Passage of Insulin and Inulin Across Vascular Membranes in the Dog

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SUMMARY

The equilibration of IRI and NSILA between arterial plasma and lymph was studied at different sites in sixteen anesthetized dogs. Lymph was collected from the thoracic duct in one group, from the main hepatic lymph vessel in another, and from the leg lymphatic in a third group. No baseline gradients were observed between arterial serum and lymph IRI concentrations. Lymph/serum NSILA concentration ratios were 0.62 ± 0.29 for leg lymph, 0.66 ± 0.20 for thoracic duct lymph and 0.84 ± 0.63 for hepatic lymph. Two tests were carried out in each dog:

(1) IVGTT (0.5 gm./kg.) Evans Blue was injected concurrently in some cases. Net glucose disappearance rate and serum IRI and NSILA patterns were similar in the three groups. Serum IRI passed rapidly into hepatic and thoracic duct lymph reaching plateau levels of 40 and 35 μU. per ml. respectively within 15 min. Leg lymph glucose concentration increased immediately but IRI appearance was delayed: A plateau of 20 μU. per ml. was reached after 30 min. No significant passage of Evans Blue into paw lymph was observed. Glucose injection was followed by a significant decrease in serum NSILA. Hepatic and thoracic duct lymph NSILA levels decreased slightly but NSILA concentration remained unchanged in leg lymph.

(2) One hour after the first test 0.2 U. per kg. of pork insulin and 25 uc ^{14}C-inulin were injected intravenously. The patterns of distribution of these two substances were similar. Both reached peak concentrations first in hepatic lymph then in thoracic duct lymph. In paw lymph their appearance was delayed and the maximum concentration achieved was lower. It is concluded that differences in capillary permeability are likely to determine in part the distribution of IRI and NSILA in body fluids and thus may influence their biological activity. DIABETES *17*:668-72, November, 1968.

The biological activity of a hormone depends on its reaching the cell membrane of the target tissue in effective concentration. Small molecules diffuse easily across vascular endothelium, and their concentrations in plasma and interstitial fluid rapidly reach equilibrium. Plasma proteins, on the other hand, are largely retained in the vascular compartment by the endothelial barrier, and their diffusion into interstitial fluid is influenced by local capillary permeability. Insulin in blood exists as an immunoreactive molecule (IRI) with a minimal molecular weight of 6,000. It is believed to circulate free or only loosely bound to plasma proteins. There is also in blood another entity which has insulin-like effects on isolated tissues in vitro and which is neither immunoassayable nor suppressible with insulin antibody: nonsuppressible insulin-like activity (NSILA). At least one component of this fraction is believed to have a large molecular weight. Experiments were designed to study the distribution of IRI and NSILA in extracellular fluids of the dog and their equilibration between the intravascular and extracellular compartments following a glucose load. In addition, the appearance of pork insulin and ^{14}C-inulin in different lymphatic beds was studied following their intravenous administration.

MATERIAL AND METHODS

Sixteen adult mongrel and bulldogs of both sexes, ranging in weight from 25 to 35 kg., were used. No food was administered after 5 p.m. on the day prior to the experiment. Anesthesia was induced with sodium pentobarbital, 30 mg. per kg. intravenously and additional doses were given as needed. The femoral artery and vein of one leg were cannulated with No. 280 polyethylene tubing. The cannula in the femoral vein was used for the infusion of isotonic sodium chloride (ca. 300 ml./hr.), glucose or insulin-inulin solutions, and the arterial cannula was used to obtain blood samples. Usually one but occasionally two lymphatic ducts in each animal were cannulated as follows: (1) Thoracic duct: Through a longitudinal left supraclavicular incision the duct was isolated, ligated at its junction with the left subclavian vein and a No. 160 or No. 200 polyethylene catheter inserted. (2) Hepatic lymphatic: The abdominal cavity was entered through a midline incision. Close to the liver hilus, the largest visible lymphatic along the portal vein was ligated in the

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proximity of the foramen of Winslow, and a No. 10 polyethylene tubing inserted and fixed with ligatures. The remaining thread-like lymphatics on the ventral and right lateral aspects of the portal vein were ligated. The cannula was brought out through a subcostal extension of the midline abdominal incision. (3) Leg lymphatic: In the lateral midportion of the lower hind leg (contralateral to the one used for placement of femoral cannulas) a longitudinal incision along the external saphenous vein was made. Usually several lymphatics from the paw were seen accompanying the saphenous vein. They were ligated proximally and one of them cannulated distally by means of No. 10 polyethylene tubing.

In some instances lymphatics were cannulated at two different sites in the same animal, however, there was no simultaneous drainage of the thoracic and hepatic lymphatics in the same experiment. Lymph collections consisted of the entire volume obtained by gravity from the catheter. Leg lymph flow was stimulated by gentle massage. Arterial blood samples were obtained at the middle of the lymph collection periods. The surgical procedures lasted usually 90 min. Another hour was thereafter allowed for stabilization prior to the experimental protocols.

Two tests were performed on each dog. First, a 30-sec. infusion of D-glucose, 0.5 gm. per kg., in the form of a 50 per cent solution in water was given. In four instances 25 mg. of Evans Blue dye were injected concurrently. Blood and lymph were sampled every 5 min. for one hour. The second test was initiated one hour after completion of the first by a 30-sec. infusion of 0.2 U. per kg. of crystalline pork insulin and 25 μc of ^{14}C-carboxyl labeled inulin (New England Nuclear Corp.). Blood and lymph were collected at 2.5 and 5-min. intervals respectively during the ensuing hour. All samples were allowed to clot at room temperature for four hours, centrifuged, and the supernatant fluids removed and stored at —20° C. Immunoreactive insulin was measured by a double antibody radioimmunoassay technic[1] in duplicate at a 1:10 dilution, except for serum collected at the 3, 5, and 7 min. following the insulin injections, which were diluted 1:100. Results were expressed in μU. per ml. of crystalline dog insulin for the first test and crystalline pork insulin for the second test. Nonsuppressible insulin-like activity was determined in samples obtained during the first test at a 1:4 dilution with the rat adipose tissue assay in the presence of an excess of anti-insulin serum.[2] Results were expressed in μU. of crystalline dog insulin per ml. of undiluted sample. For determination of radioactivity aliquots of plasma were suspended in a dioxane and CAB-O-SIL (Cabot Corp., Boston, Mass.) mixture containing naphthalene 2.5 diphenyloxazole and p-bis (2-(5-phenyloxazolyl))benzene. Evans Blue was measured by spectrophotometry at 620 mμ after extraction from serum and lymph.[3] Glucose was measured in whole blood or lymph using an AutoAnalyzer technic (Technicon, Chauncy, New York).

RESULTS

Table 1 summarizes the baseline IRI and NSILA levels found in lymph and arterial serum. IRI concentration was similar in lymph and serum except in hepatic lymph where by paired comparison it was 30 per cent higher ($p < 0.05$). On the other hand, the level of NSILA in lymph was lower than in serum at all three sites studied. This difference was statistically significant in paw lymph ($p < 0.05$), of borderline significance in thoracic duct lymph ($0.05 < p < 0.1$) and not significant in the case of hepatic lymph.

TABLE 1

Baseline values of immunoreactive insulin and nonsuppressible insulin-like activity in arterial serum and lymph

Biological fluid	No. of dogs	IRI* μU./ml.	NSILA* μU./ml.
Paw lymph	7	14.0±3.3	200±60
Serum		15.5±2.2	321±56
Thoracic duct lymph	9	16.0±1.2	173±21
Serum		14.8±1.5	262±49
Hepatic lymph	5	24.8±8.2	359±151
Serum		18.8±6.8	425±145

*Mean values ± S.E.M.

Figure 1 depicts the NSILA and IRI patterns in serum and lymph during the intravenous glucose tolerance test. In the three groups of dogs studied, net glucose disappearance rate was similar. The serum IRI pattern was also similar, with an initial peak followed by a progressive decay. On the other hand, the rates of appearance and the maximum level of lymph IRI achieved were higher in hepatic and thoracic lymph than in leg lymph. Equilibration with serum IRI was reached within 10 min. in hepatic lymph, 15 min. in thoracic duct lymph and approximately 30 min. in paw lymph. After equilibration, lymph IRI remained constant at plateau levels of 40 μU. per ml. (hepatic), 35 μU. per ml. (thoracic) and 20 μU. per ml. (paw).

In all dogs studied injection of glucose was followed by a significant reduction in serum NSILA at 5 and 30

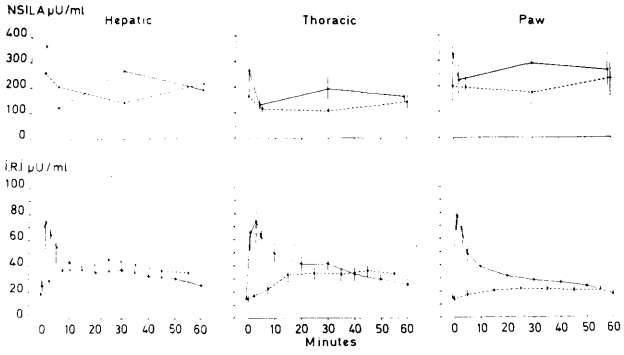

FIG. 1. Levels of NSILA and IRI in serum and lymph following rapid intravenous injection of 0.5 gm. glucose per kg. body weight.
——Arterial serum
------Lymph collected at various sites
Hepatic lymph was studied in five dogs (NSILA in three dogs S.E. not shown), thoracic duct lymph was studied in nine dogs and paw lymph in seven dogs. Figures represent mean values ± S.E.M.

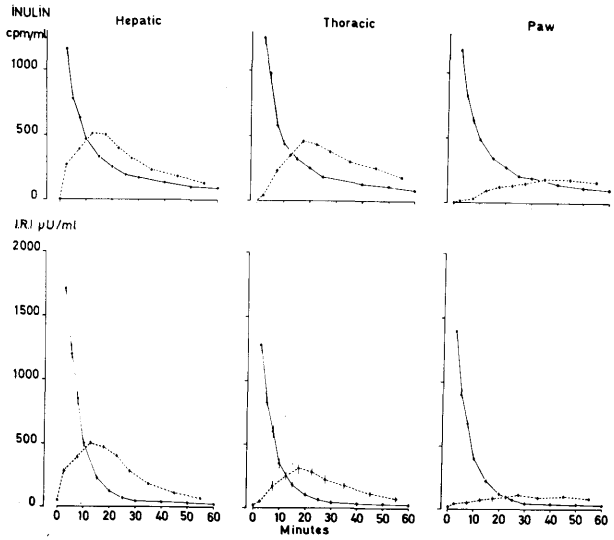

FIG. 2. Levels of radioactive inulin and IRI in serum and lymph following rapid intravenous injection of crystalline pork insulin 0.2 U. per kg. body weight and 25 μc inulin-carboxyl-^{14}C.
——Arterial serum
------Lymph
Hepatic lymph was studied in four dogs, thoracic duct lymph in five dogs and paw lymph in five dogs. Figures represent mean values ± S.E.M.

min. (figure 1). Hepatic lymph NSILA levels also decreased while only a slight drop was observed in NSILA in thoracic duct lymph. NSILA remained unchanged in leg lymph. Table 2, derived from another series of experiments with glucose injections, shows that whereas glucose rapidly diffused from blood to paw lymph, no Evans Blue protein was significantly exchanged between the two compartments.

Figure 2 summarizes data obtained during the second test, i.e., the rapid infusion of 0.2 U. per kg. of crystalline pork insulin together with 25 μC of ^{14}C-labeled inulin. The pattern of distribution of pork insulin and inulin were similar in serum and lymph collected at the three different sites. Both substances first appeared and reached a peak in hepatic lymph and then in thoracic duct lymph. In paw lymph their appearance was delayed and the maximum concentration achieved was lower.

DISCUSSION

In baseline conditions no IRI gradient was found between serum and lymph. The higher level of hepatic lymph IRI was possibly due to partial equilibration with portal serum IRI. Following glucose injection equilibra-

TABLE 2

Glucose and Evans Blue protein in blood and in paw lymph after rapid intravenous injection

	Time in minutes after injection									
Glucose (mg./100 ml.)	0	10	20	30	40	50	60	70	80	90
Blood	90±3	217±9	177±11	156±9	136±9	121±10	107±6	—	—	88±3
Paw lymph	144±4	282±18	311±11	279±15	250±15	221±14	191±15	169±11	156±7	147±5

	Time in minutes after injection				
Evans Blue protein (γ/ml. of serum or lymph)	0	60	120	180	240
Serum	0	8.3±0.6	6.0±0.5	4.5±0.4	3.3±0.4
Paw lymph	0	0.1	0.2	0.2	0.2

Figures represent mean values in four dogs ± S.E.M. At 0 time 0.5 gm. of glucose per kg. body weight and 25 mg. of Evans Blue were injected intravenously.

tion of IRI between serum and thoracic and hepatic lymph was rapid. Within ten minutes, when glucose disappearance had become proportional to its concentration in the extracellular fluid, lymph IRI levels had reached a plateau at twice the pre-injection levels. The increment in leg lymph IRI concentration was less marked and more delayed. In two dogs in which peak levels of serum IRI were low, no significant increase in paw lymph IRI was observed.

Insulin and inulin injected during the second test showed parallel patterns of equilibration across capillary beds. Again, the appearance of these exogenous substances occurred promptly in liver and thoracic duct lymph but was delayed in the leg. The parallel behavior of insulin and inulin is in agreement with previous studies that have shown IRI to be a protein with molecular weight lower than albumin.[4-5] The delayed equilibration of endogenous and exogenous serum IRI and inulin with leg lymph remains to be explained, particularly in light of the relatively rapid equilibration of glucose. A relatively smaller surface area of the vascular bed in relation to the extracellular fluid is not likely in the leg since adipose tissue and muscle are both richly supplied with capillaries.[6,7] Low velocity of lymph flow is also unlikely because Evans Blue injected into the soft tissues of the paw appeared in the leg lymphatic almost immediately in our series (data not presented) as previously reported.[8] In addition, the rapid glucose exchange as just described obviates a physiological "dead space." There are indications that permeability properties of capillaries may differ greatly in different organs. Anatomical studies have shown a higher degree of porosity in sinusoids and fenestrated capillaries of visceral organs than in muscle capillaries.[9] Moreover, the passage of dextran molecules[10] or labeled plasma proteins[11] across the blood lymph barrier was delayed in leg lymph as compared to hepatic, cervical and thoracic duct lymph. Thus, the limited exchange of IRI and inulin across the blood lymph barrier may well be due to low capillary permeability in the paw.

That serum IRI may not easily cross the capillary endothelium in fat and muscle is in keeping with the results of metabolic studies performed in the human forearm. When the intra-arterial injection of insulin transiently increased the serum IRI concentration to levels within those observed after intravenous glucose injection, there was no enhancement of glucose uptake by the forearm.[12-14]

NSILA levels in contrast to IRI are defined by biological assay. Factors capable of diminishing or enhancing this biological effect may, therefore, influence its apparent concentration. NSILA has been found in tissue extracts[15] and increased levels in lymph have been reported in the absence of increased levels in serum following tolbutamide administration to dogs.[16] Consequently, differences in NSILA levels whether static or secondary to glucose administration must be interpreted cautiously. Serum NSILA has been partially purified as an α-β globulin with molecular weight of 100-200,000.[17] In dogs, rats, and humans the distribution of NSILA between serum and extracellular fluid is in agreement with the physical-chemical characteristics of such a molecule. As with other serum proteins, the restriction of transendothelial diffusion is sufficiently great to result in NSILA sieving according to local filtration rates. Lymph/serum concentration ratios of NSILA in this study were 0.62 ± 0.29 for leg lymph, 0.66 ± 0.20 for thoracic duct lymph, and 0.84 ± 0.63 for hepatic lymph. In similarly anesthetized dogs, lymph/serum ratios of serum globulins have been reported to be 0.25, 0.59 and 0.85 respectively.[18] There is good agreement in these two sets of data except in the case of leg lymph where NSILA is considerably higher than would be expected for a serum globulin. Our data do not allow any conclusions regarding the basis for this discrepancy. Changes in permeability of vascular endothelium or release of NSILA from tissue induced by the massage of the paw are possible explanations.

A significant decrease in serum NSILA without a concurrent increase in lymph NSILA was observed following glucose administration. Similar findings have been reported for bound insulin in humans[19] and total ILA in pancreatectomized dogs.[20,21] Since the nature, origin, and fate of NSILA are unknown, the physiological significance of this observation is uncertain. It is concluded that differences in capillary permeability are likely to determine at least in part the distribution of IRI and NSILA in body fluids.

ACKNOWLEDGMENT

This work was supported in part by U.S. Public Health Grants 5 R01-AM-09584-02, R01-AM-11529, and AM-08726. The work of Dr. E. A. Rasio was supported by an International Postdoctoral Fellowship of the U.S. Public Health Service Grant IR05-TW-00289-01.

The authors gratefully acknowledge the helpful advice of Dr. George F. Cahill, Jr. in the course of this investigation and during the preparation of the manuscript. They are also indebted to Mrs. Dzidra Rumba for her valuable technical assistance.

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Plasma Growth Hormone in Childhood

(*Continued from page* 667)

only four exceptions, all had fasting plasma growth hormone levels of 1 ng. or less. One of the four had a fasting level of 3.4 ng., and a rise to 5 ng. in response to hypoglycemia; the others were below this. Another child exhibited a maximum value of 4.3 ng.; otherwise the responses were uniformly blunted, distinctly differing from the normal group, in that the maximum plasma level was less than 2.5 ng. per milliliter. Thus there is almost no overlap in plasma growth hormone levels between the normal child and the child with hypopituitarism.

Quite a different story emerges from data on children with other types of growth failure. These included instances of genetic short stature, primordial dwarfism, delayed adolescence, the maternal deprivation syndrome, Turner's syndrome, hypothyroidism, and growth retardation secondary to glucocorticoid administration. Plasma growth hormone levels were normal in almost all of these subjects, and the average maximum level during hypoglycemia was similar to that observed in normal children. Two of the subjects who had low levels subsequently responded in a satisfactory manner to arginine infusion. Three of the poor responders were children with primary hypothyroidism.

Five of the six children with Down's syndrome achieved maximum plasma growth hormone levels of 34 ng. per milliliter or more; hence the magnitude of the response exceeded that of the normal child. No explanation is at hand for this finding.

From *Nutrition Reviews*, Vol. 26, No. 8, August 1968, pp. 242-43