

## ***A Comparison of the Hypoglycemic Effect of Insulin with Systemic Venous and Portal Venous Administration***

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Among the endocrinopathies, diabetes mellitus occupies a unique position. In Addison's disease, hypothyroidism, hypoparathyroidism, hypopituitarism and other deficiency states, there is a clear correlation between a hormonal deficiency and the pathologic anatomy. In diabetes mellitus, however, this is not the case. The pancreas may be relatively normal from a morphologic point of view<sup>1, 11, 17</sup> except for minor changes which are thought by many to be due to overstimulation of the beta cells.<sup>1, 5, 40, 41, 46</sup>

One explanation for this incongruity could be that insulin is produced normally in diabetics but destroyed in an accelerated manner. Since insulin is elaborated into the portal circulation and must pass through the hepatic vascular bed, considerable attention has been focused on the liver as a site of insulin degradation.<sup>7, 24-27, 38, 43, 44</sup>

The possibility that passage through the liver obtunds or alters effectiveness of insulin has potential surgical significance. If the concept were proved, procedures for diversion of

pancreatic venous drainage into systemic venous channels could be devised, thereby making more insulin available for peripheral tissues.

The present study was undertaken to test the relative effectiveness of physiologic doses of insulin administered into the systemic circulation as compared to administration directly into the liver via the portal vein. Comparison was made of slow, constant insulin infusions alternately administered to a large series of dogs by these two routes. The results were analyzed statistically using an electronic digital computer.

### **METHODS**

A total of 83 experiments were performed in 24 adult, healthy, mongrel dogs, from 12 to 20 kg., on which portacaval transposition had been done two or more months previously. At the time of transposition, all venous tributaries to the vena cava were ligated from the inguinal ligaments to the diaphragm, excepting only the renals.<sup>34</sup> With this preparation, injections or infusions into the vena cava or its tributaries pass into the portal vein and then through the liver.<sup>34</sup> In the present study, such an infusion is termed "intraportal." The hypoglycemic response to intraportal infusions was compared to that obtained with forelimb (systemic) insulin infusions.

The animals were fed a standard kennel ration of commercial dried dog food supplemented with ground meat. Examinations were carried out after withholding food for 12 to 16 hours. In seven dogs, experiments were carried out on successive days, using a randomized order of

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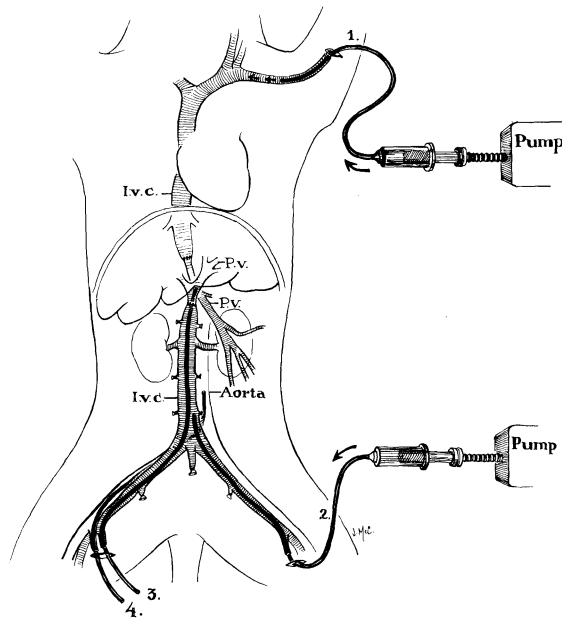


Fig. 1. Experimental preparation showing transposition of the portal vein and inferior vena cava and the positions of the catheters used. 1, Foreleg catheter for systemic insulin infusion. 2, Catheter in side branch of femoral vein for infusion of insulin via inferior vena cava which then leads into portal vein and liver (intraportal route). 3, Sampling catheter introduced through side branch of femoral vein on opposite side and threaded up the vena cava through vascular anastomosis into the portal vein. 4, Sampling catheter introduced into side branch of femoral artery and threaded into aorta.

**infusion route.** Between experiments, the catheters were left in place and protected with specially constructed jackets. After a few days, the animals became febrile, presumably owing to the continuous presence of the catheter. Because of dissatisfaction with prolonged use of

indwelling catheters, these were removed between experiments in the other 17 animals. Response to insulin in this series was observed with alternate routes of administration every five to seven days rather than daily.

Experiments were performed on unanesthetized dogs trained to lie quietly during the experiment. The infusion catheters were inserted into either the vena cava or the forelimb vein (Fig. 1) under local anesthesia.<sup>3,4</sup> Aortic samples were obtained from catheters placed through branches of the brachial or femoral arteries (Fig. 1). In the smaller series, a second sampling catheter was directed up the inferior vena cava into the hilus of the liver for collection of venous samples (Fig. 1). This permitted simultaneous evaluations of both the venous and arterial hypoglycemic curves.

After three or more control samples were taken, glucagon-free insulin\* was administered intravenously. In the first series of dogs, insulin 0.0007 unit/kg./min. was infused constantly for 50 minutes. In these animals, both venous and arterial glucose levels were obtained by sampling every 12 minutes during the insulin infusion. Insulin, 0.0012 unit/kg./min., was also administered in the other 17 dogs as a 50 minute constant infusion. In this group, arterial samples only were taken every 10 minutes.

Blood samples were placed in chilled heparinized test tubes and centrifuged immediately. Plasma glucose was determined with an auto-analyzer,<sup>†</sup> using a manifold which required approximately 0.5 ml. plasma for analysis. The blood loss from sampling, about 30 ml., was replaced either 12 hours before or just after the experiment.

\*Supplied by Dr. W. R. Kirtley, Eli Lilly Co., Indianapolis.

†Technicon Corporation, Chauncey, New York.

Table 1. *T* Values and *P* Values for Control Versus Time Periods in Dogs Receiving 0.0007 Unit of Insulin/kg./min. Minutes are Time after Onset of Insulin Infusion

Group	Degrees of Freedom	<i>t</i>	8 min.*	20 min.*	32 min.*	44 min.*	74 min.	104 min.	148 min.
			Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7
Arterial glucose concentration with systemic insulin infusion	10	<i>t</i>	5.12	9.36	7.25	9.30	4.71	3.48	2.16
		<i>p</i>	0.05%	0.05%	0.05%	0.05%	0.5%	0.5%	5%
Arterial glucose concentration with portal insulin infusion	9	<i>t</i>	3.19	5.77	9.95	12.53	5.12	2.67	1.82
		<i>p</i>	1%	0.05%	0.05%	0.05%	0.05%	2.5%	5%

\*During 50 minute infusion.

Table 2. Significance of Route of Administration of Insulin in Dogs Receiving 0.0007 Unit/kg./min.

Group	Interaction between times	Degrees of freedom	Interaction between treatment	Degrees of freedom	Total interaction	Degrees of freedom
Arterial glucose, F observed values P (systemic vs. portal)	47.115 1%	7/133	0.325 Not significant	1/19	0.530 Not significant	7/133

Table 3. T-Values and P Values for Control Versus Time Periods in Dogs Receiving 0.0012 Unit of Insulin/kg./min., Expressed as Observed Values of Arterial Glucose Concentration (mg./100 ml.) and as a Per cent of Control Values Minutes are Time after Start of Insulin Infusion

Group	Degrees of freedom	6 min.* Time 1	16 min.* Time 2	26 min.* Time 3	36 min.* Time 4	46 min.* Time 5	76 min. Time 6	106 min. Time 7
Arterial glucose concentration, observed values with systemic infusions	30	t p 0.994 15-20%	4.058 0.05%	7.681 0.05%	10.17 0.05%	12.818 0.05%	6.652 0.05%	0.937 15-20%
Arterial glucose concentration, observed values with intraportal infusion	30	t p 2.408 1%	6.295 0.05%	9.740 0.05%	12.609 0.05%	13.013 0.05%	7.487 0.05%	1.630 5%
Arterial glucose concentration as a % of control value with systemic insulin infusion	30	t p 1.114 10-15%	4.165 0.05%	8.453 0.05%	11.264 0.05%	14.61 0.05%	7.735 0.05%	1.020 15%
Arterial glucose concentration as a % of control value with portal insulin infusion	30	t p 2.437 1%	6.311 0.05%	10.196 0.05%	14.255 0.05%	10.824 0.05%	7.591 0.05%	1.724 5%

\*During 50 minute insulin infusion.

Table 4. Significance of Route of Administration of Insulin in Dogs Receiving 0.0012 Unit/kg./min.

Group	Interaction between times	Degrees of freedom	Interaction between treatment	Degrees of freedom	Total interaction	Degrees of freedom
Arterial glucose, F observed values P (systemic vs. portal)	108.28 1%	7/419	0.928 Not significant	1/60	0.319 Not significant	7/419
Arterial glucose as % of control value P	130.2 1%	7/419	1.119 Not significant	1/60	0.417 Not significant	7/419

Two statistical analyses were employed to determine if the response to physiologic doses of insulin administered into the systemic circulation was significantly different from the response to insulin administered into the liver via the intraportal route. The data from each set of experiments were first analyzed to determine means, standard deviations and standard errors. Then a matched, paired comparison was performed between the control data and the data from each sampling time. The resulting *t* values were then examined to determine the degree of probability that the data representing each time period was from the same population as the control data (Tables 1 and 3). The amplitude of the *t* value may also be used to determine the validity of the response. Hence, in this series of experiments, the *t* values for each time period provide an indication of the significance of the hypoglycemia (Tables 1 and 3).

Sets of data, in which only one variable was different, were analyzed using a standard analysis of variance. The Fischer ratios, *F*, for the interaction\* between times, the interaction between treatments, and the total interaction (interaction between times multiplied by interaction between treatments) were determined (Tables 2 and 4).

The interaction between times represents the pooled time interaction of the two sets of data, each representing a different treatment. The Fischer ratio is an indication of the probability that the interaction did not occur. That is, if the interaction between times was significant at the 1 per cent level, there would be only one chance in a hundred that the time interaction could be attributed to chance.

In order to predict that a given treatment was significantly different from the other, it is necessary to examine the interaction between treatments. If the interaction between treatments is significant at the 1 per cent level, then there was only one chance in a hundred that the sample taken from each treatment group was from the same population.

Thus, it may be deduced that although the two given treatment groups vary significantly in time, they may not be significantly different as far as their mode of treatment is concerned. The converse is also true.

The total interaction should also be significant (Tables 2 and 4) if the interaction between times and between treatments is significant. If

the total interaction is found to be significant when both interaction between times and between treatments are not significant, then it is possible that the one curve had an effect that was cancelled by the other (as would be the case when two linear curves with slopes of the opposite sign were compared). If, however, the interaction between times and the interaction between treatments are both significant and the total interaction is not significant, then the results are uninterpretable and the data must be reevaluated.

## RESULTS

### Arterial and Venous Response to Insulin 0.0007 unit/kg./min.

Matched experiments were performed on seven dogs, using alternate routes of administration on successive days. Each dog was used at least once for each route of administration.

The mean control arterial plasma sugar for the portal infusion series was  $94 \pm 3.4$  (S.E.) mg./100 ml. and for the systemic series the average was  $95 \pm 3.3$  (S.E.) mg./100 ml.

With both routes, the maximum arterial hypoglycemia occurred 44 minutes after onset of the insulin infusion (Fig. 2). Blood sugar dropped to  $69 \pm 3.1$  (S.E.) mg./100 ml. with portal, and  $66 \pm 2.4$  (S.E.) mg./100 ml. with systemic infusion. The configuration of the hypoglycemic curves showed a slightly attenuated response with portal infusion (Fig. 2). Analyses of variance were made using both the observed values (Tables 1 and 2) and the values represented as a per cent of their own control arterial plasma glucose concentration. No statistically significant difference between the responses to the two routes of administration was found by either method of computation.

Arterial and venous samples were obtained simultaneously from the femoral artery and the portal vein, just above the caval-portal anastomotic junction (Fig. 1). Venous blood from the hindquarters and kidneys contributes to this sample. Venous plasma glucose fall closely paralleled the arterial response. The difference between arterial and vena caval plasma glucose concentrations averaged  $2.8 \pm .43$  (S.E.) mg./100 ml. in the control period and ranged between  $2.9 \pm .54$  (S.E.) and  $1.3 \pm .54$  (S.E.) mg./100 ml. during insulin infusions. The concentration differences were not significantly different with the two routes of insulin infusion.

\*If two populations are hypothesized to be different, the degree to which they overlap is referred to as interaction.

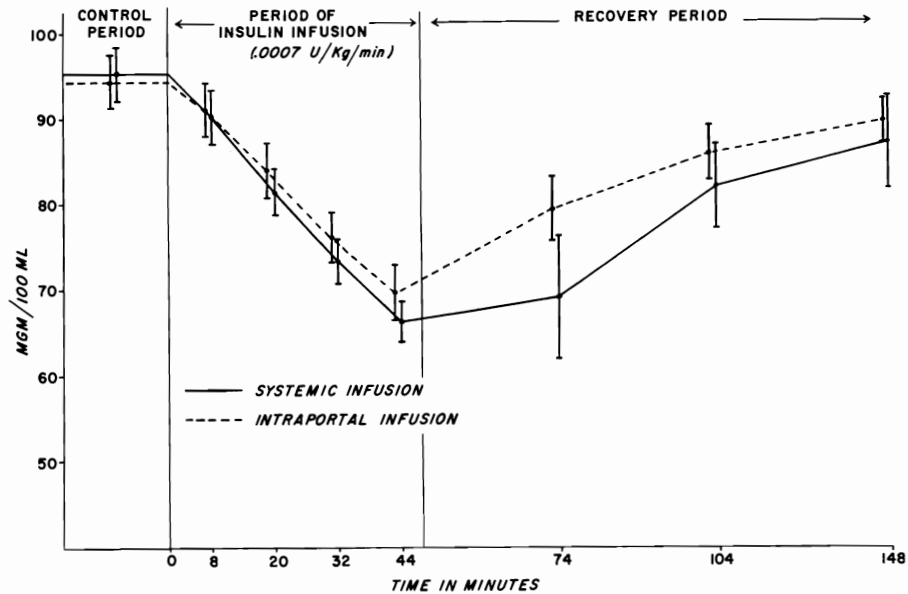


Fig. 2. Effect of insulin infusion (0.0007 unit/kg./min.) upon arterial plasma glucose concentration given by systemic and intraportal routes. Mean values and standard errors are represented by dots and vertical bars respectively. The apparent difference in late effect with the two routes was not statistically significant.

**Arterial Response to Insulin 0.0012 unit/kg./ml.**

Sixty-two paired experiments were performed on 17 dogs; in 31 insulin was infused by systemic route and in 31 by intraportal route. The mean control plasma glucose concentration was 92 mg./100 ml.  $\pm$  1.9 (S.E.) and 93 mg./100 ml.

$\pm$  2.3 (S.E.) before intraportal and systemic insulin administration respectively.

With intraportal infusion, the maximum hypoglycemia was 65 mg./100 ml.  $\pm$  1.7 (S.E.) and occurred 46 minutes after the onset of insulin infusion. With systemic infusion, the lowest plasma glucose concentration was 68 mg./100 ml.  $\pm$  2.3 (S.E.), and also occurred at 46 minutes (Fig. 3).

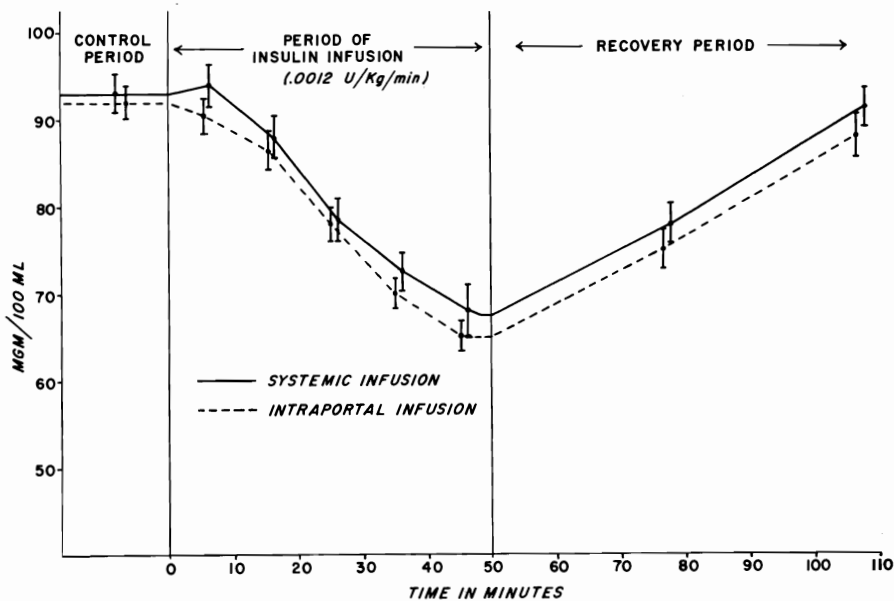


Fig. 3. Effect of insulin infusion (0.0012 unit/kg./min.) upon arterial plasma glucose with systemic compared to portal administration of insulin. Mean values and standard errors are represented by dots and vertical bars respectively. There was no significant difference in effect with the two routes of infusion.

The configuration of the curves was almost identical (Fig. 3). Analyses of variance using both observed values and percentage of control values indicated no significant difference in the effects of insulin given by the two routes of administration (Tables 3 and 4).

### DISCUSSION

For a number of years, the concept has been gaining popularity that the liver moderates or partially controls the effect of insulin by virtue of its capacity for inactivation of the hormone.<sup>23,24,36</sup> Metz and Best have suggested that therapeutically administered insulin may be the "right drug by the wrong route."<sup>23</sup> Stetten<sup>36</sup> postulated the role of the liver as a guardian mechanism shielding the organism from "unwanted hormone." Egdahl and his associates<sup>6</sup> have provided evidence that the liver traps insulin (or insulin-like activity) after glucose infusions and later releases it.

Several considerations have made such a hypothesis attractive. First is the anatomic fact that endogenous insulin is all elaborated into the portal circulation. Reports by Mirsky and others<sup>15,24-27,29,38,39,45</sup> of semi-specific enzyme systems in hepatic parenchyma which inactivated insulin provided a possible degradation mechanism. The later discovery that I<sup>131</sup> insulin is selectively concentrated in liver<sup>7,12,16,19,28,44</sup> could be interpreted along similar lines. Finally, Madison and his associates<sup>18,20,21</sup> have detected differences in the mechanism of hypoglycemia with portal as opposed to systemic injection of insulin.

Essential to confirmation of such a theory of hepatic inactivation is the demonstration of a difference in physiologic effect of insulin infused directly into the liver via the portal vein as opposed to systemic administration. A large but inconsistent bulk of evidence has accumulated on this subject in the last thirty years. About half the investigations have suggested that the two routes of injection result in an identical hypoglycemic response.<sup>2,3,10,14,20,30,31,33</sup> The other half indicate that portal injection is less effective than systemic.<sup>4,8,9,14,22,37,42</sup> The disparate results may be due to differences and difficulties in experimental methods. These include the use of glucagon-contaminated insulin, anesthesia, recent surgery, insufficient numbers of experiments, comparative testing at too short time intervals, testing with large or rapidly given doses, variations in fasting, use of subcutaneous injection techniques and failure to subject results to adequate statistical evaluation.

The experimental protocol followed in the present study was designed to avoid or evaluate these objections. Many of the advantages of the experimental model are due to the use of dogs with transposition. Although the vascular flow is altered in these animals, hepatic blood flow and liver function are essentially normal.<sup>13,32,35</sup> Previous studies have demonstrated that the operation of portacaval transposition does not by itself cause any change in insulin responsiveness.<sup>33</sup>

In earlier studies, it was shown that relatively large and rapidly injected doses of insulin resulted in the same hypoglycemic response, whether given by a transportal or systemic route.<sup>31,33</sup> The present study, employing small doses of insulin with prolonged constant infusion, has yielded similar results. Comparability of effect with the two routes was present in both the arterial and venous samples.

The present study does not provide corroboration for the belief that the liver influences the action of insulin under the conditions and circumstances of these experiments. The data provide little hope that diversion of endogenous insulin into systemic venous channels would enhance its effectiveness. Neither potentiation nor obtundation of hypoglycemic effect was demonstrated with primary passage of insulin through the liver. These findings would appear to be important in evaluating the significance of related research. For example, the selective fixation of I<sup>131</sup> insulin by the liver may not necessarily be equated with a loss of physiologic activity. If hepatic detoxifying mechanisms involving insulinase or other enzymes exist they are not apparent under the circumstances of these experiments.

### SUMMARY

The hyperglycemic effect of insulin by prolonged intraportal and systemic infusion was measured in unanesthetized dogs with a modified portacaval transposition. There was no significant difference in response with the two routes of administration. The relation of these results to research directed to surgical therapy of diabetes is discussed.

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