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Research Article

Extraction of insulin by the liver after administration of glucose in the duodenum has been studied in fourteen anesthetized dogs. Plasma insulin and glucose were measured in the portal vein hepatic vein and hepatic artery. During the control period $40 \pm 3\%$ of the approximately 11 mU of insulin presented to the liver/min was removed during a single transhepatic passage. Within 5 min after glucose administration, the amount of insulin reaching the liver increased significantly. In some animals this increase preceded any significant increase in the glucose concentration of the femoral artery. After glucose administration, hepatic extraction of insulin remained unchanged in five animals and rose significantly in nine. In five of the latter animals, the increase may have been more apparent than real due to nonrepresentative sampling of hepatic venous blood. However, for the whole group of animals, comparison of arterial insulin levels with the amount of insulin delivered to the liver suggested a transient increase in insulin extraction between 5 and 50 min after glucose administration. In no animal was there a decrease in the proportion of insulin extracted by the liver after glucose administration. The results indicate that the extraction process is not saturable at physiological insulin levels. Prior to glucose administration, net hepatic glucose output averaged between 30 and 40 mg/min. After glucose administration, the liver began to [...]

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ABSTRACT Extraction of insulin by the liver after administration of glucose in the duodenum has been studied in fourteen anesthetized dogs. Plasma insulin and glucose were measured in the portal vein hepatic vein and hepatic artery. During the control period $40 \pm 3\%$ of the approximately 11 mU of insulin presented to the liver/min was removed during a single transhepatic passage. Within 5 min after glucose administration, the amount of insulin reaching the liver increased significantly. In some animals this increase preceded any significant increase in the glucose concentration of the femoral artery. After glucose administration, hepatic extraction of insulin remained unchanged in five animals and rose significantly in nine. In five of the latter animals, the increase may have been more apparent than real due to nonrepresentative sampling of hepatic venous blood. However, for the whole group of animals, comparison of arterial insulin levels with the amount of insulin delivered to the liver suggested a transient increase in insulin extraction between 5 and 50 min after glucose administration. In no animal was there a decrease in the proportion of insulin extracted by the liver after glucose administration. The results indicate that the extraction process is not saturable at physiological insulin levels. Prior to glucose administration, net hepatic glucose output averaged between 30 and 40 mg/min. After glucose administration, the liver began to take up glucose and there was a significant correlation between hepatic glucose uptake and the amount of insulin reaching the liver. However, since the amount of glucose presented to the liver also increased, it is not established that the insulin was responsible for the change in hepatic carbohydrate metabolism.

The data demonstrate an increase in the absolute amount of insulin extracted by the liver after glucose administration and an important role for the liver in regulating peripheral insulin concentrations.

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INTRODUCTION

The anatomic location of the liver, receiving the entire pancreatic output of insulin prior to its entry into the systemic circulation, and its ability to degrade insulin permits it to play an important role in regulating peripheral insulin concentrations (1). Although increases in peripheral insulin levels have been assumed to reflect increased pancreatic secretion of insulin, Stern, Farquhar, Silvers, and Reaven (2) emphasized the paucity of experimental evidence to support this assumption. They pointed out that their measurements of the rate of delivery of insulin into the general circulation in humans was not necessarily equivalent to the total insulin secreted by the pancreas. They also criticized the concept that the metabolic clearance rate of insulin measured insulin secretion because it does not include that fraction of the insulin secreted by the pancreas which is removed by the liver prior to its entry into the general circulation. Wright (3) also indicated that hepatic extraction of insulin could influence significantly peripheral insulin concentrations.

Hepatic extraction of insulin has been measured by several different techniques. Mortimore, Tietze, and Stetten reported that the isolated perfused rat liver removed 40% of [125 I]insulin (25 mU/ml) in a single passage (4). Insulin uptake was significantly less during perfusion of the hind limb. Madison and Kaplan estimated that approximately 54% of the 0.65 U of insulin injected into the portal vein of anesthetized patients was extracted by the liver during a single transhepatic passage (5, 6). Insulin extraction in these studies was evaluated using trichloroacetic acid precipitable radioactivity. However, Marshall, Gingerich, and Wright have emphasized the hazards of using [125 I]insulin and measurement of trichloroacetic acid precipitable radioactivity as an indication of insulin metabolism (7). Samols and Ryder measured insulin concentrations by immunoassay in the hepatic vein and a systemic artery in patients with portal-caval

shunts or thrombosis of the portal vein (8). Estimation of hepatic blood flow permitted calculations of insulin flux across the liver but the results may have been influenced by the liver disease. In four such patients, hepatic extraction averaged 41% of the 0.2 mU/kg per min endogenous insulin.

Although these results indicated basal hepatic extraction of approximately 50% of the insulin presented, conflicting data exists concerning hepatic extraction of insulin after administration of glucose. Kaplan and Madison reported that glucose decreased the fraction of insulin presented to the liver which was removed by that organ (6). Infusion of 9 g of glucose over 17 min decreased hepatic extraction of insulin from an average of 54 to 38%. When 52 g of glucose was infused over 165 min, only 7.8% of the administered [¹²⁵I]insulin was removed by the liver during the initial passage. However, the studies of Waddell and Sussman suggested contrary findings (9). In unanesthetized dogs, glucose administration increased the discrepancy between portal and peripheral vein insulin concentrations. When the portal vein insulin concentrations were above 500 μU/ml, almost all of the insulin was removed by the liver in contrast to when the portal vein insulin concentration was below 50 μU/ml when almost none was extracted. However, since appropriate blood flows were not measured, hepatic extraction of insulin could not be quantitated.

The present studies were undertaken to examine hepatic extraction of insulin in a more quantitative fashion and to determine whether this process is modified by glucose administration into the duodenum with its resultant increase in pancreatic insulin secretion. The results confirm that about 50% of the insulin presented to the liver in the basal state is removed during a single passage; following glucose administration there is no decrease in the percent of insulin removed by that organ, and some evidence to suggest a transient increase.

METHODS

Both male and female dogs of mixed breed weighing between 20 and 25 kg were used. After an overnight fast, the animals were anesthetized with barbital (30 mg/kg). The abdomen was opened and the portal vein and hepatic artery were exposed. External electromagnetic flow probes (Carolina Medical Electronics, Inc., King, N. C.) of the appropriate size were placed around these vessels. The portal vein probe was positioned 1 cm below the bifurcation of the vessel just before it entered the liver. The hepatic artery probe was placed approximately 3 cm from its origin. The gastroduodenal branch of the hepatic artery was ligated. The flow probes were calibrated by timed in vitro measurement of blood flow from a reservoir through segments of dog portal vein and hepatic artery. Once calibrated, the probes gave accurate readings which were reproducible to ±2% and were not affected by variation in the hematocrit

within the range 35–48%. Polyethylene catheters with multiple sideholes were placed in the portal vein, in the femoral artery and in an hepatic vein. The tip of the catheter in the portal vein was just below the site of the flow probe and therefore above the gastro-duodenal vein, the most cephalad of the veins draining the pancreas. The hepatic vein catheter was introduced through the superior vena cava and the tip advanced about 0.5–1 cm into the hepatic vein. Since the dog has several hepatic veins and the results of Kanazawa, Kuzuya, Ide, and Kosaka indicated that the insulin concentrations might vary in the different hepatic veins (10), the experimental technique was modified in dogs number 2 and 3 in an attempt to mitigate this problem. In these two dogs, the inferior vena cava was ligated beneath the diaphragm but above the entry of the renal veins. A catheter was placed in the inferior vena cava above the ligature and in the vicinity of the entry of the hepatic veins so that mixed hepatic venous blood was sampled. A flow probe was also placed on the inferior vena cava 3 cm above the diaphragm. The flow in this vessel did not differ from the sum of portal vein and hepatic artery flows by more than 10%. In the 12 other dogs, total hepatic blood flow was calculated as the sum of the blood flows in the portal vein and hepatic artery. Blood samples for insulin and glucose were obtained simultaneously from the portal vein, femoral artery, hepatic vein, or inferior vena cava at 5-min intervals during a 20–30 min control period. Blood flow measurements were made just prior to obtaining the blood samples. Following the control period, 50 g of glucose, as a 50% solution in water, were injected into the duodenum. Immediately after glucose administration, blood samples and flow measurements were obtained at 1, 3, 5, 7, 9, 11, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, and 120 min. It was not always possible to obtain all of these samples in every dog. During the experiment the dogs received an intravenous saline infusion to replace the blood removed and maintain a normal blood pressure. In four of the dogs, the hematocrit of the blood obtained from each vessel during the experiment was determined. These values ranged from 48 to 41% with a mean hematocrit of 43%. The hematocrit tended to be higher at the beginning of the experiment, but a significant change did not occur in every case. Since the insulin concentration was determined using plasma, but the blood flow measurements represented whole blood, the product of the insulin concentration in a vessel times its blood flow was multiplied by 0.57 to correct for the hematocrit.

The insulin reaching the liver was the sum of the contribution of the portal vein (portal vein insulin concentration × portal vein blood flow corrected for hematocrit) and the hepatic artery (femoral artery insulin concentration × hepatic artery blood flow corrected for hematocrit). The insulin leaving the liver was the product of the hepatic vein or inferior vena cava insulin concentration and the sum of the portal vein and hepatic artery blood flows corrected for hematocrit. The percent hepatic retention was determined by the formula:

$$\frac{\text{insulin to the liver} - \text{insulin leaving the liver}}{\text{insulin to the liver}} \times 100\%.$$

It is recognized that this formula is only strictly applicable to steady-state conditions which no longer exist after glucose has been administered and the concentration of insulin and glucose are changing. However, appropriate formulae for such non-steady-state conditions do not exist and fur-

TABLE I
Mean Results of Intraduodenal

	Time									
	-20	-15	-10	-5	0	1	3	5	7	9
Portal vein blood sugar, mg/100 ml	93	83	86	83	84	84	94	109	98	103
SEM	±10	±8	±8	±7	±7	±7	±9	±10	±8	±8
Range	56-153	58-135	54-148	54-133	55-133	64-114	71-126	71-170	73-133	73-133
Portal vein insulin, μU/ml	37	38	38	41	43	39	47	79	82	93
SEM	±14	±13	±9	±11	±10	±9	±13	±21	±26	±29
Range	15-164	14-182	14-117	18-124	13-126	17-81	22-105	29-237	29-260	28-303
Portal vein flow, ml/min	498	488	474	459	455	527	479	443	466	496
SEM	±66	±51	±55	±48	±42	±67	±60	±43	±51	±56
Range	220-898	275-898	184-898	215-843	220-734	265-816	306-734	210-734	300-762	285-762
Femoral artery blood sugar, mg/100 ml	92	87	86	85	85	85	89	100	95	97
SEM	±9	±7	±7	±7	±7	±7	±9	±8	±7	±7
Range	64-155	48-140	42-133	44-131	53-130	62-119	64-124	69-140	69-131	73-131
Femoral artery insulin, μU/ml	15	14	16	15	16	17	19	20	23	25
SEM	±2	±1	±2	±1	±2	±2	±4	±3	±5	±5
Range	6-24	6-22	7-31	6-30	6-27	11-30	9-43	10-47	13-62	14-57
Hepatic artery flow, ml/min	185	180	166	166	160	159	145	150	139	146
SEM	±37	±28	±16	±16	±18	±63	±21	±17	±18	±21
Range	50-474	55-474	60-296	65-296	60-296	89-296	74-237	55-237	58-207	94-237
Hepatic vein blood sugar, mg/100 ml	95	91	90	90	89	92	92	104	100	101
SEM	±7	±7	±7	±7	±7	±9	±11	±30	±8	±24
Range	66-127	60-127	60-131	57-134	58-125	62-128	69-138	57-138	71-140	70-140
Hepatic vein insulin, μU/ml	14	15	16	16	16	14	14	20	17	21
SEM	±1	±1	±1	±2	±1	±2	±2	±2	±2	±4
Range	6-36	6-17	7-34	6-30	8-30	7-21	7-19	7-57	13-28	11-55
Insulin to liver, mU/min	8.4	10.8	11.6	11.2	11.6	13.3	13.7	22.2	24.0	29.5
SEM	±1.1	±2.8	±2.5	±2.4	±3.0	±3.3	±3.3	±7.2	±7.9	±10.2
Range	3.9-15.1	5.2-41.5	3.7-32.1	5.0-29.8	4.7-34.8	4.6-31.9	5.5-30.2	4.7-90.6	8.4-84.3	8.5-102.9
Insulin from liver, mU/min	5.3	5.3	5.3	5.2	5.2	5.8	4.7	6.5	6.5	7.5
SEM	±1.0	±0.6	±0.8	±0.8	±0.6	±1.0	±0.9	±0.6	±0.6	±1.4
Range	1.9-13.0	2.2-10.8	2.4-10.8	2.0-11.3	2.8-8.9	2.0-11.7	1.8-7.5	1.7-13.2	3.8-9.0	2.7-16.6
% Hepatic retention	34	39	43	42	41	48	55	56	63	60
SEM	±5	±7	±6	±8	±7	±9	±12	±7	±7	±8
Range	-20-67	-3-90	14-76	5-81	3-85	6-78	14-81	7-92	33-92	23-93
<i>P</i> value for change in % hepatic retention from control value of 40±3%						NS	NS	<0.05	<0.01	<0.02
Net hepatic glucose output, mg/min	47	40	27	39	31	38	30	-10	14	5
SEM	±15	±17	±21	±17	±12	±17	±16	±18	±12	±13
Range	-18-144	-105-156	-179-149	-107-156	-64-89	-23-108	-9-47	-138-75	-28-51	-67-69
Number of dogs	11	14	14	14	14	8	7	13	8	9

thermore the influence of the transit time of insulin through the liver has not been taken into consideration in such calculations. The net hepatic glucose output of the liver has been calculated in a similar fashion except no

correction was made for hematocrit. The same reservations applicable to the non-steady state are appropriate for this determination. The hepatic plasma insulin clearance was determined by multiplying the percent hepatic retention of

Glucose Administration in 14 Dogs

(minutes)											
11	15	20	25	30	40	50	60	70	80	90	120
113 ±8 75-159	138 ±12 82-208	146 ±13 100-240	155 ±14 94-235	157 ±16 90-265	143 ±12 84-260	152 ±12 81-272	170 ±19 81-310	152 ±11 89-304	148 ±9 81-333	162 ±19 89-272	163 ±22 88-249
97 ±17 24-219	120 ±19 28-217	180 ±39 28-380	187 ±56 28-550	231 ±65 28-701	320 ±129 28-1010	289 ±115 35-1040	279 ±124 36-1571	288 ±197 43-1862	268 ±110 35-1083	377 ±153 40-1577	472 ±235 56-1831
427 ±49 210-734	418 ±34 195-626	407 ±31 195-571	397 ±32 200-598	393 ±33 195-598	365 ±34 225-490	369 ±36 175-571	352 ±29 143-490	322 ±48 122-653	350 ±59 170-762	354 ±52 170-789	355 ±63 190-816
104 ±8 71-135	120 ±9 76-157	126 ±10 78-175	125 ±12 71-190	129 ±12 67-203	124 ±11 76-178	130 ±12 76-203	146 ±14 79-230	121 ±11 75-198	123 ±11 72-210	141 ±15 84-241	136 ±16 74-218
25 ±5 14-62	30 ±6 14-84	30 ±5 14-60	39 ±8 13-112	44 ±9 15-128	56 ±14 17-139	61 ±13 21-139	73 ±17 21-202	72 ±20 16-176	73 ±22 17-211	105 ±32 21-371	132 ±50 18-431
135 ±22 50-248	147 ±15 55-223	146 ±17 50-252	146 ±16 50-252	144 ±13 45-230	130 ±18 55-237	121 ±17 45-222	136 ±16 50-266	122 ±19 55-237	116 ±21 59-237	124 ±15 55-207	107 ±15 15-160
108 ±7 80-140	128 ±12 82-208	131 ±11 89-191	137 ±12 86-203	139 ±11 84-205	133 ±10 84-228	137 ±10 84-236	152 ±14 84-247	131 ±11 81-245	132 ±11 81-253	141 ±16 92-237	139 ±16 88-231
27 ±6 14-75	30 ±4 17-56	32 ±5 16-70	37 ±6 16-88	38 ±7 9-98	45 ±11 9-102	58 ±16 8-153	76 ±27 14-349	58 ±18 10-184	59 ±18 14-190	114 ±67 11-771	175 ±101 13-923
24.4 ±5.5 7.5-58.9	31.2 ±6.0 7.6-64.8	38.2 ±8.9 7.7-96.8	40.6 ±9.7 7.5-108.3	53.3 ±12.3 9.1-136.8	60.5 ±18.4 7.2-172.3	55.2 ±15.7 9.8-141.6	55.5 ±18.3 10.8-216.8	47.5 ±26.4 8.1-89.0	45.6 ±17.0 8.0-156.3	79.2 ±40.3 10.5-418.8	97.7 ±51.7 11.8-466.0
8.3 ±1.7 3.6-20.4	9.0 ±1.1 4.8-16.8	13.3 ±3.9 4.6-51.8	11.0 ±1.9 4.7-28.3	11.6 ±2.7 2.3-37.0	12.4 ±3.4 2.5-34.2	15.7 ±4.7 2.0-47.4	21.5 ±8.8 3.6-114.4	13.6 ±4.0 2.0-42.5	14.2 ±4.6 3.2-42.0	36.3 ±25.0 1.9-261.7	50.7 ±31.8 1.5-295.4
56 ±11 3-87	63 ±6 30-87	65 ±8 21-91	65 ±7 21-92	68 ±7 19-92	69 ±9 20-95	62 ±11 6-92	59 ±6 26-85	53 ±8 -28-86	63 ±7 33-90	59 ±8 23-85	55 ±9 30-87
NS	<0.01	<0.02	<0.01	<0.01	<0.01	NS	<0.01	NS	<0.01	<0.05	NS
-6 ±10 -40-50	-15 ±18 -113-76	-35 ±18 -163-62	-46 ±17 -138-96	-50 ±24 -256-68	-20 ±16 -98-101	-34 ±15 -110-40	-51 ±39 -443-76	-46 ±19 -157-17	-32 ±25 -187-66	-53 ±34 -268-53	-62 ±39 -321-79
11	13	13	13	14	11	11	14	11	10	12	9

insulin by the hepatic plasma flow. The limitations of non-steady state also apply to this calculation, but it does eliminate changes in blood flow as a determinant in hepatic extraction of insulin.

Glucose was measured using whole blood by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N. J.) and insulin by immunoassay using dextran-coated charcoal.

TABLE II
Amount of Insulin Coming to and Leaving the

Dog	Insulin	Time											
		-20	-15	-10	-5	0	1	3	5	7	9	10	11
	<i>mU/min</i>												
1	To liver	5.6	9.1	7.2	5.0	8.8	16.5	20.2	27.1	29.6	18.0	23.8	
	Leaving liver	3.7	4.1	4.5	4.7	4.1	4.6	4.2	4.5	4.6	4.7	4.3	
	% Hepatic retention	33	56	38	5	53	72	79	83	84	74	82	
2	To liver	5.6	6.7	7.9	5.1	5.0			4.7			10.3	
	Leaving liver	6.7	6.4	6.0	4.8	4.8			4.5			5.2	
	% Hepatic retention	-20	-3	23	5	3			7			49	
3	To liver	3.9	6.7	6.6	11.2	7.9			19.2			37.5	
	Leaving liver	2.9	5.0	5.0	4.6	4.8			13.2			12.3	
	% Hepatic retention	26	26	25	59	38			31			68	
4	To liver		6.7	9.1	7.1	5.7	7.8	8.8	11.9	15.6	23.1	21.0	
	Leaving liver		6.7	7.9	6.1	5.1	5.7	6.7	8.3	9.0	16.6	20.5	
	% Hepatic retention		-0.5	14	14	11	27	29	31	43	28	3	
5	To liver	15.1	9.9	10.2	10.5	34.8	31.9	30.2	27.2	24.1	52.5	28.5	
	Leaving liver	6.1	4.8	5.1	4.1	5.2	7.0	5.8	6.6	7.0	9.4	8.6	
	% Hepatic retention	60	51	50	62	85	78	81	76	71	83	70	
6	To liver	8.6	11.4	8.4	9.7	9.7			11.1			11.9	
	Leaving liver	4.7	3.9	3.7	3.2	3.3			3.6			6.7	
	% Hepatic retention	46	49	55	67	66			68			43	
7	To liver	8.8	8.8	15.4	10.5	15.9			18.5			16.9	
	Leaving liver	5.0	5.5	6.0	5.6	7.1			8.6			11.1	
	% Hepatic retention	43	38	62	47	55			54			35	
8	To liver	5.6	5.2	5.6	5.8	4.7	4.8	7.4	9.5	8.4	8.6	12.7	
	Leaving liver	1.9	2.2	2.4	2.0	2.8	2.0	1.8	1.7		2.7	3.6	
	% Hepatic retention	67	58	56	66	42	59	76	83		70	72	
9	To liver	15.1	13.1	13.2	14.9	10.8	19.3	15.1	15.8	16.0	20.2	20.3	
	Leaving liver	13.0	10.8	10.8	11.3	8.9	11.7		7.8	7.9	9.4	10.0	
	% Hepatic retention	14	18	18	24	18	39		50	51	53	51	
10	To liver		12.2	17.8	23.2				90.6	84.3	102.9	58.9	
	Leaving liver		4.3	4.2	4.3				7.1	7.0	7.5	7.7	
	% Hepatic retention		64	76	81				92	92	93	87	
11	To liver	7.4	9.4			6.1	14.0			20.5	22.6	39.2	
	Leaving liver	5.2	6.0	5.0	4.8	4.3	5.2			6.0	6.5	6.5	
	% Hepatic retention	30	37			29	63			72	71	83	
12	To liver	8.0	6.6	3.7	5.0	5.1	4.6	5.5	8.5	8.4	8.5	9.0	
	Leaving liver	5.0	5.2	2.4	3.1	2.9	2.9	2.8	3.6	3.8	4.5	5.0	
	% Hepatic extraction	40	22	33	38	43	37	49	58	55	48	46	
13	To liver	8.0	8.1	8.0	7.9	8.1	8.0	8.8	9.1	9.3	8.9	7.5	
	Leaving liver	5.3	5.7	4.7	7.2	6.4	7.5	7.5	7.5	6.2	6.9	7.0	
	% Hepatic retention	33	30	41	7	22	6	14	19	33	23	6	
14	To liver		41.5	32.1	29.9	28.6			36.6			43.3	
	Leaving liver		4.3	8.5	7.9	7.6			7.0			11.2	
	% Hepatic retention		90	74	74	74			81			74	

Liver and Percent Hepatic Extraction in 14 Dogs

(minutes)														
15	20	25	30	40	45	50	60	70	75	80	90	100	110	120
22.6	55.0	73.5	96.6	141.6		141.6	216.8	257.6		156.3	165.1			235.1
4.8	5.2	5.8	7.5	8.3		21.8	33.4	42.6		42.0	36.8			83.4
79	91	92	92	94		85	85	83		73	78			65
10.4	16.9	7.1		20.8	14.2	17.0	16.4	8.1		48.8	67.8	32.0		
6.1	6.4	6.9	6.9	7.6	6.7	8.1	9.5	10.4		13.7	13.0	24.8		
41	63	65		64	53	52	42	-28		72	81	22		
37.2	47.5	18.9	68.8	55.9	84.2	56.5	47.1	89.0	92.2	81.9				
11.4	11.6	13.0	15.0	20.8	23.4	24.6	26.3	30.4	29.7	25.2				
69	76	31	78	63	72	56	44	66	68	69				
39.5	30.4		55.4	59.1		50.3	70.7	11.2		14.5	10.5			11.8
16.8	19.6	28.3	37.0	34.2		47.4	25.5	8.0		8.5	8.1			6.1
58	36		33	42		6	64	29		41	23			49
48.0	68.6	59.6	67.1	55.5		62.1	58.9	29.0		26.7	24.9			22.9
8.8	8.8	11.8	15.6	21.5		22.4	22.0	14.7		12.6	10.8			11.7
82	87	80	77	61		64	63	49		53	56			49
16.1	17.7	18.8	20.5		20.1		23.5		20.5		14.9			17.3
8.4	10.3	10.3	8.6		10.0		10.4		11.7		8.6			9.9
48	42	46	58		50		56		45		42			43
21.7	18.3	21.8	27.1		26.4		39.0		41.4		47.2			45.0
10.7	8.6	11.8	11.1		15.6		15.5		23.2		22.0			23.8
51	53	46	59		41		60		44		54			47
			9.1	53.1		21.6	18.8	14.4		15.8	12.7	22.0	19.7	12.1
			2.3	2.5		2.0	3.6	2.0		3.2	1.9	1.9	1.5	1.5
			75	95		91	81	86		80	85	91	92	87
18.3	23.6	12.1	22.1	20.7		30.4	24.5	21.1		34.2	36.6	32.1	32.1	40.9
8.4	7.8	8.4	9.7	8.9		17.8	14.9	11.1		10.9	5.7	6.0	4.3	5.2
54	67	66	56	57		42	39	48		68	84	82	87	87
64.8		108.3	136.8	172.3		133.9	25.7	31.6						
8.6	13.0	14.5	19.4	17.4		11.3	5.2	7.0						
87		87	86	90		92	80	78						
56.4	96.8	58.5	98.7	29.7		35.3	25.7	13.5		8.0				
7.1	10.8	10.0	10.3	6.0		5.5	5.2	4.2		4.1				
87	89	83	90	80		85	80	69		49				
11.7	22.9	32.3	12.2	48.6		49.0	41.5	28.8		42.7	38.1			
5.6	4.6	4.7	3.1	4.5		5.1	6.5	5.8		4.2	5.6			
52	80	85	75	91		90	85	80		90	85			
7.6	7.8	7.5	7.5	7.2		9.8	10.8	17.9		26.9	34.3	33.3	34.0	27.9
5.3	6.2	5.8	6.1	5.8		7.5	8.0	14.1		17.9	24.9	23.9	20.5	19.7
30	21	21	19	20		24	26	22		33	27	28	40	30
51.4	53.7	44.3	71.6		146.6		157.4		353.2		418.8			466.0
14.9	14.0	12.0	9.0		37.0		114.4		77.1		261.8			411.0
71	74	73	87		75		27		78		38			37

TABLE III
Change in Hepatic Plasma Insulin Clearance after

Dog	Control hepatic plasma insulin clearance	Change in hepatic plasma insulin minutes after							
		1	3	5	7	9	11	15	20
	<i>ml/min</i>								
1	98±24	109	126	149	144	107	124	112	137
2	2±13			9			71	57	86
3	74±17			-2			73	79	12
4	40±14	56	61	66	98	45	-32	133	60
5	262±31	103	54	15	14	23	-11	25	56
6	196±19			74				-20	-42
7	212±16			19				-31	-22
8	195±13	-28	-4	-6		-28	-12		
9	111±10	107		149	157	165	145	127	147
10	243±16			192	133	214	153	153	
11	160±22	118			143	168	197	201	196
12	128±19	-10	23	44	32	14	10	17	96
13	113±25	-88	-58	-46	1	-35	-93	-23	-48
14	246±12			10				-31	-26
Mean ±SEM	147±22	46±28	34±26	52±20	90±23	75±31	57±28	61±22	63±24
<i>P</i>				<0.02	<0.01	<0.05		<0.02	<0.02

The mean ±SEM control hepatic plasma insulin clearance for each dog is based on five determinations during a 20 min period prior to the administration of glucose. The *P* value is calculated on the basis of the change in the mean value at any given time from the control value.

RESULTS

The mean responses and the ranges of the 14 dogs given glucose into the duodenum are presented in Table I.¹ During the control period, the mean hepatic vein glucose concentration of approximately 90 mg/100 ml exceeded both the portal vein and hepatic artery glucose values. The mean portal vein insulin concentration was approximately 40 μU/ml, significantly greater than that found in the hepatic vein or the femoral artery. Prior to glucose administration, about 11 mU/min of insulin reached the liver with a range from 3.7 to 41.5 mU/min. This predominantly represented pancreatic insulin secretion since blood flows and insulin concentrations were significantly greater in the portal vein than in the hepatic artery. At this time, 5.3 mU/min (range 1.9–13 mU/min) of insulin was leaving the liver indicating an average extraction of 40±3% of the insulin during a single transhepatic passage. The range of hepatic extraction of insulin was from -20 to 90% during the control period.

Within 5 min after the administration of glucose into the duodenum, the average portal vein insulin concentration and the total amount of insulin presented to the liver increased significantly. The values for the

¹The individual values for the 14 dogs can be obtained from ASIS/NAPS c/o Microfiche Publications, N. Y. (document no. 02137).

amount of insulin coming to and leaving the liver and the percent hepatic retention in each individual dog is presented in Table II. Portal vein blood flow was unchanged. In three dogs (nos. 1, 9, and 11) increased insulin reached the liver within 1 min after glucose administration and preceded any detectable rise in the femoral artery blood glucose concentration in two of them. In four additional dogs (nos. 6, 8, 12, and 13), significantly more insulin reached the liver before femoral artery blood glucose concentration increased. Subsequently augmented pancreatic insulin secretion was usually associated with increased arterial glucose concentrations. Although the mean values for insulin presented to the liver (Table I) do not indicate the biphasic insulin secretory response to glucose described by Curry, Bennett, and Grodsky (11), 10 of the 14 dogs (nos. 1, 2, 3, 4, 5, 8, 10, 11, 12, and 14), demonstrated two peaks of insulin secretion (Table II). The earliest peak was seven minutes after glucose administration in dog no. 1, while in six others (nos. 3, 4, 5, 10, 11, and 14) it was at 20 min or earlier. In the three remaining dogs, this initial peak was at 40 min. The peak portal vein insulin concentration during this initial pancreatic response to glucose varied from 138 to 317 μU/ml and the amount of insulin presented to the liver ranged from 20.8 to 102.9 mU/min. In most of the animals demonstrating two peaks, the second one oc-

Glucose Administration into the Duodenum

clearance from control value (ml/min)—
glucose administration:

	25	30	40	50	60	70	80	90	120
	134	133	125	101	104	94	64	78	45
	91		88	63	54	-41	93	102	27
	-6	108	77	99	26	76	75		
		79	100	-21	134	30	69	27	74
	32	5	-58	-42	-46	-135	-117	-141	-161
	-28	3			-39			-97	-101
	-18	13			8			-27	-61
		-6	74	38	9	-25	-44	-48	-93
	153	124	83	51	5	25	74	88	68
	14	-1	-10	-7	-105	-118			
	172	209	122	111	-99	34	-27		
	120	64	143	142	158	119	140	110	
	-51	-55	-55	-38	-27	-14	56	30	46
	-19	16			-157			-117	-128
	49±23	53±21	63±21	45±20	2±24	4±25	38±24	0±28	-29±28
		<0.05	<0.01	<0.05					

occurred at 60 min or later and was more prolonged. It was characterized by much higher portal vein insulin concentrations (range 142–1,862 μ U/ml) and greater amounts of insulin reaching the liver (range 22–257.6 mU/min.). In four dogs (nos. 6, 7, 9, and 13), only a single peak was observed and it came 60 min or later after administration of glucose.

The marked increase in insulin reaching the liver was associated with higher insulin concentrations in the hepatic vein and femoral artery. However, in seven of the animals, the increased insulin presented to the liver was not immediately reflected in elevated insulin concentrations in the hepatic vein and femoral artery. Usually the insulin concentration rose in the hepatic vein before the femoral artery. In an occasional animal (nos. 5, 9, and 10), the insulin concentration increased in the femoral artery before the hepatic vein. In dog no. 5, the insulin concentration increased in both the hepatic vein and femoral artery before it rose in the portal vein. In 6 of the 14 dogs (nos. 5, 8, 9, 10, 11, and 12), the insulin concentration in the femoral artery exceeded that in the hepatic vein.

Following glucose administration, the increment in the amount of insulin reaching the liver exceeded that leaving that organ, indicative of augmented hepatic extraction. Within 1 min after glucose administration, the mean percent of insulin presented which was removed

rose to 48±9% from the control value of 40±3%. At 5 min, when the amount of insulin reaching the liver had significantly increased, hepatic extraction was also significantly greater at 56±7% ($P < 0.05$). This mean percent increased to a maximum of 69±9% at 40 min after glucose administration ($P < 0.01$) and was associated with increasing amounts of insulin reaching the liver. However, after this time, the percent of the insulin presented which was removed by the liver declined toward control values even though the average amount of insulin presented to the liver continued to increase and was maximal at the end of the experiment. Of the 14 animals, nine (nos. 1–4 and 8–12) demonstrated a definite increase in hepatic extraction of insulin after glucose administration. In these dogs, the value during the control period varied from -20 to 81% and reached a peak of 64–95% after glucose administration. Increased hepatic extraction of insulin was present 1 min after glucose administration in four dogs (nos. 1, 4, 9, and 11) and within 5 min in three others (nos. 8, 10, and 12). In the remaining two dogs, it was not apparent until 11 min after glucose. The peak hepatic extraction usually occurred later than this although in dog no. 10 it was at 9 min when 93% of the insulin presented was removed in a single transhepatic passage. In five of the remaining eight dogs, peak extraction was at 30 or 40 min after glucose. In these dogs, the fraction of insulin

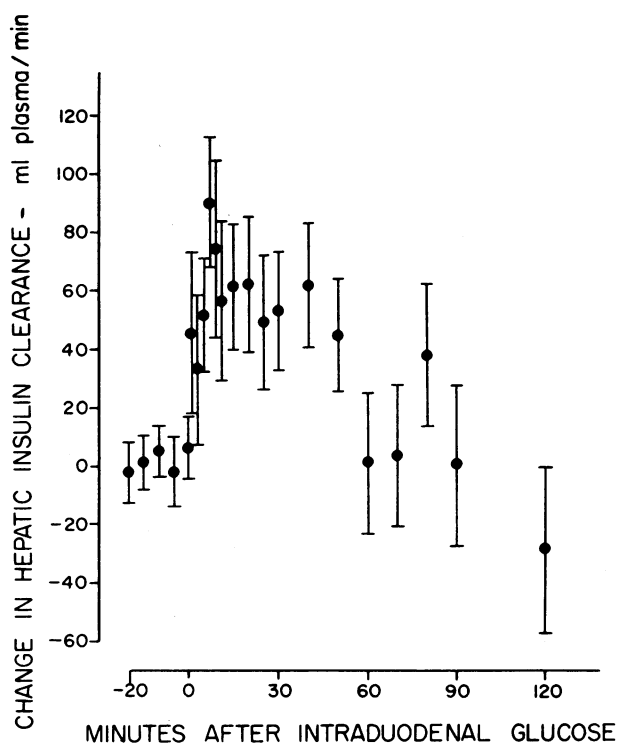


FIGURE 1 Change in hepatic plasma insulin clearance after glucose administration. Glucose was administered at 0 time. The results are the mean \pm SEM. The mean clearance during the control period was 147 ± 22 ml/min.

extracted then declined toward the end of the experiment. In five of the nine dogs demonstrating increased hepatic extraction of insulin, the peak coincided with the time when the largest amount of insulin reached the liver (nos. 2, 8, 9, 11, and 12). In all of the others, except dog no. 4, the maximum amount of insulin reached the liver after the peak of insulin extraction. Despite the increased extraction rate, arterial insulin concentrations still rose, reflecting the very marked increase in the absolute amounts of insulin reaching the liver.

During the experiments, hepatic blood flow tended to decrease progressively and it is possible that this might spuriously influence the results. In order to obviate this possibility, hepatic plasma insulin clearances were calculated. The data in Table III represent the mean control hepatic plasma insulin clearance in each of the 14 dogs and the increment of change from the control value after the administration of glucose. The average change in hepatic plasma insulin clearance for the entire group of 14 dogs is graphed in Fig. 1. The mean hepatic plasma insulin clearance during the control period was 147 ± 22 ml/min with a range from 2 to 262 ml/min. By 5 min after glucose administration,

hepatic plasma insulin clearance had significantly increased over the control values ($P < 0.02$). Except for the results at 11 and 25 min, this significant increase persisted until 60 min when it returned to control values. In general, the results obtained calculating hepatic plasma insulin clearance supported those based on calculation of percent hepatic retention of insulin.

An alternative method of examining the data is presented in Fig. 2, where the amount of insulin presented to the liver is plotted against the simultaneous arterial insulin concentration. If the liver removed a constant percent of the insulin reaching it, a straight line relationship should exist between these two variables assuming that the fractional removal in the peripheral circulation remained unchanged. The points representing the times from 5 min to 50 min after glucose administration clearly are above this line indicating that the augmented insulin reaching the liver was not reflected by commensurate elevations of the arterial insulin concentrations. From 60 min to the end of the experiment, the points are again on the line which goes through the origin and directly relates these two variables. During this same period, hepatic plasma insulin clearance had also returned to the values obtained before administration of glucose.

Five of the 14 dogs had no change in insulin extraction by the liver after glucose administration. Four of these

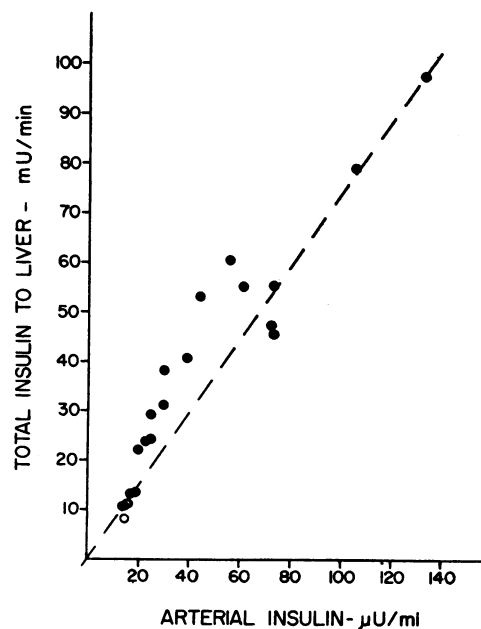


FIGURE 2 Relationship between the arterial insulin concentration and the total amount of insulin presented to the liver. The \circ represent the points obtained during the control period while the \bullet are the points obtained after the administration of glucose. The data are taken from Table I.

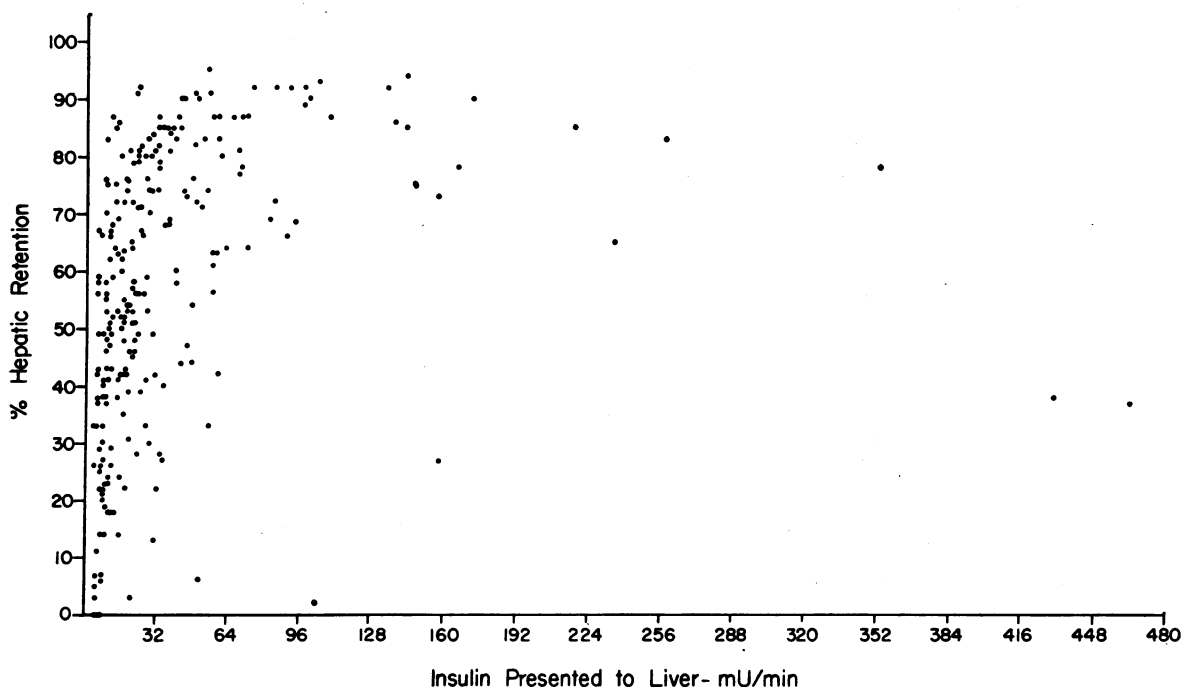


FIGURE 3 Relationship between the amount of insulin presented to the liver and the fraction of it which was extracted by that organ. The individual values from each time point from each of the 14 dogs have been plotted.

(nos. 6, 7, 13, and 14) were the same whether the data was calculated on the basis of percent hepatic retention of insulin or hepatic plasma insulin clearance. By the former parameter, dog no. 5 had no response to glucose administration while during the 30 min after glucose there was an increase in hepatic plasma insulin clearance except at eleven minutes. Following this period, the clearance decreased considerably. The magnitude of this decrease might make the significance of the earlier positive values open to question. Dog no. 8 did not have any increase in hepatic plasma insulin clearance, but there was a significant increase in percent hepatic retention of insulin. This discrepancy probably represents the decreased hepatic blood flow toward the end of the experiment when the percent hepatic retention of insulin was augmented. The effect of changes in hepatic blood flow are mitigated when clearances are calculated. Four of the five dogs without change in percent extraction (nos. 5-7 and 14) had control values for hepatic insulin extraction over 60% and two of them exceeded 70%. Although these values were considerably higher than in the dogs exhibiting augmented hepatic insulin extraction after glucose, dogs nos. 8 and 10 had basal extraction of 66 and 81% of the insulin presented, respectively. As would be expected, dogs with a high percent of hepatic retention of insulin also had high hepatic plasma insulin clearance rates. Using this latter mea-

surement, four of the five dogs which did not change their clearance after glucose administration had control clearance values of 195 ml/min or above. However, dogs nos. 5 and 10 had basal values exceeding 240 ml/min and they increased after glucose administration. The increase in insulin presented to the liver after glucose was similar in the dogs with and without increased hepatic extraction of insulin.

Prior to the administration of glucose, net hepatic output of glucose averaged between 30 and 40 mg/min. During the control period, some of the values in four dogs (nos. 1, 2, 9, and 14) indicated hepatic uptake of glucose, but usually of small magnitude except in dog no. 14 who had consistent and significant hepatic glucose uptake. This dog also had higher portal vein glucose and insulin concentrations than the other dogs suggesting that maybe it was not fasting. Within 5 min after glucose administration, 7 of the 14 dogs (nos. 1-3, 7-9, and 13) demonstrated significant hepatic uptake of glucose. In all these dogs except no. 1, this change to hepatic glucose uptake was associated with a significant increase in the portal vein glucose concentration which then usually exceeded the femoral artery glucose concentration by 10-20 mg/100 ml. In dog no. 13, the liver began to take up glucose within one minute after glucose administration. The peak of hepatic glucose uptake was more delayed, occurring within 15 min in three dogs and

within 30 min in three others. In seven of the remaining eight dogs, it occurred 60 min or more after glucose administration. The maximum uptake of glucose varied from 9 mg/min in dog no. 8 to 443 mg/min in dog no. 14. This latter dog also had the largest amount of insulin coming to the liver (466 mU/min). There was a significant correlation ($P < 0.01$) between the amount of insulin reaching the liver and hepatic glucose uptake. There was not a very consistent relationship between the percent of the insulin presented to the liver which was removed in a single passage and net hepatic glucose uptake.

In Fig. 3 every individual value of percent insulin extracted by the liver from all 14 dogs is plotted as a function of the amount of insulin reaching the liver. When less than 32 mU/min of insulin reached the liver, there was great variability (range 0–90%) in the fraction which was extracted. This percent increased rapidly when more than 32 mU/min reached the liver and, except for one point, exceeded 60% between 128 mU/min and 352 mU/min. When over 400 mU/min insulin reached the liver, hepatic extraction decreased to 30–40% suggesting saturation of the process.

DISCUSSION

During the control period, about 0.5 mU/kg/min insulin reached the liver. This is very similar to previous estimates of basal pancreatic insulin production (12–15). In anesthetized dogs, Kanazawa, Kuzuya, and Ide (12) reported a value of 0.2 to 0.3 mU/kg per min while values of 0.2 mU/kg per min were obtained by Rappaport et al. (13), Ishiwata, Hetenyi, and Vranic (14) and Campbell and Rastogi (15). Furthermore, infusion of 0.2 mU/kg per min insulin into the portal vein maintained normal plasma glucose values in depancreatized dogs (14, 16). Our somewhat higher values reflect inclusion of the hepatic artery component of insulin reaching the liver. This close agreement for pancreatic insulin production as measured by a variety of procedures provides additional assurance that we measured all the insulin reaching the liver.

The results of the control observations in this study support the previous concept that about 50% of the insulin presented to the liver in the basal state is removed (4–6, 8). However, interpretation of the extraction and clearance values found during the phase of increased insulin secretion following glucose administration is hampered by the variability of responses found in different animals, as well as by the point-to-point variability during the control period in some of the dogs (Table II). Part of the variability of response between dogs is due to the finding that in six animals (nos. 5, 8–12) hepatic vein insulin levels were lower than the

simultaneous arterial levels, a situation which is not tenable during active insulin secretion. The glucose levels, however, were above arterial values, confirming that hepatic vein blood was indeed being sampled. This problem has been encountered by other investigators (8, 10, 17) and is probably due to large differences in insulin concentration in the various hepatic veins consequent upon differential distribution of pancreatic venous blood within the major branches of the portal vein, as demonstrated by Kanazawa et al. (10). The effect of a spuriously low hepatic vein insulin concentration would be to falsely elevate hepatic insulin extraction or clearance, and it is possible that the augmented insulin extraction seen after glucose administration in dogs no. 8–12 is on this basis. However, in the remaining four dogs (nos. 1–4) which showed a clear rise in hepatic insulin extraction following glucose administration, these reservations do not apply and the portal, arterial, and hepatic venous insulin concentrations maintain their expected relationship. This group includes the two dogs in which a mixed hepatic venous effluent was collected from the distally ligated vena cava. Two additional dogs (nos. 7 and 14) exhibited the phenomenon of hepatic vein insulin being apparently less than arterial insulin only during the control period. This was more marked in dog no. 14, which has the highest control value for insulin extraction of all the animals studied.

In the dogs which showed no change in insulin extraction following glucose administration, there are no obvious factors in the experimental data to account for their variance in response from dogs nos. 1–4, except that in two of them (nos. 7 and 14) the spurious hepatic vein levels obtained during the control period may have masked a subsequent rise in percent extraction of insulin. Both of these dogs together with nos. 6 and 13 showed no change in insulin extraction after glucose administration whether on the basis of percent extraction or plasma clearance. If the between-animal variability is on the basis of random errors in the sampling of hepatic veins with a spectrum of relative insulin concentrations, it is perhaps significant that none of the experiments revealed spuriously high hepatic vein levels which might lead to the observation of a decreasing percent extraction of insulin with increasing delivery of insulin to the liver. Indeed, of all dogs studied, none showed such a decrease in percent extraction, and only one (no 5) showed a decrease in plasma insulin clearance starting 30 min after the administration of glucose. In this particular case, there was a marked decrease in hepatic blood flow during the experiment from a high initial level. As blood flow itself may be a rate determinant of clearance, this single finding may not be significant.

Our results, therefore, suggest that with the increasing delivery of insulin to the liver following glucose administration, there is no decrease in the percent of insulin removed by that organ and therefore no evidence for saturation of the process of extraction by these physiological amounts of insulin. Indeed, within the limits of the variability observed, it is possible that a transient increase in percent extraction occurs, and this point deserves further study. These results are in marked contrast with the observations of Madison and Kaplan (5, 6) who reported that glucose administration to humans caused a marked reduction in the percent of [¹²⁵I]insulin which was extracted by the liver. Our results are more consistent with the studies of Waddell and Sussman comparing portal and inferior vena cava insulin levels in unanesthetized dogs (9). During the control period, the portal vein insulin concentrations were approximately twice those of the inferior vena cava. However, following glucose administration, portal vein insulin levels increased to a mean of three times those in the inferior vena cava. In some experiments, the portal vein insulin concentration was 10 times that in the inferior vena cava. Portal vein and inferior vena cava insulin concentrations were very similar when portal vein insulin levels were less than 50 μ U/ml. In contrast, when the portal vein insulin concentration was greater than 500 μ U/ml, almost all of the insulin was removed by the liver. Blackard and Nelson also obtained data consistent with increased hepatic extraction of insulin after glucose administration (18). In the patients studied by Samols and Ryder, infusion of insulin did not influence the percent of insulin extracted by the liver (8).

The observation of an increase in hepatic insulin clearance after glucose administration in some of our experiments may be related to the questions of transit time across the liver and equilibration with tissue compartments within the liver. Transit time of insulin through the liver is not known, but in experiments currently in progress where insulin is infused into the portal vein, significant elevation of hepatic vein insulin levels usually occurs within 2 min. Failure to allow for this time might give spuriously high values for insulin clearance during the first few minutes of rising insulin levels coming to the liver; however, the values for clearance remain significantly elevated (Fig. 1) during the period 5–15 min after glucose administration when afferent insulin levels are at a fairly stable level (Table I). If the observed increase of clearance were purely due to delayed transit, it should have returned to control levels during this period.

The time required for equilibration of the increasing insulin levels with extravascular compartments within the liver also remains an unknown factor in these stud-

ies. It should, however, be emphasized that, provided allowance is made for the effect of transit time as outlined above, our direct measurements of clearance based on the measurement of blood flow and arteriovenous difference will remain valid in terms of clearance of insulin from the blood whether such clearance represents removal of that insulin from the vascular compartment for equilibration purposes or for any other reason. Whatever the mechanism for insulin clearance during transit through the liver, the observation remains valid that the liver removes a proportion of it which is not decreased and may be transiently increased. This observation is strengthened by the data as plotted in Fig. 2. If there were no alteration in hepatic extraction of insulin, the straight-line relationship between arterial insulin concentrations and the total amount of insulin presented to the liver established during the control period should persist throughout the entire experiment. The validity of this line is strengthened by the fact that it goes through the origin and the points obtained 1 and 3 min after glucose administration also fall on it as do the points from 60, 90, and 120 min after glucose. However, during the period from 5 min to 60 min following glucose, the amount of insulin reaching the liver increased more rapidly than did the arterial insulin concentration consistent with increased uptake by the liver. This observation is not affected by the spuriously low hepatic vein insulin levels found in the six dogs referred to earlier, as hepatic vein levels are not utilized when plotting the data in this fashion.

The results obtained by Madison et al. suggest a limited ability of the liver to extract insulin and saturation of this process at relatively low insulin concentrations since their patients were not given inordinately large amounts of insulin (5, 6). Our results (Fig. 3) indicate the hepatic capacity to extract insulin may not be saturated until 400 mU/min or more reaches the liver. Turner, Grayburn, Newman, and Nabarro (19) and Silvers, Farquhar, Lerner, and Reaven (20) also concluded that the liver had a large capacity to remove insulin. Waddell and Sussman found much higher hepatic extraction ratios when portal vein insulin concentration was 500 μ U/ml than when it was 50 μ U/ml (9). Samols and Ryder reported that raising insulin concentrations to 250 μ U/ml did not decrease hepatic extraction of insulin (8).

Irrespective of whether there is an increase in the percent of insulin removed, these observations permit the conclusion that there is a large increase in the absolute amount of insulin taken up by the liver after glucose administration. This is consistent with the observation that as much as half such glucose is metabolized by the liver and that this process is facilitated by insulin (21, 22). Thus, the additional insulin removed

by the liver could mediate the increased hepatic uptake of glucose and the sharp reduction in hepatic glucose output in the present experiments and extensively studied by Madison and colleagues (21, 22). However, the increased hepatic uptake of glucose could also be correlated with the augmented amounts of glucose presented via the portal vein. Thus, these data do not differentiate between insulin and glucose as the regulators of carbohydrate balance across the liver. In addition, the present results provide no data as to the fate of the insulin removed by the liver. It may contribute to the changes in hepatic carbohydrate metabolism, but it is also possible that it is degraded (1) without exerting any physiological action.

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