine or nervous control. Certain tissues release into, while others remove FFA from circulation. Gordon(1) found both myocardial and visceral extraction of FFA in unanesthetized patients. Liver and heart of dogs remove FFA(2,3,4,5,6) while adipose tissue has been described in dogs and humans(1,2,3,4) as supplying FFA to plasma. The action of various normally secreted hormones, as well as the autonomic nervous system on FFA plasma levels has been widely investigated (7,8,9,10,11). The relation of cellular output to cellular utilization is of prime importance in depicting the integration of forces acting to set FFA plasma concentration at a particular level. Cellular uptake of FFA might be considered more tangibly if one studies the reaction of such cells to *in vivo* changes in plasma FFA concentration, this alteration brought about without stimulating the output mechanism in adipose tissue. The present investigation reports canine hepatic reactions to changes in portal vein FFA content induced by infusion of FFA-loaded serum into portal tributary.

Methods. Adult mongrel dogs were anesthetized with intravenously administered pentobarbital sodium (.03 g kg). Hepatic vein samples were obtained from polyethylene cannulae placed in these vessels through jugular vein. The position of each cannula was checked before experiment was begun, by manual recognition through midline abdominal incision. A smaller catheter was then passed through mesenteric vein until its tip closely approximated junction of portal vein with liver. From this cannula, samples representing mixed portal blood were obtained. For sampling A-V differences in leg tissue cannulae were placed in femoral vein and artery through small side branches. A large bore cannula for rapid infusion of serum into portal vein was then inserted a short distance into a more caudal mesenteric vein, but not allowed to enter the portal vessel itself. All cannulae were maintained patent by a slow infusion of physiological saline. To obtain quantitative data regarding liver behavior, estimated hepatic plasma flow (EHPF) was determined by BSP dilution method of Bradley *et al.*(12). A priming dose of 50 mg of dye was administered followed by constant infusion rate of .060-.070 mg/kg/min into a vein of upper extremity. After arterial BSP concentrations had stabilized (usually about one hour) samples were drawn into chilled syringes and prepared for determination of FFA and glucose concentrations and EHPF. Free fatty acids were determined in duplicate analyses by Dole's method(7); Nelson's colorimetric adaptation of Somogyi's glucose determination was carried out in quadruplicate determinations(13). Serum was prepared by obtaining serum from donor dog the previous day, and adding to a particular volume enough sodium oleate so that the concentration added would be at least 2 mg/ml serum. After loading with oleate, the serum stood overnight in the cold. Just before infusion, serum was filtered for removal of particulate matter. A volume of serum from original pool was treated in an identical fashion except that sodium oleate was not added. This serum served as "control serum" infused prior to infusion of loaded serum. To both sera was added an amount of BSP which gave a concentration equal to portal BSP level at time

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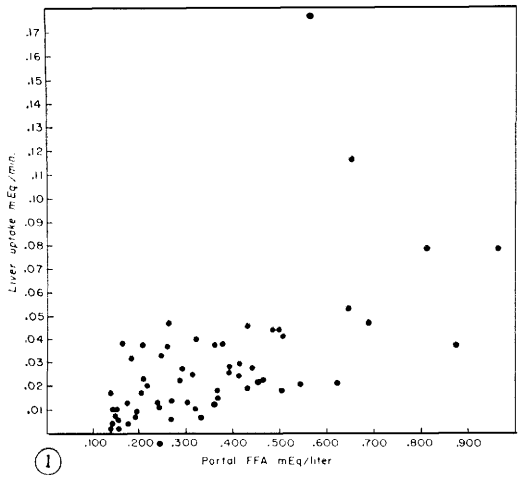

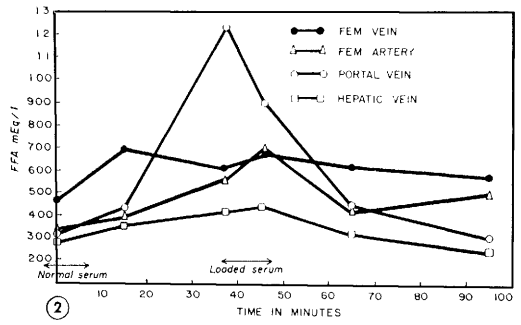

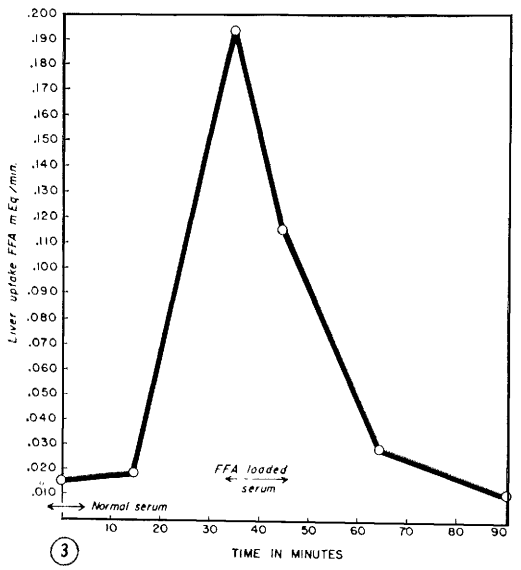

FIG. 1. Scattergram of portal FFA *vs* uptake of FFA by the liver.

FIG. 2. Effect of increased portal FFA concentration on plasma FFA levels.

FIG. 3. Hepatic FFA uptake as a function of portal concentration.

of infusion, as determined just before infusion was started.

Results. Analysis of many studies on liver uptake of FFA in dogs has given us the impression that this organ has a tendency to remove a quantity of FFA in proportion to portal concentration. A scattergram of portal FFA levels *versus* liver uptakes is presented in Fig. 1. Each point was obtained from a different animal in anesthetized but otherwise untreated state. No such suggestion of proportionality can be obtained if one plots EHPF *versus* liver uptakes of FFA in the same animals. In another group of dogs, portal FFA concentration was increased in each animal by rapid infusion of serum rich in sodium oleate. Fig. 2 shows FFA concentrations existing in hepatic, portal and femoral veins as well as femoral artery during sampling periods in 1 experiment. Fig. 3 relates liver uptake of FFA as a function of portal FFA concentration in the same animal. Liver uptake values were obtained by multiplying FFA differences between portal and hepatic vessels by estimated hepatic plasma flow. Data on both FFA, glucose and EHPF of all experiments are presented in Table I.

Results show that intraportal infusion of normal serum at rate employed for experimental serum (8-14 ml/min) had little effect on EHPF or, portal hepatic FFA differences, and values for 2 control determinations (first 2 samples of each experiment) are almost identical for absolute FFA hepatic removal. Rapid infusion of serum rich in FFA (Na oleate) however, produced expected increases in portal FFA presentation to the liver; at the same time, hepatic vein concentration of this substance increased very little, thus creating vastly elevated portal-hepatic differences.

Discussion. In discussing sequence of events occurring in humans given glucose and insulin, Gordon(1) hypothesized that transport of fat is controlled primarily by adipose tissue, and that glucose injection causes first a decrease in FFA liberation from depots; this in turn lowers arterial levels, and lastly there is cessation of extraction by other tissues. This implies, as suggested by Dole(14,15) that cells will remove FFA in a passive man-

TABLE I. Results of Portal Infusion of FFA-Loaded Serum on FFA Uptake and Glucose Output by the Liver.

Sample	1	2	3	4	5	6	7
Min. from infusion		+15			+5	+20	+40
Dog 319 (20.8 kg)	Control serum		—8 cc/min.—		14 cc/min.		
△ PH FFA*	.110	.099	.470	.349	.910	.124	
" glucose†	33	—	21	30	30	50	
EHPF‡	410	400	280	310	374	250	
Hepatic glucose output§	135	—	59	100	111	125	
" FFA uptake‖	.045	.040	.135	.108	.338	.031	
Dog 320 (15.5 kg)	Control serum		14 cc/min.	10 cc/min.			
△ PH FFA	.049	.067	.819	.462		.113	.061
" glucose	7	22	36	42		44	13
EHPF	310	293	236	252		262	175
Hepatic glucose output	22	64	82	105		114	23
" FFA uptake	.015	.019	.194	.116		.029	.011
Dog 322 (19.8 kg)	Control serum		8 cc/min.	10 cc/min.			
Arterial FFA	.302	.318	.406	.592	.410	.316	
Venous "	.403	.427	.597	.630	.501	.705	
Hepatic "	.334	.290	.395	.600	.245	.290	
Portal "	.278	.308	.524	.809	.336	.333	
Arterial glucose	79	87	89	88	112	99	
Venous "	84	86	88	92	104	100	
Hepatic "	127	120	158	153	116	131	
Portal "	85	82	104	109	101	98	
△ PH FFA	—.056	.018	.129	.209	.091	.043	
" glucose	42	38	54	44	15	33	
EHPF	440	470	425	495	550	350	
Hepatic glucose output	184	178	232	220	82	113	
" FFA uptake	—.024	.009	.055	.105	.050	.043	

Serum infusions (for loaded and control serum) are shown above the sample columns in each experiment.

* meq/l. † mg %. ‡ ml/min. § mg/min. ‖ meq/min.

ner, depending on plasma concentration and that FFA regulatory mechanisms act on cells which produce FFA. Actually, our work is, in a fashion, a test of this hypothesis, but the reverse condition (increasing FFA liberation as simulated by injection) is used as the first change. It would, if sequence is correct, follow that (1) increased FFA liberation leads to (2) increased plasma levels and as a result (3) increased removal by the liver (and perhaps other organs) takes place. Indeed, an increased removal was demonstrated.

In Dogs 319 and 322, the third samples were obtained while infusion rate was about 8 ml/min of loaded serum; during sample 5 the rates increased to 14 and 10 ml/min respectively. Sample 3 from Dog 320 (portal FFA 1.23 meq/l) represented a portal infusion rate of 14 ml/min, and sample 4 an infusion rate of 10 ml/min (portal FFA .900 meq/l). Thus, different portal concentrations were achieved by merely adjusting rate of portal infusion. Within 20 minutes after infusions were stopped, both venous and arterial levels had fallen to control levels, or, slightly below. Femoral V-A differences were decreased during infusion of loaded serum.

It appears that amount of FFA removed by liver is a function of concentration presented, implying that a local mechanism may exist in hepatic cells which can vary the amount of FFA removed from portal plasma.

Sudden elevation of FFA concentration appeared to have little effect on glucose output by liver, as compared to injection of control serum. The slight rise in glucose levels may have been the result of action on liver of amines introduced with injected sera, since preparation of serum might release serotonin from platelets, or, adrenalin may be released

during exsanguination of the donor animal.

Summary. Behavior of liver to changes in portal concentration of FFA was studied. Rapid infusion of serum containing large amounts of FFA into portal veins of dogs caused increased hepatic uptakes of this metabolite. Uptake of FFA was a function of portal concentration. Liver cells behave in a passive manner to fluctuations in serum FFA concentration, and local hepatic mechanisms are responsible for changing degree of FFA uptake.

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Growth of SE Polyoma Virus in Primary and Established Cell Cultures.* (25713)

PIERO BALDUZZI AND HERBERT R. MORGAN

Louis A. Wehle Virus Research Lab., Dept. of Bacteriology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The SE polyoma virus multiplies *in vitro* with cytopathic effect (CPE) in primary tissue cultures from mouse embryo(1) and monkey kidney(2) and in an established cell line (P388 D_1) derived from a murine lymphoma(3), but fails to grow in rat embryo tissue cultures(4). This study reports results of experiments to determine if polyoma virus multiplies in other primary and established cell cultures to determine further the host range spectrum of this virus *in vitro*.

Materials and methods. The SE polyoma virus (strain 210-22) was obtained from Dr. Bernice Eddy and propagated in mouse embryo tissue cultures. Presence of virus was determined by infectivity titrations as described previously(1). Cultures were observed 3 weeks for CPE and fed at weekly intervals. $TCID_{50}$ end points were calculated according to method of Reed and Muench (5). Four different tissue culture systems were used: mouse embryo cells, L cells, rabbit embryo cells and HeLa cells. Tissue culture tubes of mouse embryo cells were prepared with trypsin-dispersed cells from primary cultures of 14- to 18-day-old mouse embryos by modification of trypsin digest method of Youngner(6). Eagle's medium(7) modified by using Hanks(8) BSS and 10% bovine fetal serum was used as growth medium. Medium consisting of 8 parts Eagle's medium with double concentration of amino acids and vitamins, one part tryptose phosphate broth and 2% horse serum was used as maintenance medium. L cells were maintained in Hanks BSS with lactalbumin hydrolysate 0.5%, yeast extract 0.01% (HLYE) and 2% horse serum. In one experiment, an additional set of cultures of L cells was maintained in syn-

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