Absorption of Insulin from Perfused Rabbit Small Intestine in Vitro

Motoaki Shichiri, M.D., Nobuaki Etani, M.D., Ryuzo Kawamori, M.D., Kenkichi Karasaki, M.D., Akira Okada, M.D., Yukio Shigeta, M.D., and Hiroshi Abe, M.D., Osaka, Japan

SUMMARY

The intestinal absorption of insulin has been studied in a preparation of isolated rabbit intestine in which artificial plasma is circulated through normal vascular channels. After insulin in doses of 100, 200, and 440 U. was introduced into the intestinal lumen, its appearance in the venous effluent was measured immunologically during perfusion for two hours.

Insulin gradually appeared in the venous effluent and reached a peak fifty to ninety minutes after the perfusion was started. The proportion of insulin absorbed was 6.2 to 9.2 per cent with a dose of 100 U., 5.9 to 15.6 per cent with 200 U., and 8.9 to 15.9 per cent with 440 U. The amount of insulin added significantly correlated with the total amount of insulin absorbed from the intestine. The biologic activity in the fraction absorbed was equivalent to that obtained immunologically. In the venous effluent, insulin component was also identified by the relative mobility similar to crystalline insulin on acrylamide gel electrophoresis.

Although the intestinal absorption in this system does not duplicate physiologic absorption, these results indicate that a considerable portion of insulin can be absorbed from the intestine in a physiologically active form. DIABETES *22*: 459-65, June, 1973.

In the past, the enteral absorption of insulin has been measured only indirectly by assessing the hypoglycemic response.[1-4] Measuring plasma insulin concentration constitutes a more direct approach. In a previous study,[5] a considerable increase in plasma immunoreactive insulin was observed after administration of insulin into the Thiry-Vella loop of the jejunum of rabbits, into which no pancreatic secretion entered, but the fraction absorbed was considered to be small. It is important, therefore, to assess quantitatively the amount of insulin which can be absorbed from the intestine.

From the First Department of Medicine, Osaka University Medical School, Osaka, Japan.

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The difficulty in assessing the fraction of insulin absorbed from the intestine in vivo has led to the development of alternative in vitro methods. In one such system, segments of intestine are everted so that the mucous membrane lies on the outside.[1,6,7] In the intestinal everted sac, net transport is measured by the movement of insulin across the whole thickness of the small intestinal sac, however, and not into blood vessels or lymphatics. A preparation of isolated intestine in which fluid is circulated through the normal vascular channels[8-10] allows study of the intestinal absorption of insulin in vitro. In this system, when insulin is introduced into the intestinal lumen, it gradually appears in the venous effluent. The fraction of insulin absorbed can then be approximated by measuring insulin concentrations in the fluid immunologically and biologically at regular intervals. The results obtained with this isolated intestinal perfusion experiment are reported in the present paper.

MATERIALS AND METHODS

Preparation of isolated intestinal loop. Postabsorptive male white rabbits weighing 2.0 to 2.5 kg. were anesthetized with intravenous pentobarbital sodium. The abdomen was opened by a midline incision. The ileum about proximal to the ileocecal junction was identified, and a branch of superior mesenteric artery (ileocolic) was exposed.

The artery was then cannulated with a polyethylene catheter with a 1 mm. outer diameter. At the moment of cannulation the artery was gently flushed through with heparinized Ringer's solution. Following this procedure, the vascular perfusion was commenced at the rate of 0.5 ml. per minute from a reservoir of Tyrode-dextran solution oxygenated with 95 per cent oxygen and 5 per cent carbon dioxide at 37° C. The vein was then cannulated with a polyethylene catheter with a 1.5 mm. outer diameter and secured in position with ligatures. All blood vessels not draining the isolated loop were

tied. This procedure allowed the perfusion fluid to replace the natural circulation with no interruption.

The ileal segment, about 15 cm. in length, supplied by the cannulated artery was dissected. The two glass cannulas with 9 mm. inner diameters were inserted into the cut peripheral and proximal ends of the isolated ileum and tied. The ileal lumen was washed out gently with about 40 ml. of Ringer's solution at 37° C. During this preparation, the ileal segment was prevented from drying by covering it with gauze moistened in Ringer's solution.

The ileal segment was then freed from the remaining tissues in the abdomen, lifted, and immersed in 200 ml. Tyrode-dextran solution at 37° C.

Experimental procedure. A schematic diagram of the intestinal perfusion system is shown in figure 1. Adjustments and regulation of the perfusion flow were made with a peristaltic pump (SIP-11, Sharp Co. Ltd., Japan). The perfusion flow was adjusted to 0.5 ml. per minute for the first ten minutes and then increased to 1.0 ml. per minute. When the flow became constant, no further manipulation was necessary.

The isolated intestine was immersed in the Tyrode-dextran solution[11] (pH 7.4, 6 per cent dextran, average molecular weight about 50,000, Pharmacia Laboratories, Inc., New York City). The artificial plasma was Tyrode-dextran solution containing 0.1 per cent glucose, 0.1 per cent bovine serum albumin powder and 1,000 U. heparin. It has an osmolarity of 297 milliosmols per liter. The artificial plasma and the intestinal bathing fluid were warmed at 37° C. and oxygenated by continuously bubbling through them a mixture of 95 per cent oxygen and 5 per cent carbon dioxide (figure 1).

Insulin (Shimizu Chem. Co., Japan) in a dose of 24.5 U. per milligram was dissolved in Tyrode's solution in concentrations of 25, 50, and 100 U. per milliliter, and was introduced into the ileal lumen in a volume of 4 ml.

During the experiments, the venous effluent was collected every ten minutes for two hours and the volume of each sample was recorded. Every thirty minutes, 0.1 ml. of the intestinal lumen fluid and 2 ml. of the intestinal bathing fluid were withdrawn and the amount of insulin transferred through the intestinal wall was measured. All samples were kept frozen (—20° C.) until the time of analysis.

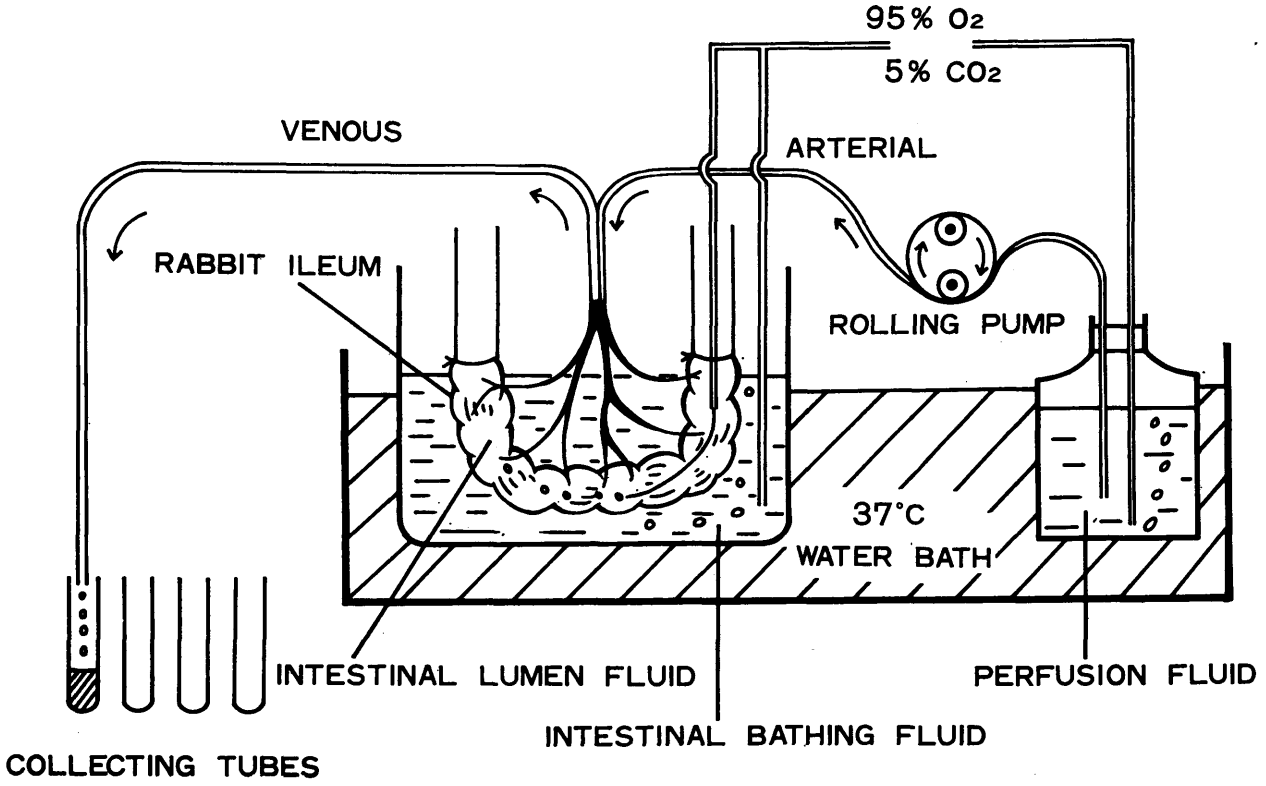

FIG. 1. Schematic diagram of intestinal perfusion method in vitro.

Analytical methods. Polyethylene glycol (PEG), an inert nonabsorbable marker, was used to indicate extent of change of concentration of insulin by movements of water across the intestinal mucosa.[9,12-14] PEG, average molecular weight 4,000 (Wako Chem. Co., Japan), was added to give a final concentration of 120 mg./100 ml. PEG concentration was estimated by the method of Hyden.[15] Insulin concentration was determined by the two-antibody method of Randle and Hales[16] and by a biological assay. For the latter analysis, six male rabbits weighing 2.0 to 2.5 kg. were injected intravenously with the venous effluent at a dose of 0.5 to 1.0 U. per kilogram. The blood glucose lowering effect of samples was compared with that of standard insulin. The blood glucose determination was made by the method of Somogyi-Nelson.[18] The immunological and biological active substance in the venous effluent was identified by the relative mobility on acrylamide gel electrophoresis.[19]

RESULTS

The mean arterial flow was kept constant during the time of intestinal perfusion at the rate of 0.9 to 1.1 ml. per minute. As shown in table 1, in each experiment a considerable portion (up to 90 per cent) of the fluid infused in the artery could be collected from the cannulated vein. In all perfusion experiments, however, there was a net movement of water from the blood vessel into the intestinal lumen. In experiment 6, the ratio of initial to final concentration of PEG was 1.90 (table 1), indicating the greatest water movement into the lumen.

The net water movement was incorporated into the calculation for insulin disappearance from the intestinal lumen. After perfusion for two hours, a mean of 17.2 per cent of the added dose had disappeared from the lumen in the experiment with a 100 U. dose of insulin (group 1). Expressed in this way the mean figures were

TABLE 1

Insulin disappearance from the intestinal lumen after addition of insulin into the isolated perfused intestine

	Exp. No.	Arterial flow	Total fluid infused	Total fluid recovered	PEG_i/PEG_o*	Net water balance†	Insulin added	Initial insulin conc.	Final insulin conc.	Per cent insulin disappearance‡ from intestinal lumen
		(ml./min.)	(ml./120 min.)			(ml.)	(U.)	(U./ml.)	(U./ml.)	
Group 1	1.	0.9	108.0	98.7	1.62	−3.0	100.8	21.0	10.9	15.7
	2.	0.9	108.0	96.3	1.59	−2.6	103.4	23.5	11.9	19.5
	4.	1.0	120.0	111.3	1.54	−2.8	98.4	19.3	10.5	16.3
										(17.2)
Group 2	5.	1.0	120.0	109.4	1.64	−2.9	195.3	43.4	20.0	24.3
	6.	0.9	108.0	98.6	1.90	−3.7	213.2	52.0	24.4	10.8
	7.	1.0	120.0	111.2	1.43	−2.2	192.5	38.5	21.5	20.3
										(18.5)
Group 3	8.	1.0	120.0	105.3	1.62	−2.9	448.9	95.5	51.2	13.1
	9.	0.9	108.0	93.5	1.61	−2.6	465.3	108.2	48.5	27.8
	11.	1.1	132.0	119.3	1.41	−1.8	415.3	92.5	45.3	30.9
										(23.9)

The ileum of rabbit (about 15 cm. in length) was perfused for two hours. Insulin concentration was measured by the method of Randle and Hales.

* Ratio of initial to final concentration of PEG (poylethylene glycol) in the intestinal lumen.

† Calculated from the equation: Net water balance $= [(PEG)\ added/(PEG)_i] \times [1-(PEG)_i/(PEG)_o]$; a minus sign indicates net entry of water into the intestinal lumen.

‡ Per cent insulin disappearance from the intestinal lumen $= 100-100\ [(n)_o/(n)_i] \times [(PEG)_i/(PEG)_o]$, where $(PEG)_i$ = initial conc. of PEG, $(PEG)_o$ = final conc. of PEG, $(n)_i$ = initial conc. of insulin, $(n)_o$ = final conc. of insulin.

Mean of three rabbits in each group is given in parentheses.

TABLE 2

Absorption of insulin from the isolated perfused intestine of rabbit in vitro

	Exp. No.	Dry weight	Insulin added	Insulin concentration in each fraction (mU./ml.)												Insulin absorbed* (U./ 120 min.)	(%)
		(mg.)	(U.)	10′	20′	30′	40′	50′	60′	70′	80′	90′	100′	110′	120′		
Group 1	1.	532	100.8	3	39	125	111	170	40	70	36	87	39	25	23	6.234	6.2
	2.	725	103.4	8	20	67	121	122	250	138	102	105	112	68	65	9.511	9.2
	4.	650	98.4	10	92	146	111	149	116	87	60	46	31	31	19	8.479	8.6
																	(8.0)
Group 2	5.	634	195.3	10	125	180	235	359	804	495	367	430	124	112	73	30.371	15.6
	6.	530	213.2	3	26	39	80	90	120	126	175	312	207	203	188	12.521	5.9
	7.	677	192.5	22	26	51	75	89	135	280	221	170	170	125	100	13.500	7.0
																	(9.5)
Group 3	8.	625	448.9	10	23	46	127	144	222	338	543	465	1,010	650	532	40.171	8.9
	9.	508	465.3	91	165	359	600	470	694	761	1,049	1,511	818	1,310	645	72.294	15.5
	11.	753	415.3	107	281	290	290	485	630	1,372	815	1,040	470	509	320	66.122	15.9
																	(13.4)

The ileum of rabbit (about 15 cm. in length) was perfused for two hours, and the venous effluents were collected every ten minutes.

* Total amount of insulin in the venous effluent: Sum of [(insulin concentration) x (effluent volume) in each fraction]. Mean of three rabbits in each group is given in parentheses.

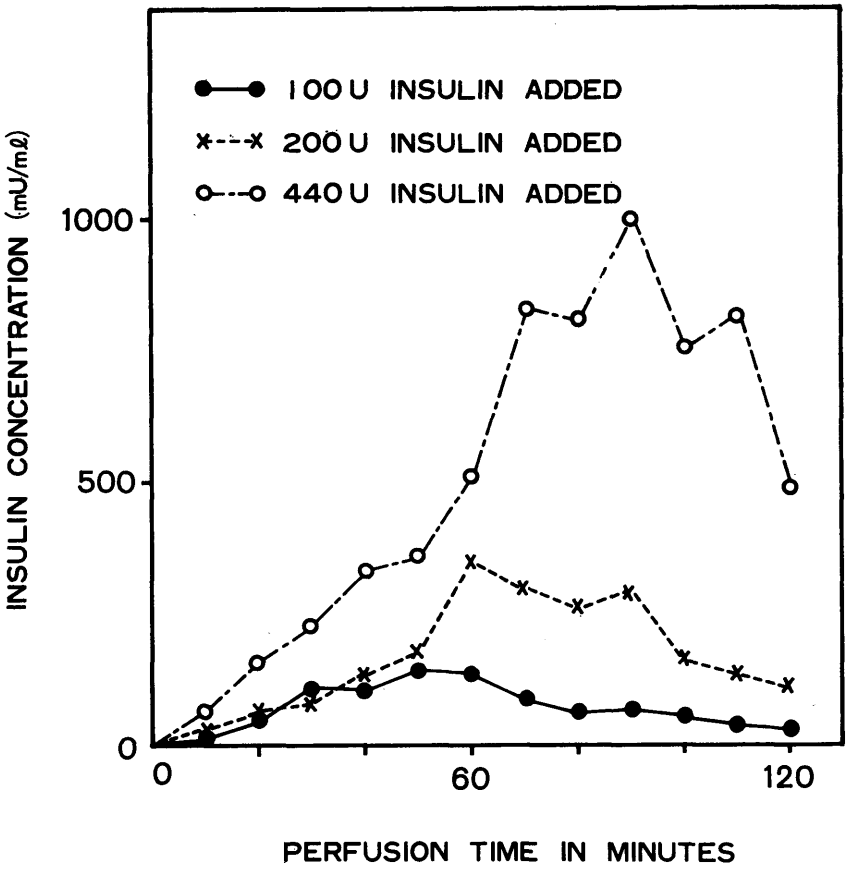

FIGURE 2

The concentration of immunoreactive insulin in the venous effluent after addition of insulin into the isolated perfused intestine. Mean of three rabbits in each group.

18.5 per cent with a dose of 200 U. (group 2) and 23.9 per cent with a dose of 440 U. (group 3).

Table 2 presents the time course of insulin appearance in the effluent and the total amount of insulin absorbed from the intestine. Mean variations of insulin concentrations in the effluent are shown in figure 2. Insulin gradually appeared in the effluent and reached a peak at fifty to ninety minutes after the perfusion was started. Insulin concentration tended gradually to decrease thereafter. With a larger dose, a more delayed absorption peak was observed. In the first group, with a dose of 100 U., an average amount of insulin absorbed was 8.1 U., equivalent to 8.0 per cent of the added dose. In the 200 U. and 440 U. groups, mean values of insulin absorbed were 18.8 and 59.5 U., being 9.5 and 13.4 per cent, respectively. There was a significant correlation between the amount of insulin added and the total amount absorbed from the intestine (figure 3).

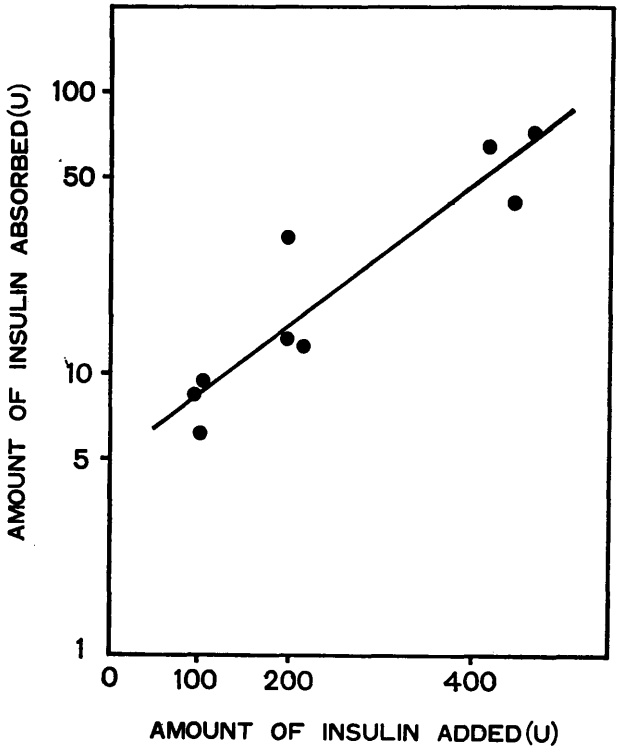

FIG. 3. Effect of different doses of insulin added on the amount of insulin absorbed from the isolated perfused intestine. Statistical analysis shows $r = 0.928$, $P < 0.01$.

The insulin transference through the intestinal wall to the intestinal bathing fluid was very small, corresponding to about 0.05 to 0.09 per cent of the added dose.

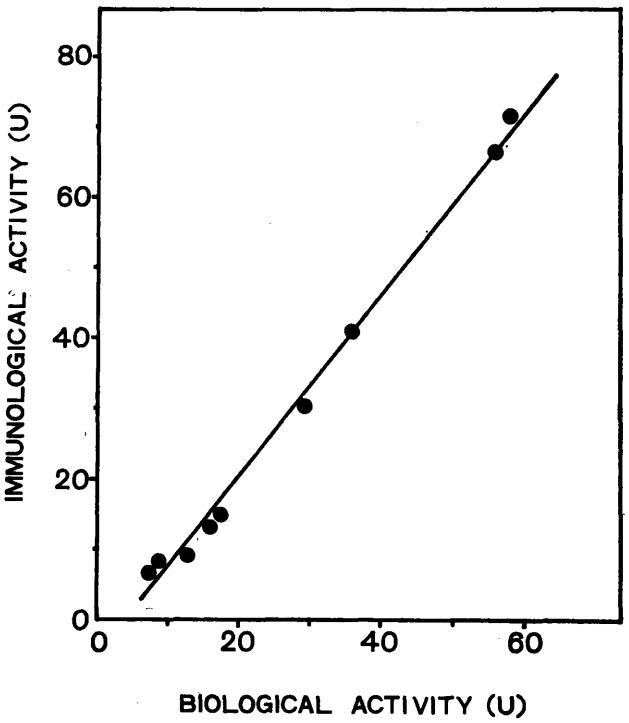

FIG. 4. Comparison of immunologic and biologic activities in the fraction of insulin absorbed from the isolated perfused intestine. Statistical analysis shows $r = 0.996$, $P < 0.01$.

Figure 4 shows the biologic activity of insulin absorbed from the intestine, in comparison with the immunological method. The biologic activity in the fraction absorbed was equivalent to that obtained immunologically.

By the acrylamide gel electrophoresis of the effluent,

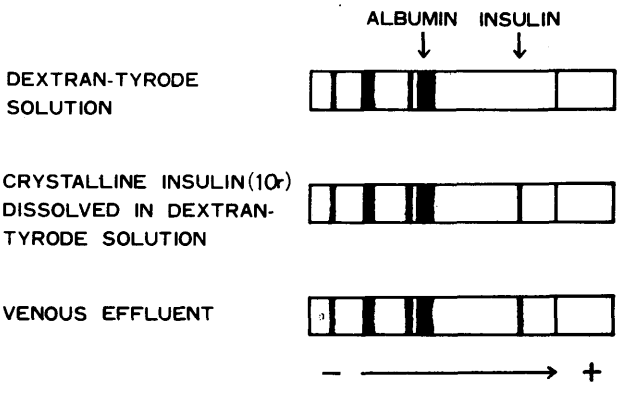

FIG. 5. Schematic representation of acrylamide gel electrophoretic components of the venous effluent. Electrophoresis was carried out on 10.0 per cent polyacrylamide gels (5 mm.) at pH 8.9. Gels were removed from the tubes, and stained with 1 per cent amido schwarz in 7.5 per cent acetic acid.

a component with mobility similar to crystalline insulin was clearly demonstrated (figure 5).

DISCUSSION

The viability of the intestine for the two hour period was assessed by measuring glucose utilization and the active transport of glucose from the intestinal lumen. In those experiments, glucose was taken up at the rate of 0.032 ± 0.002 mg./100 mg. dry weight per minute for as long as two hours. Active transport against the concentration gradient was observed. Peristalsis also was observed during the perfusion. Therefore, the viability of the isolated intestine was considered to be preserved. In the present perfusion experiments, however, there was almost inevitably a net movement of water into the intestinal lumen. As calculated from the ratio of initial to final concentration of PEG, this occurred to the extent of 1.8 to 3.0 ml. (3.7 ml. in experiment 6) during two hour perfusion. Accumulation of fluid in the lumen and in the tissue became significant during the period 90 to 120 minutes after the commencement of perfusion. Mild edema of the intestine also was noted at the end of perfusion.

In the experiments in vivo, between 40 and 250 U. of insulin per kilogram weight are required to produce a predictable degree of hypoglycemia following a single administration of insulin into the gastrointestinal tract.[1-5] Judging from hypoglycemia and the plasma insulin responses in vivo, it has been concluded that a small fraction of insulin (0.3 to 3 per cent) can be absorbed from the intestine in the absence of pancreatic proteolytic enzymes.[2,4,5] In the present intestinal perfusion experiments in vitro with the same doses of insulin as in vivo, on the other hand, a considerable amount of insulin disappeared from the intestinal lumen (10.8 to 30.9 per cent) and was recovered from the venous effluent (5.9 to 15.9 per cent) during the two hour perfusion. In these experiments, the amount of insulin absorbed was directly correlated with the dose of insulin added. The disagreement in the results related to the fraction of insulin absorbed in vitro and in vivo might be due to the differences in the fluid (blood and artificial plasma) circulated through the intestinal vascular bed, in its flow rate, in the peristaltic mobility, and in the enzymatic activity inside the intestine.

The amount of insulin that disappeared from the intestinal lumen exceeded the amount of insulin recovered in the venous effluents. Since the lower molecular weight proteins can enter the blood directly as well as via the lymph,[20,21] the lymph could constitute another route of insulin transport. Pierce et al.[22] demonstrated that the insulin-like activity rose in both lymph and blood after administration of insulin through the cannula placed in the duodenum. In lymph, however, the rise in activity was very small as compared to that in blood. Proteolytic enzymes might be responsible for the destruction of a portion of insulin. When insulin with I-125-insulin as a tracer was incubated in intestinal fluid in vitro, however, only a small quantity of the radioactivity (1 to 2 per cent) was found in the supernatant of the 5 per cent TCA precipitation. It has been established that the intestinal brush border membrane of the epithelial cell is an important digestive surface.[23-25] Ugolev,[26] Goldberg et al.,[27] and Greenberger[25] have demonstrated that pancreatic enzymes are selectively adsorbed on the intestinal mucosa, resulting in "membrane or contact digestion." In the present perfusion experiments, therefore, such surface hydrolytic activity might be quantitatively more responsible for the destruction of a portion of insulin than luminal digestive activity.

On the basis of the present results, we have concluded that a considerable amount of insulin can be absorbed from the intestine in a biologically and immunologically active form. The finding on acrylamide gel electrophoresis supports this conclusion. However, precise information regarding the peak absorption and the rate of insulin absorption could not be obtained, since the concentration of insulin in the intestinal lumen decreased markedly with time. Another technic, in which an insulin solution is allowed to flow through a cannulated intestinal segment with a constant concentration might provide more precise information on the rate of insulin absorption. The questions now arise whether insulin in concentrations considerably lower than those used in the present study can produce the same effect with the same percentages of intestinal absorption and whether insulin is absorbed by simple diffusion or by means of a specialized transport mechanism. Further studies are necessary to elucidate these points and clarify the absorption of the large molecular substances, such as peptide hormone.

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