

The Role of the Liver in Glucagon Metabolism

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ABSTRACT Total plasma immunoreactive pancreatic glucagon (IRG) was measured in samples taken simultaneously from the proximal portal vein and superior vena cava of 26 healthy rats. The portal-peripheral ratio of IRG was 2.80 ± 0.25 , the portal-peripheral difference (Δ) 124 ± 15 pg/ml, and percentage extraction 58 ± 3 . Gel filtration of paired portal and peripheral vein samples showed that reduction in the 3,500-dalton IRG component (glucagon) in peripheral samples accounted for almost all the differences, there being minimal and inconsistent changes in the high molecular weight ($>40,000$) fraction. The portal-peripheral ratio of the 3,500-dalton glucagon was 5.24 ± 1.10 , the portal-peripheral difference 130 ± 33 pg/ml, and the percentage extraction 81 ± 5 .

To study the transhepatic differences in the 9,000-dalton "proglucagon-like" material, the experiment was repeated in nine rats 24 h after bilateral nephrectomy, a procedure which increases plasma levels of this fraction. The portal-peripheral ratio for plasma IRG in these rats was 1.48 ± 0.12 , the portal-peripheral difference 140 ± 29 pg/ml, and percentage extraction 28 ± 5 . Gel filtration revealed no consistent differences between portal and peripheral concentrations of the 9,000- and $>40,000$ -dalton components, which comprised 40 and 13%, respectively, of the mean IRG level of 492 ± 35 pg/ml. In contrast, there were marked differences between portal and peripheral levels of the 3,500-dalton component, the ratio being 3.42 ± 0.63 , the portal-peripheral difference 182 ± 32 pg/ml, and percentage extraction 64 ± 5 . Similar studies in a healthy dog, in which species there are significant circulating levels of the 9,000-dalton IRG component, confirmed the selective hepatic extraction of the 3,500-dalton fraction.

We conclude that the various IRG fractions are metabolized differently by the liver, and that portal-peripheral ratios based on direct assay of plasma IRG will vary depending on the percentage glucagon immunoreactivity in each fraction; the greater the combined contribution of fractions other than the 3,500-dalton component to total plasma IRG, the lower will be the ratio. Because of the heterogeneity of circulating IRG and significant differences in the metabolism of its various components, gel filtration of plasma samples is necessary for precise quantitation of the hepatic uptake of each particular fraction.

INTRODUCTION

The role of the kidney in the metabolism of glucagon in both man and animals (1-6) is well established. Total plasma immunoreactive glucagon (IRG)¹ levels are elevated in chronic renal failure (7) and consist of increases in both the 3,500- and 9,000-dalton components (4). Studies in rats have suggested that the 3,500-dalton fraction is handled by both glomerular filtration and uptake from the postglomerular peritubular blood, while the 9,000-dalton fraction is mainly metabolized by the latter process (1). On the other hand, although plasma IRG levels are elevated in many patients with compensated cirrhosis, hepatic failure with portosystemic shunting (8-10) and in rats after 70% hepatectomy (11), there is still controversy regarding the role of the liver in glucagon metabolism. Some investigators have reported (12-14) that the liver plays an unimportant role in the degradation of glucagon; others have suggested that the liver is a major site of glucagon metabolism (15-17). In support of the latter conclusion, Blackard et al. (18) reported that portal-peripheral glucagon ratios in eight fasting nondiabetic subjects were 1.7 ± 0.5 , and Dencker et al. (19) found similar values in three normal sub-

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¹Abbreviations used in this paper: IRG, immunoreactive glucagon; Pe, peripheral; Po, portal.

jects after a meal. This study describes experiments in rats in which the hepatic handling of IRG and its various fractions was investigated. The results indicate that the liver is a major site of metabolism of the 3,500-molecular weight glucagon, but that this organ has little effect on the other IRG components.

METHODS

Animals. 26 Sprague-Dawley rats (250–300 g) were submitted to laparotomy under ether anesthesia, and blood was withdrawn simultaneously from the proximal portal vein and superior vena cava by two individuals and placed in chilled tubes containing 500 U/ml Trasylol (FBA Pharmaceuticals Inc., New York) and EDTA (1.2 mg/ml). The tubes were centrifuged at 4°C, and the plasma was stored at –20°C until assayed. The animals were divided into three groups: group 1 ($n = 9$) was allowed free access to food and water; group 2 ($n = 8$) was fasted overnight, but allowed access to water, and group 3 ($n = 9$) was infused for 15 min with intravenous arginine hydrochloride (500 mg/kg) after an overnight fast. Blood samples were taken 15 or 30 min after the arginine infusion was started. An additional nine rats underwent bilateral nephrectomy, and 24 h later, portal and peripheral samples were obtained and processed as described above.

After an overnight fast, a laparotomy was performed on a 25-kg dog, and the portal vein and inferior vena cava were cannulated. An intravenous infusion of arginine hydrochloride (500 mg/kg) was administered over 30 min. Simultaneous samples were withdrawn from the proximal portal vein in the porta hepatis and the inferior vena cava above the entry of the hepatic vein during a 90-min period.

Assay procedure. IRG was measured by a double-antibody radioimmunoassay using 30K antiserum (kindly provided by Dr. R. Unger, Dallas, Tex.) as previously described (4). The sensitivity of the assay is 3–4 pg/tube.

Gel filtration. Gel filtration was carried out on portal and peripheral plasma samples from seven control rats (three fed, one fasted, and three arginine stimulated) and all the nephrectomized animals, as well as on portal and peripheral samples taken from the dog at 15 and 40 min. Plasma (0.5–1.0 ml) was applied to 50 × 1-cm Bio-Gel P-30 columns (Bio-Rad Laboratories, Richmond, Calif.) and eluted under gravity at room temperature with a 0.2 M glycine buffer, pH 8.8, containing 0.25% human serum albumin, 1% lamb serum, and 500 U/ml Trasylol. 1.0-ml fractions were collected, and the total volume was assayed. Four peaks were identified (1, 4) according to their molecular size: >40,000 daltons (peak A), 9,000 daltons (peak B), 3,500 daltons (peak C), and approximately 2,000 daltons (peak D).

Statistical methods. Results are presented as mean ± SEM. The statistical significance of differences between group means was assessed by Student's *t* test, *P* values of <0.05 being considered significant.

RESULTS

To facilitate comparison of the hepatic handling of the various IRG components, simultaneously drawn portal and peripheral vein levels are expressed as portal-peripheral ratios (Po:Pe), absolute portal-peripheral differences (Δ pg/ml), and percentage extraction [(portal-peripheral) portal × 100].

Portal and peripheral plasma IRG. Table I presents the IRG results in the three subgroups of control rats. The mean portal IRG concentration for the whole group was 203 ± 17 pg/ml, and the peripheral IRG was 79 ± 6 pg/ml. Po:Pe was 2.80 ± 0.25, Δ was 124 ± 15 pg/ml, and percentage extraction 58 ± 3. However, there were significant differences between the fed, fasted, and arginine-stimulated rats. Thus, while the portal vein IRG levels in the three groups did not differ, there was a significant difference between the fed and fasted groups peripheral concentrations (85 ± 5 vs. 61 ± 9 pg/ml, $P < 0.05$) resulting in a greater Δ value in the fasted rats (169 ± 34 vs. 93 ± 10 pg/ml, $P < 0.05$). There was, in addition, a significant difference in the Po:Pe ratio between the fasted and fed group (4.00 ± 0.54 vs. 2.08 ± 0.32, $P < 0.005$), and the

TABLE I
Portal and Peripheral Vein IRG Levels, Portal-Peripheral Ratios, Portal-Peripheral Differences, and Percentage Extraction in Three Subgroups of Control Rats

Group	IRG		Po/Pe	Δ	Extraction
	Portal vein	Peripheral vein			
	pg/ml			pg/ml	%
Fed					
1	267	119	2.24	148	55
2	170	100	1.70	70	41
3	204	78	2.62	126	62
4	130	70	1.86	60	46
5	140	85	1.65	55	39
6	170	80	2.13	90	53
7	190	80	2.24	110	58
8	155	75	2.07	80	52
9	180	80	2.25	100	56
Mean ± SEM	178 ± 14	85 ± 5	2.08 ± 0.10	93 ± 10	51 ± 3
Fasted					
1	378	95	3.98	283	75
2	177	71	2.49	106	60
3	296	84	3.52	212	72
4	112	41	2.73	71	63
5	369	74	4.98	295	80
6	284	58	6.36	226	80
7	115	20	5.75	95	83
8	112	48	2.33	64	57
Mean ± SEM	230 ± 41	61 ± 9	4.00 ± 0.54	169 ± 34	71 ± 4
Arginine					
1	115	74	1.55	41	36
2	153	141	1.09	12	5
3	304	148	2.05	156	51
4	295	77	3.83	218	74
5	144	79	1.82	65	45
6	365	107	3.41	258	71
7	189	69	2.73	120	63
8	112	56	2.0	56	50
9	146	40	3.65	106	73
Mean ± SEM	203 ± 31	88 ± 12	2.45 ± 0.32	114 ± 28	52 ± 7
Overall $n = 26$	203 ± 17	79 ± 6	2.80 ± 0.25	124 ± 15	58 ± 3

percentage extraction was also higher in the fasted compared to the fed group (71 ± 4 vs. $51 \pm 3\%$, $P < 0.001$).

Portal and peripheral IRG components. The results of the gel filtration experiments using plasma from control ($n = 6$) and nephrectomized rats ($n = 8$) are shown in Table II and Figs. 1 and 2. Portal and peripheral IRG levels were higher in nephrectomized animals than in controls ($P < 0.001$). Although there was marked extraction of C peak material in both groups of rats as assessed by all three indices, there was minimal and inconsistent extraction of A, B, and D peak components. As the concentrations of the A and D peak components were low in most animals, the Δ value is the most meaningful. In many instances it was not possible to calculate the Po:Pe ratio or percentage extraction, because peripheral levels exceeded those in the portal vein or were undetectable in one or both samples. The Δ value indicates no consistent extraction of either A or D peak material. It should be appreciated that the results involving the A and D peak components may be less accurate than those of total IRG or the C peak material, because of their relatively low concentrations. However, by the same token their contribution to the total IRG extraction will be small.

Because B peak levels were very low in control animals, nephrectomy was performed to elevate this component sufficiently to enable its extraction to be monitored accurately. As assessed by all three parameters, the hepatic removal of B peak material was insignificant in these rats.

Comparison of the extraction of B and C peak material in individual animals was only possible in the nephrectomized groups, where the B peak component was elevated. C peak extraction was significantly higher than that of the B peak ($P < 0.001$) as assessed by all three indices. The A peak component in this group of animals was also moderately increased permitting comparison of its extraction to that of C peak glucagon. Both portal-peripheral differences (Δ) and percentage extraction were significantly less than the corresponding C peak values ($P < 0.001$), but the A peak Po:Pe, although lower than that of the C peak, did not reach statistical significance.

The magnitude of portal-peripheral IRG differences depends mainly upon the contribution of C peak material to the plasma IRG concentration. Thus, there was no significant difference in the absolute portal-peripheral values (Δ) of total plasma IRG (97 ± 24 vs. 140 ± 29 pg/ml) or of C peak material between the control and nephrectomized animals (130 ± 33 vs. 182 ± 32 pg/ml). In contrast, the Po:Pe ratio (1.48 ± 0.12 vs. 2.29 ± 0.35) and percentage extraction (28 ± 5 vs. 52 ± 7) of total plasma IRG in the latter rats was lower than in the former group ($P < 0.05$ and $P < 0.02$, respectively).

This result reflects the increased contribution of the B peak component to the plasma IRG in the nephrectomized animals.

Regression analysis (Fig. 3) was performed on portal and peripheral IRG concentrations in the control groups of rats ($n = 26$; see Table I) and the nephrectomized animals ($n = 8$; see Table II). The portal and peripheral values correlated in the control group ($y = 0.14x + 49$, $r = 0.44$; $P < 0.05$), but the correlation was of borderline significance in the nephrectomized animals ($y = 0.72x - 5$, $r = 0.68$; $P = 0.05-0.1$). Portal and peripheral concentrations of C peak material correlated on pooling the results of the control and nephrectomized animals, although not in either group alone. Portal and peripheral levels of the B peak component in nephrectomized rats were closely correlated ($P < 0.001$, $r = 0.85$), in keeping with the similar concentrations of this component in portal and peripheral plasma.

Portal and peripheral plasma IRG and components in the dog. The results obtained in the dog experiment are shown in Table III and Fig. 4. Appreciable quantities of B peak component were present as reported by Valverde et al. (20). There were only small differences in portal and peripheral B peak concentrations, while the C peak levels differed markedly.

DISCUSSION

Circulating total plasma IRG is composed of at least four fractions which differ in their distribution in healthy subjects and in patients with various diseases. The pattern may vary in an individual subject under different nutritional conditions, and there is in addition a species difference as evidenced by the observation that dogs have a larger proportion of IRG in the 9,000-dalton fraction (20). The 3,500-dalton component is the biologically active form of the hormone, and the 9,000-dalton moiety is probably a biosynthetic precursor with very low or absent biological activity. The origin and nature of the 2,000-dalton and void volume fractions ($\approx 160,000$ daltons) are still uncertain. The former may represent an in-vivo or in-vitro degradation product, while the latter may be a protein which cross-reacts immunologically with glucagon, or may represent the association of glucagon with another large molecular weight protein. Srikant et al. (21) showed that the void volume material was equipotent to 3,500-dalton glucagon on a molar basis. Although it is possible that only the 3,500-dalton material is of physiological importance in the circulation, the various plasma components contribute to the total plasma glucagon immunoreactivity and must therefore be taken into account when interpreting the significance of plasma IRG concentrations.

The results of this study indicate that there are

TABLE II
 Portal and Peripheral Vein IRG Levels and the Distribution of Portal and Peripheral Vein Glucagon Immunoreactivity in Nonfasted Control Rats and a Group of Rats 24 h after Nephrectomy

Group	Whole plasma						A Peak				B Peak				C Peak				D Peak				Column recovery									
	Po	Pe	Δ	Po/Pe	Ext*	%	Po	Pe	Δ	Po/Pe	Ext	%	Po	Pe	Δ	Po/Pe	Ext	%	Po	Pe	Δ	Po/Pe	Ext	%	Po	Pe	%					
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml				
Control																																
1	175	65	110	2.69	63	20	22	-2	0.91	-10	11	0	11	0	11	0	11	0	11	166	30	136	5.53	82	5	5	0	1.0	0	115	88	
2	119	82	37	1.45	31	35	22	13	1.59	37	4	4	0	1	0	68	23	45	2.96	66	12	17	5	17	12	17	-5	0.71	-29	100	80	
3	130	55	75	2.36	58	14	39	-25	0.36	-178	4	0	4	0	4	64	0	64	—	100	11	0	11	0	11	0	11	—	100	72	71	
4	220	114	106	1.93	48	0	30	-30	—	—	0	4	-4	—	—	232	42	190	5.52	82	0	0	0	0	0	0	0	0	—	0	105	97
5	270	71	199	3.80	74	0	16	-16	—	—	0	0	0	—	—	290	32	258	9.06	89	0	0	0	0	0	0	0	0	—	0	107	68
6	144	92	52	1.56	36	10	28	-18	0.35	-180	43	0	43	0	43	127	43	87	3.14	69	5	10	5	10	5	10	-5	0.5	-100	128	88	
Mean	176	80	97	2.29	52	13	26	-13	0.80	-83	10	1	9	1	60	158	28	130	5.24	81	6	5	6	5	6	5	0	0.73	-5	105	82	
±SEM	±24	±9	±24	±0.35	±7	±5	±3	±7	±0.29	±56	±7	±0.8	±7	±25	±37	±6	±33	±1.10	±5	±2	±3	±2	±3	±2	±3	±2	±0.14	±26	±8	±4		
Nephrectomy																																
1	494	282	212	1.75	42	11	11	0	1.0	0	157	107	50	1.46	31	227	66	161	4.19	71	0	0	0	0	0	0	0	—	0	94	68	
2	400	285	105	1.54	26	52	12	40	4.33	76	159	212	-53	0.75	-33	236	63	173	3.70	73	12	0	12	0	12	0	12	—	100	114	110	
3	551	254	297	2.16	53	36	5	31	7.20	86	221	183	38	1.20	17	447	61	386	7.32	86	4	4	4	4	4	4	0	1.0	0	128	99	
4	470	338	122	1.39	28	33	19	14	1.73	42	261	251	10	1.03	4	213	69	144	3.08	68	12	15	3	15	12	15	-3	0.8	-25	110	104	
5	495	465	30	1.07	6	33	33	0	1.0	0	288	338	-50	0.85	-17	205	100	105	2.05	51	7	11	7	11	7	11	-4	0.63	-57	107	104	
6	325	241	84	1.33	25	52	26	26	2.0	50	183	163	20	1.12	11	199	108	91	1.84	46	28	29	28	29	28	29	-1	0.96	-4	142	135	
7	656	534	122	1.23	18	228	243	-15	0.94	-7	129	132	-3	0.98	-2	293	90	203	3.26	69	0	19	19	0	19	19	—	—	99	91		
8	541	393	148	1.37	27	0	0	0	—	0	160	177	-17	0.90	-11	395	206	189	1.92	48	0	8	8	0	8	8	—	—	103	100		
Mean	492	350	140	1.48	28	56	44	12	2.60	31	195	195	0	1.03	0	277	95	182	3.42	64	8	11	8	11	8	11	-3	0.84	2	112	101	
±SEM	±35	±37	±29	±0.12	±5	±25	±29	±7	±0.88	±13	±20	±26	±14	±0.07	±6	±33	±17	±32	±0.63	±5	±3	±4	±3	±4	±3	±4	±3	±0.08	±22	±6	±6	

A peak, 160,000-dalton component; B peak, 9,000-dalton component; C peak, 3,500-dalton component; D peak, 2,000-dalton component.
 * Ext, extraction.

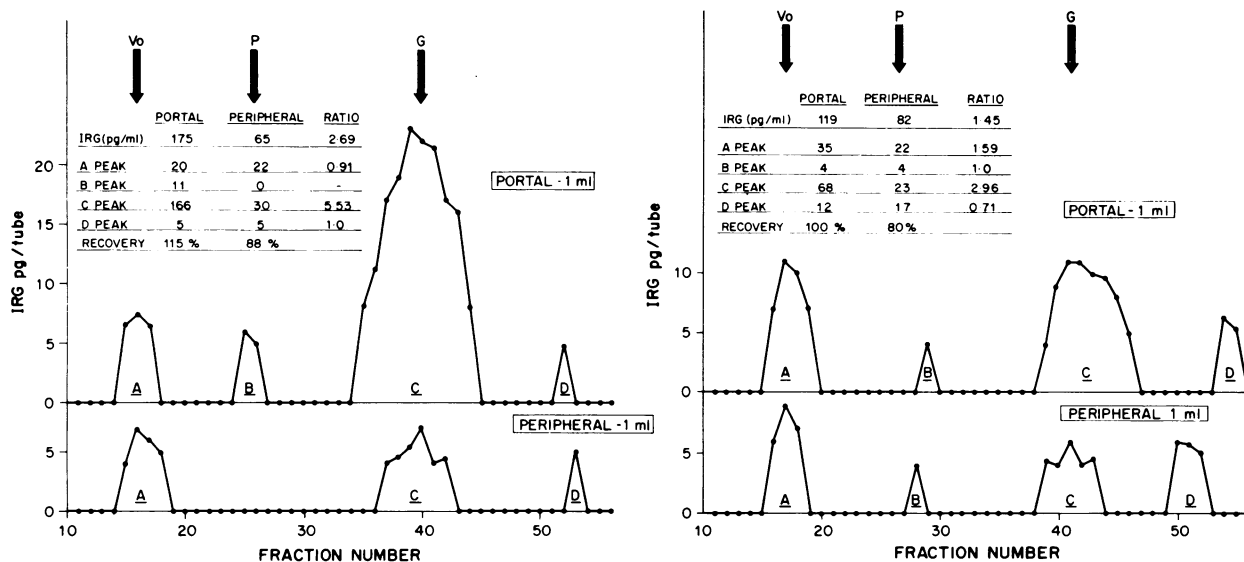


FIGURE 1 Gel filtration profiles of plasma IRG in simultaneously drawn portal and peripheral samples of control rats 1 and 2 of Table II. Vo = void volume (mol wt >40,000); P = bovine proinsulin (mol wt 9,000); G = glucagon (mol wt 3,500). Columns were calibrated with ^{125}I -gamma globulin, ^{131}I -proinsulin, and ^{125}I -glucagon.

differences in the portal and peripheral concentrations of the various IRG components in the rat and dog. The 3,500-dalton fraction is extracted by the liver, while the other three molecular species are not removed to any extent by this organ. Thus, with very low plasma concentrations of 9,000- and 2,000-dalton components, the presence of significant quantities of IRG in the void volume will result in an underestimation of the hepatic extraction of the 3,500-dalton glucagon component, if direct measurements of total plasma IRG are interpreted as being equivalent to the 3,500-dalton hormone. As the concentration of void volume material increases, and it may comprise as much as 80% of plasma IRG in the fasting state, the extent to which the hepatic extraction of 3,500-dalton glucagon will be underestimated will become greater. In situations where there are large amounts of circulating 9,000-dalton material, such as in chronic renal failure and in some patients with diabetes and glucagonomas, measurements of IRG may similarly underestimate hepatic extraction of 3,500-dalton glucagon, particularly if high concentrations of the void volume component coexist (22). These findings may account for the varying conclusions which have been reached with regard to the hepatic extraction of glucagon and, in particular, may provide an explanation for those experiments in which portal-peripheral measurements indicate a small or insignificant extraction of glucagon by the liver (12-14).

A greater hepatic extraction of plasma IRG was noted in fasted rats compared to fed and arginine-

stimulated animals. Although we have not gel-filtered sufficient samples to interpret this finding in terms of the individual IRG components, it seems probable that hepatic extraction may vary depending on the metabolic status of the animal and the absolute concentration of 3,500-dalton glucagon in the portal vein. With regard to the latter possibility, the percentage extraction of the 3,500-dalton material tended to be greatest in those rats, both control and nephrectomized, with the highest absolute portal concentrations of this fraction (Table II, rats 1, 4, and 5 in the control group; rats 3, 7, and 8 in the nephrectomy group, and Fig. 2c). The portal-peripheral difference in 3,500-dalton material in the animal shown in Fig. 2c was 2,272 pg/ml, suggesting that the liver may have a large capacity for removing this component. Similar observations were made with respect to insulin by Kaden et al. (23), who demonstrated an increase in the proportion of insulin extracted by the liver in dogs after intraduodenal glucose administration compared to the fasting state. These authors suggested that the liver may play an important role in regulating peripheral insulin concentrations.

Although there is a correlation between portal and peripheral plasma IRG levels, it is not possible to predict accurately portal vein concentrations of 3,500-molecular weight glucagon on the basis of peripheral IRG concentrations or vice versa. Caution must therefore be shown in equating posthepatic delivery rates with pancreatic secretory rates, particularly when only total peripheral IRG levels are measured.

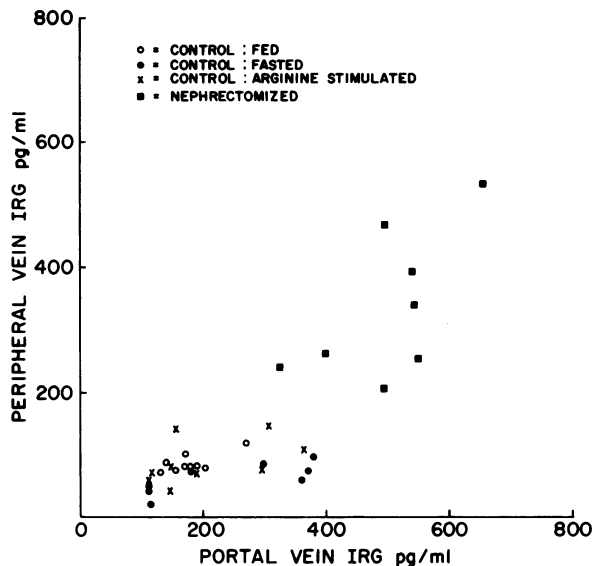
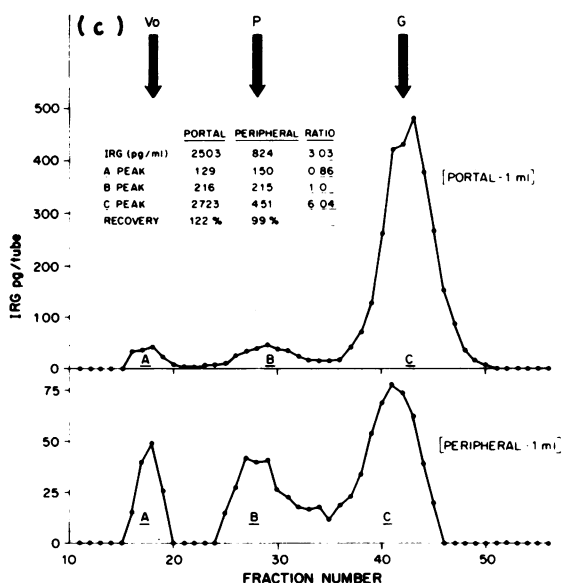
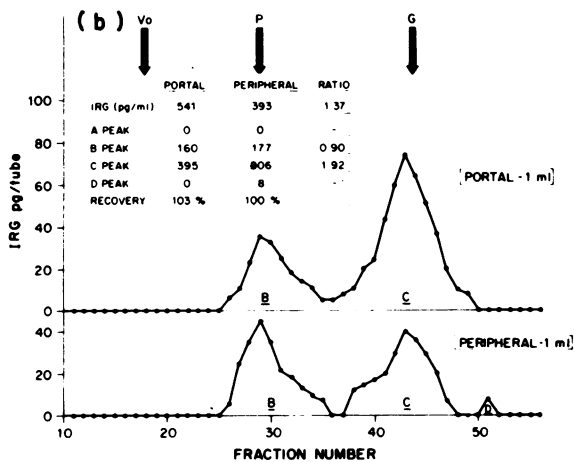
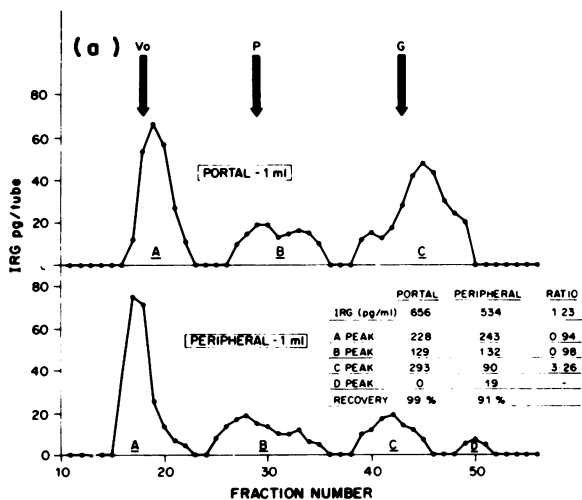


FIGURE 3 Correlation of portal and peripheral vein IRG in control ($n = 26$) and nephrectomized rats ($n = 8$).

The findings in this study, as well as those concerned with the metabolism of the various plasma IRG components by the kidney, closely resemble those described for insulin and proinsulin. These experiments demonstrated that the liver was a major site of insulin metabolism (24), and that the kidney also degraded appreciable amounts of this hormone (25). In contrast, the hepatic extraction of proinsulin was very small, the major degradative organ being the kidney (25, 26). In this regard, Duckworth et al. (27) have postulated that closely related, possibly identical, enzyme systems are responsible for the degradation of insulin and glucagon. Recently a correlation between the levels of circulating proinsulin and proglucagon has been demonstrated in patients in renal failure,² providing further evidence in favor of the closely related metabolic handling of these peptide hormone precursors.

We recognize that the approach used in this study has certain limitations. Measurements made in samples taken from portal and peripheral veins do

² J. B. Jaspan. Unpublished data.

FIGURE 2 Gel filtration profiles of plasma IRG in simultaneously drawn portal and peripheral samples in three rats 24 h after nephrectomy. Fig. 2a and b represent rats no. 7 and 8 of Table II. Fig. 2c shows the profiles in a nephrectomized animal with extremely high IRG values. Because the IRG concentrations in this rat were approximately five times greater than those in the other nephrectomized animals, the results were omitted from the calculations shown in Table II. Columns were calibrated as in Fig. 1.

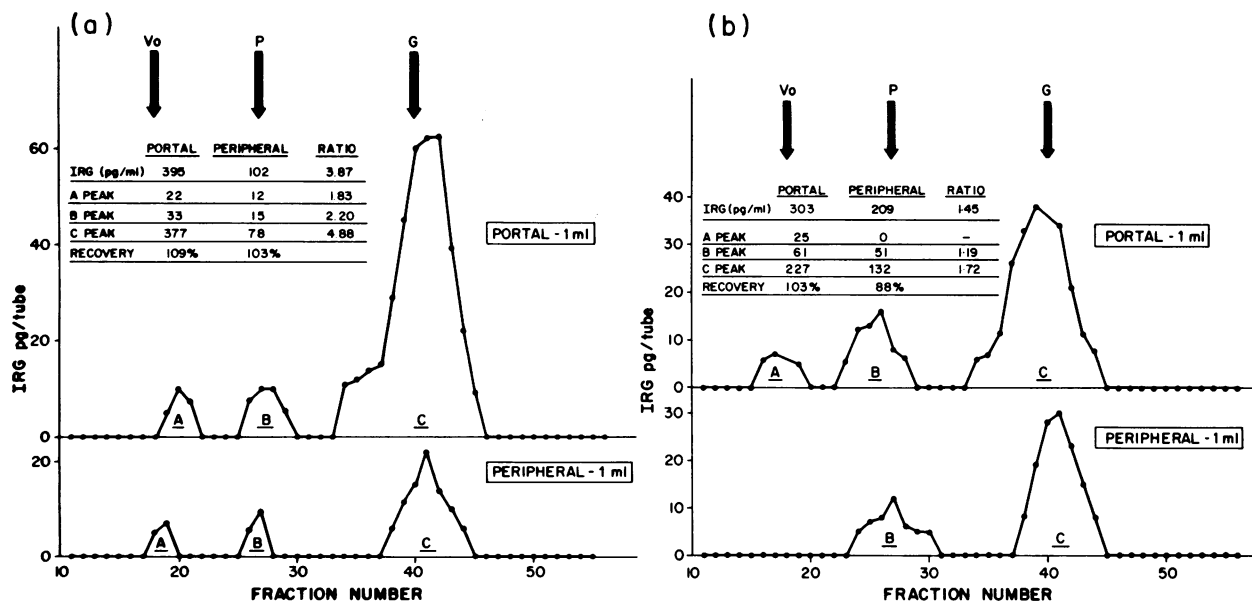


FIGURE 4 Gel filtration profiles of plasma IRG in simultaneously drawn portal and peripheral samples taken from the dog. Samples were taken at 15 min (a) and 40 min (b) after starting arginine infusion. Columns were calibrated as in Fig. 1.

TABLE III
Portal and Peripheral Vein IRG Levels, Portal-Peripheral Ratios, Portal-Peripheral Differences, and Percentage Extraction in a Dog after Arginine Infusion

Time	IRG		Po/Pe	Δ	Extraction
	Portal	Peripheral			
min	pg/ml			pg/ml	%
0	186	99	1.87	87	47
15	395	102	3.87	293	74
30	388	284	1.36	104	27
35	410	271	1.51	139	34
40	303	209	1.44	94	31
60	497	308	1.61	189	38
90	374	260	1.43	114	30
Mean	364	219	1.86	146	40
\pm SEM	\pm 37	\pm 33	\pm 0.32	\pm 28	\pm 6

not provide one with absolute quantitative data regarding the hepatic uptake of a substance. To determine the uptake of the various IRG components by the liver, we will need to measure hepatic blood flow derived from both the portal vein and hepatic artery, estimate the transit time of the hormone through the liver, obtain representative samples from the various hepatic veins, and deal with the problem of streaming in the portal vein. Kaden et al. (23) have succeeded in overcoming many of these difficulties in carrying out this type of experiment in dogs, and we

now plan to pursue this approach. Nevertheless, our results clearly indicate large differences in the concentrations of 3,500-molecular weight glucagon in portal and peripheral plasma samples. The absence of such differences in the concentrations of the 9,000- and 160,000-dalton components measured in the same samples strongly suggests that only the 3,500-molecular weight material is removed by the liver.

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