

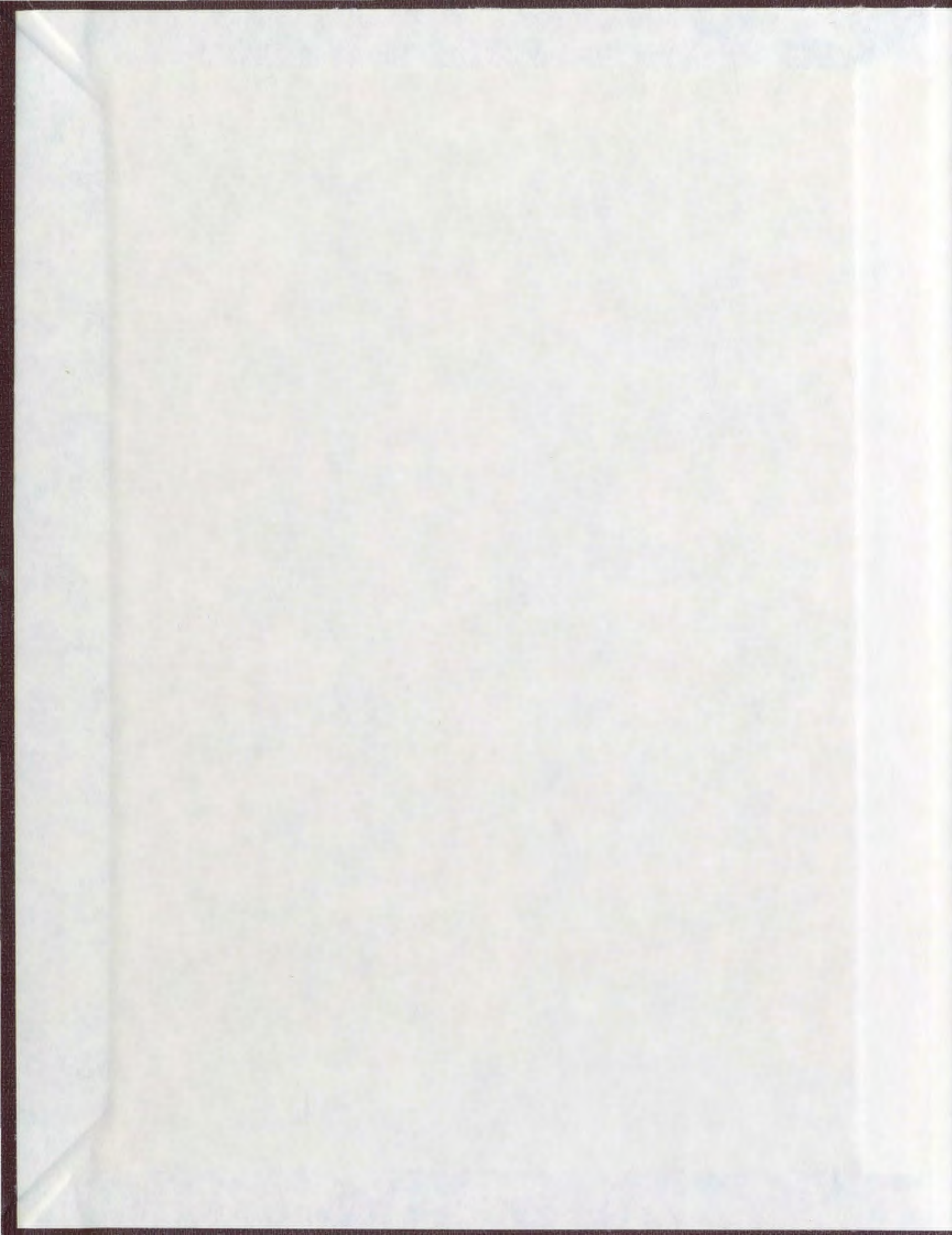
**HORMONAL FACTORS INFLUENCING
INTESTINAL LYMPH FLOW**

CENTRE FOR NEWFOUNDLAND STUDIES

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HORMONAL FACTORS INFLUENCING INTESTINAL LYMPH FLOW

by

©Catherine Jo-Anne Lawrence, B.Sc.

A Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Faculty of Medicine
Memorial University of Newfoundland
March 1980

St. John's

Newfoundland

"11"

ABSTRACT

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Splanchnic blood flow is known to increase in the postprandial state though the mechanisms are unclear. Gut hormones are probably at least partially responsible. Intestinal lymph is mainly derived from the splanchnic blood via capillary filtration. As alterations in the splanchnic microcirculation are likely to be associated with alterations in intestinal lymph flow this study was devised to examine the effects of two hormones, namely secretin and vasoactive intestinal polypeptide, on intestinal lymph flow and composition.

Conscious restrained male Sprague-Dawley rats with intestinal lymphatic fistulae, duodenal cannulae, and tail-vein cannulae were studied.

Natural secretin at doses from 0.25 - 5 U/ml and a saline control were administered intravenously in ten animals. Lymph flow, protein concentration and output were determined. To exclude the possibility of a vasoactive contaminant causing the observed effects, three rats were studied similarly with synthetic secretin.

Analysis of the results gave polynomial curves with 95% confidence bands for each set of data. Comparison of the equations between natural and synthetic secretin for lymph flow and protein output showed no significant difference ($P > .01$). These results indicate that secretin is a powerful stimulant of intestinal lymph flow and protein output in the conscious rat. However, the mechanism by which secretin produced these effects is unclear.

A steady-state of enhanced lymph flow was established. Protein concentration of successive lymph samples taken throughout the infusion showed no measurable change. Examination of gradient polyacrylamide

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electrophoretic gels showed no evidence of change in the protein pattern as a result of alterations in lymph flow suggesting that secretin does not alter capillary permeability. It is proposed that secretin may open up previously non-perfused vessels.

Vasoactive intestinal polypeptide was studied in three animals in which 0.1 μ g, 0.5 μ g and a saline control were administered. Unlike secretin, no alteration in intestinal lymph flow or protein output was observed.

In conclusion, while natural and synthetic secretin are potent intestinal lymphagogues, vasoactive intestinal polypeptide seems to have no intestinal lymphagogue effect.

ACKNOWLEDGEMENTS

My gratitude is expressed to my supervisor Dr. J. A. Barrowman for his guidance throughout this work. His encouragement and expertise helped to give me the motivation and peace of mind needed.

Dr. K. B. Roberts and Dr. C. R. Triggler, the other members of my supervisory committee also have my thanks for their able direction. I would like to thank Dr. D. Bryant for the benefit of his statistical knowledge and Dr. R. Payne for his advice regarding electrophoresis. To the Audio Visual staff, - Carmel Murphy, Eugene Ryan, Gareth Gauthier, and in particular Clifford George goes my appreciation for their cooperation in the production of certain facets of this work. Also, thanks go to Jennifer Dawe for typing this thesis.

Finally, I thank my husband, John and my family for their love and support throughout.

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INTRODUCTION

Earnest Henry Starling (Fig. 1) a nineteenth century English physiologist made two important contributions to the science which are central to the substance of this thesis.

In 1896 he proposed a theory which was known as "Starling's Hypothesis" but which can now be called "Starling's Principle" since it has received extensive experimental substantiation. This Principle explains the forces which control fluid movement across the capillary wall. Starling maintained that the direction and rate of fluid transfer was proportional to the algebraic sum of the effective hydrostatic pressure in the capillaries and the osmotic pressure of the plasma proteins, (Zoffey, 1970).

The principal involves three extracellular fluids: blood plasma, interstitial fluid, and lymph. Blood flowing through the blood capillaries filters out into the interstitial fluid. This filtration occurs because of Starling's Principle and the physical properties of the capillary wall. The capillary wall, although permeable to crystalloids, is relatively impermeable to larger plasma protein molecules; thus these proteins cannot be absorbed back into the blood capillary.

One could describe the lymphatic system as an auxillary return route for the cardiovascular system. The lymphatic system commences with blind-end initial lymphatic capillaries whose functional microanatomy makes lymph formation possible. Lymph is a derivative of the interstitial fluid, formed from it by forces which can only be speculated upon. It is interesting that the closer a lymphatic vessel is to a capillary vessel the more likely the lymph in it will resemble

Figure 1

E.H. Starling (1866-1927; British physiologist and eminent teacher).



the capillary filtrate, (Clark & Clark, 1937).

Realizing the relationship between the three extracellular fluids, experiments can be done using lymph flow as an indirect measure of blood flow in any particular region with its composition reflecting the composition of interstitial fluid in that region. In a general way, alterations in blood flow are likely to be reflected as alterations in lymph flow.

The second contribution of Starling which I wish to refer to is his work with W.M. Bayliss on secretin. This work initiated the science of Endocrinology.

On January 16th, 1902, they denervated a jejunal loop of an anaesthetized dog, applied a small amount of weak hydrochloric acid, and observed an increased flow of pancreatic juice. Realizing that this must be a chemical reflex they called the chemical mediator "secretin". Starling introduced the Greek word hormone ($\sigma\eta\mu\epsilon\rho\omega$) meaning 'I arouse to activity'; a word classifying all bloodborne chemical messengers.

The mucosa of the gastrointestinal tract is the largest endocrine organ in the body with a large mass of endocrine cells scattered throughout it. Although secretin was discovered in 1902, half a century elapsed before the importance of gastrointestinal hormones was realized. Presently, enormous advances have occurred in the understanding of the endocrine system due to the isolation and purification of gastrointestinal hormones and to the introduction of new immunological methods.

There are two groups of gastrointestinal hormones, those with

true hormone status including secretin, cholecystokinin, gastrin, and possibly gastric inhibitory polypeptide and those which are putative or "candidate" hormones including vasoactive intestinal polypeptide, motilin, substance P, and somatostatin. A hormone must fulfill four criteria to achieve true hormonal status, i.e.;

1) one must demonstrate that a physiological stimulus applied to one part of the gut changes the activity in another part. A physiological stimulus being one which would be normally present during the ingestion and digestion of a meal;

2) the effect must persist after all nervous connections between the two parts are interrupted;

3) one must isolate a substance from the site of application of the stimulus which when injected into the bloodstream mimics the effect of the stimulus;

4) one must identify the substance chemically and confirm its structure by synthesis.

It has been shown that the gastrointestinal hormones exert a wide spectrum of effects and play a major part in the control of all gastrointestinal physiology. The problem now is to discern which effects are physiological.

One area of research involves studying the effect of gastrointestinal hormones upon the splanchnic circulation. Splanchnic blood flow accounts for more than one fourth of the cardiac output, yet the mechanism controlling postprandial hyperemia is poorly understood.

Postprandial hyperemia was first described by Herrick (1934). Using thermostromuhr's in dogs he showed increases in superior mesenteric

arterial blood flow postprandially. Reininger (1957) using anaesthetized rats confirmed the work of Herrick. Later, Brandt (1968) and Vatner (1970) again confirmed the existence of postprandial hyperemia.

Although the phenomenon is not disputed, the ill-understood mechanism is. It has been proposed to be neural, mechanical, and humoral; most probably a combination of all three. Biber et al., (1973) proposed that the vasodilation depends partly upon a release of intestinal hormones and partly upon local mechanisms evoked by mechanical and possibly chemical stimulation of the intestinal mucosa. Thus, the contribution of each mechanism will have to be studied.

The humoral role has been attributed to many gastrointestinal hormones including secretin (Ross, 1970; Fara et al., 1972); cholecystokinin (Fara et al., 1973); gastrin (Bowen et al., 1975); vasoactive intestinal polypeptide (Said et al., 1970); and gastric inhibitory polypeptide (Fara, 1978). All, excluding vasoactive intestinal polypeptide, are known to be powerful and rather selective splanchnic vasodilators. The vasomotor properties of vasoactive intestinal polypeptide are less selective (Thulin, et al., 1973).

This study is concerned with two of these gastrointestinal hormones, secretin and vasoactive intestinal polypeptide.

Ross (1970) showed that secretin is a splanchnic vasodilator. He demonstrated that in the cat intravenous infusions of small doses of secretin increased superior mesenteric arterial flow without altering systemic pressure. In 1972, Fara et al., implicated secretin as well as cholecystokinin in the hyperemia occurring in the gut following a meal. They showed that intraduodenal agents mediated the mesenteric

vascular response through the release of gastrointestinal hormones.

Fara et al., (1975) showed that secretin not only increases superior mesenteric arterial flow but redistributes blood away from the jejunal mucosa to the submucosa.

Vasoactive intestinal polypeptide was discovered by Said and Mutt in 1970, so named because of its potent vasodilator and hypotensive actions. They showed that local injections increased superior mesenteric arterial flow by thirty-four percent. However, Thulin and Olsson (1973), showed that in the dog vasoactive intestinal polypeptide had virtually no effect on the superior mesenteric arterial flow. This was confirmed by Coupar (1976) who showed that a continuous infusion of vasoactive intestinal polypeptide did not significantly affect the mesenteric vascular bed of the rat, even though the amounts infused were biologically active. Instead, vasoactive intestinal polypeptide caused a large fluid secretion into the lumen of the jejunum; another important physiological action of the hormone.

If a gastrointestinal hormone is to be involved in postprandial hyperemia, then it must be released in the period following a meal. Evidence of postprandial release of secretin can easily be shown by bioassay methods even though the radioimmunoassay methods that have been reported to date have had difficulty in consistently demonstrating this release. However, Chey et al., (1977) showed a significant increase in plasma secretin concentrations of normal healthy human subjects as well as in patients with duodenal ulcer disease following a meal.

The postprandial release of vasoactive intestinal polypeptide has been studied to a lesser extent with conflicting results as to whether

vasoactive intestinal polypeptide is released following a meal. A recent study by Burhol et al., (1979) showed a late but significant increase in plasma vasoactive intestinal polypeptide after the ingestion of a test meal.

Few experiments have looked at the effect of gastrointestinal hormones on lymph flow. In the rat both cholecystokinin and glucagon were shown to be intestinal lymphagogues (Turner et al., 1977; Barrowman et al., 1978a & b). However, the effect of secretin and vasoactive intestinal polypeptide as intestinal lymphagogues has not been studied.

Razin et al., (1962) demonstrated that impure preparations of secretin and cholecystokinin enhances thoracic lymph duct flow in anaesthetized dogs, while Granger et al., (1979) showed that vasoactive intestinal polypeptide decreases the lymph flow response of the cat ileum.

The present study was devised to examine the effect of two gastrointestinal hormones, secretin and vasoactive intestinal polypeptide, on intestinal lymph flow and protein output in the rat. As previously discussed, alterations in lymph flow should reflect alterations in blood flow; in particular here alterations in intestinal lymph flow should reflect alterations in intestinal blood flow. It is hoped that there will be some insight into whether the vasodilatory properties of secretin and vasoactive intestinal polypeptide are physiologically important in the digestive process, particularly in postprandial hyperemia.

MATERIALS AND METHODS

ANIMALS

Experiments were done on male Sprague-Dawley rats weighing between 200 - 300 grams. The rats were chosen randomly from the Animal House at the Health Sciences Complex, Memorial University of Newfoundland, St. John's, Newfoundland.

Until the time of the experiment these animals were maintained on an animal diet of Rat Chow #5012, Ralston Purina Company.

ANAESTHESIA

Material

Ether anhydrous, Fisher Scientific.

Method

A large glass jar is used for inducing anaesthesia in the rats. Ether is applied to gauze pads, attached to the cover of the jar, and left for thirty seconds allowing vapors to build up in the jar. Then, the rat is placed in the jar for one to three minutes, until anaesthesia occurs. A limp body and tail are signs of anaesthesia.

Anaesthesia is maintained by a face mask containing ether; a small beaker containing gauze pads is soaked in ether. The beaker is placed over the nose of the rat periodically during the procedure. The effect of ether wears off three to five minutes post-surgery.

SURGERY

Laparotomy

Material

small animal clipper with head size 10, Canlab
scalpel, Bard-Parker Scalpels, #10

Method

The fur on the ventral portion from the xyphisternum down is removed with the clippers. Fur is also removed from the right side stab wound area.

A mid-line incision along the skin is made for one and one half to two inches from the xyphisternum using a scalpel. Blunt-ended scissors is used to cut through the abdominal muscle layers.

Surgical Procedures

Material

a) Lymphatic fistula:

PE 50 intramedic polyethylene tubing (I.D. .58mm (.025"), O.D. .965mm (.038")), Clay Adams.

Yale 25 gauge needle, Becton Dickinson

Yale 16 gauge needle, Becton Dickinson

Plastipak 3cc syringe, Becton Dickinson

Heparin (1000 U/ml), Hepalean, Harris Laboratories.

The PE 50 tubing is fed through the gauge 16 needle and bevelled at the end. The syringe is filled with heparin and the gauge 25 needle is attached to it and then inserted into the PE 50 tubing which is subsequently filled with heparin.

b) Duodenal fistula:

Plastipak 3cc syringe, Becton Dickinson

Bardic premature infant feeding tube, 5 French, 15 inches, C.R.

Bond (Canada) Ltd.

The syringe is filled with 0.85% sodium chloride and attached to the infant feeding tube.

c) Adhesive:

isobutyl-2-cyanoacrylate monomer, Ethicon Ltd., Edinburgh

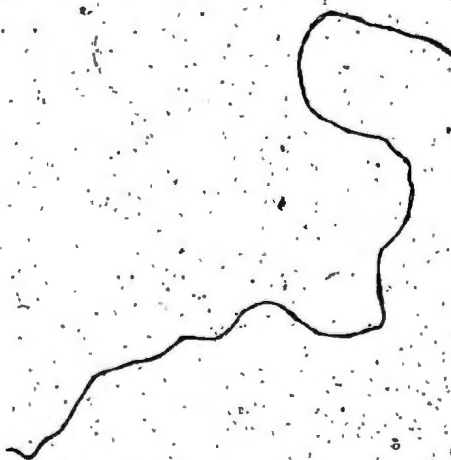
A Pasteur pipette is dipped into the adhesive which is blown onto the desired area.

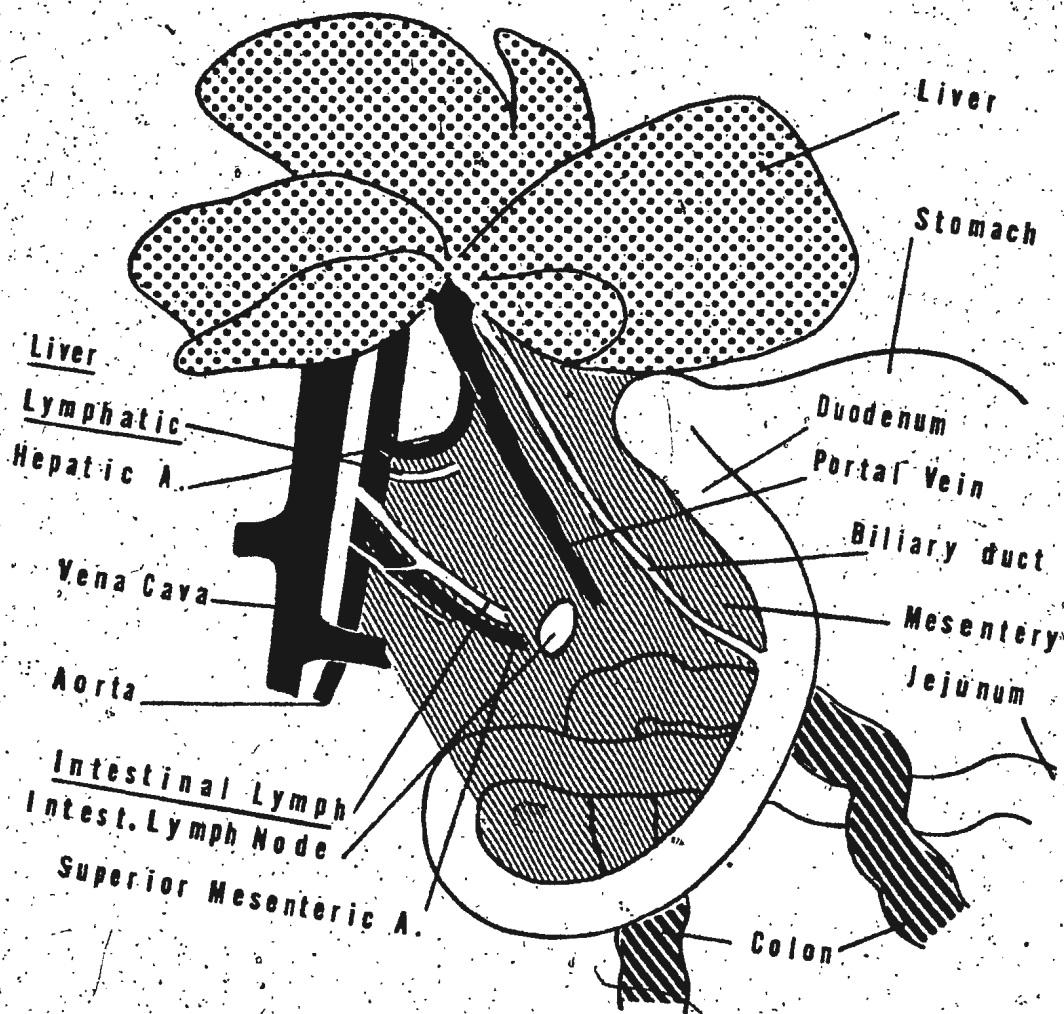
Method

The duodenum is reflected to the left exposing the relevant anatomy (Fig. 2). The superior mesenteric artery branches off the aorta. Above and below it are the main intestinal lymphatic vessel and an accessory vessel, respectively.

Figure 2 Relevant Anatomy

Note the anatomical relationship between the superior mesenteric artery and the main intestinal lymphatic vessel.





A stab wound is made into the right flank of the rat with a 16 gauge needle and the heparized PE 50 is fed through it. Forceps placed under the vena cava and the right kidney pull the tubing next to the intestinal lymphatic vessel thus the tube is now aligned with the lymphatic vessel.

The peritoneum covering the lymphatics is gently teased away with a fine pointed glass probe. This exposes the vessel which is slightly bluish-white in color.

A small incision is made in the main intestinal lymphatic and the PE 50 tubing inserted for approximately 3 mm. A drop of isobutyl-2-cyanoacrylate monomer secures the tubing in place. This material polymerizes rapidly on contact with moisture to form a hardened layer. It should be noted that only a drop be applied to the area since a thin layer results in a firmer bond than a thicker layer.

The isobutyl-2-cyanoacrylate monomer is also used to interrupt flow through the accessory lymphatic vessel and to secure the cannula at the site of the stab wound.

Another incision is made in the duodenum about 1 cm from the pyloric sphincter. The infant feeding tube is introduced for 2 cm through the incision in a caudal direction. It is secured with a drop of isobutyl-2-cyanoacrylate monomer, and exits through the abdominal wound.

Closing the wound

Material

nonabsorbable surgical suture U.S.P. (4-0), Ethicon Inc.

Method

The muscle layers are sutured together with a continuous stitch.

Then the skin is sutured together with interrupted reef knots.

Tail-vein

Material

PE 10, intramedic polyethylene tubing (I.D. .28mm (.011"), O.D. .66mm (.024")), Clay Adams.

Yale 20 gauge needle, Becton Dickinson

Yale 30 gauge needle, Becton Dickinson

Plastipak 3cc syringe, Becton Dickinson

0.85% sodium chloride, Fisher Scientific

The PE 10 tubing is fed through the gauge 20 needle and bevelled at the end. A 3cc syringe which is half-filled with 0.85% sodium chloride and attached to a gauge 30 needle is inserted into the tubing.

Method

As soon as the abdominal incision is closed the tail-vein is cannulated. A tourniquet is applied around the tail and the gauge 20 needle is put into one of the three tail-veins. This is done by inserting the needle in between two of the scales on the rat tail skin. The PE 10 tubing is pushed into the vein through the needle. The needle is withdrawn, the tourniquet released and the tubing secured with isobutyl-2-cyanoacrylate monomer. To keep the tubing patent 0.85% sodium chloride is infused.

POST-OPERATIVE CARE

Material

a) restraint cage

Bollman-type restraint cage

b) pumps

Harvard Parallel/Reciprocal Infusion/Withdrawal Pump (Series 940), Harvard Apparatus Company, Inc.

Harvard compact infusion pump (Series 975), Harvard Apparatus Company, Inc.

c) syringes

plastipak 5cc syringe, Becton Dickinson

plastipak 10cc syringe, Becton Dickinson

plastipak 50cc syringe, Becton Dickinson

Method

The rat is placed in the restraint cage (Fig. 3) with the tail, along with the cannula, passing through a hole in the back. Steel rods are placed around the rat limiting its activity. Endpieces are placed at both ends of the cage to prevent the rat from displacing the steel rods.

To further limit activity thread ties are made. Thread through both the skin of the nape of the neck and the lower dorsal area of the rat is tied to the top two steel rods.

The animal is placed in a warm room (28°C), covered by a cloth and given free access to water. It is left there for at least 18 hours to recover from the surgery.

Throughout the post-operative period the animal receives 0.85% sodium chloride infusion. Two types of Harvard infusion pumps are used. The syringes are filled with 0.85% sodium chloride.

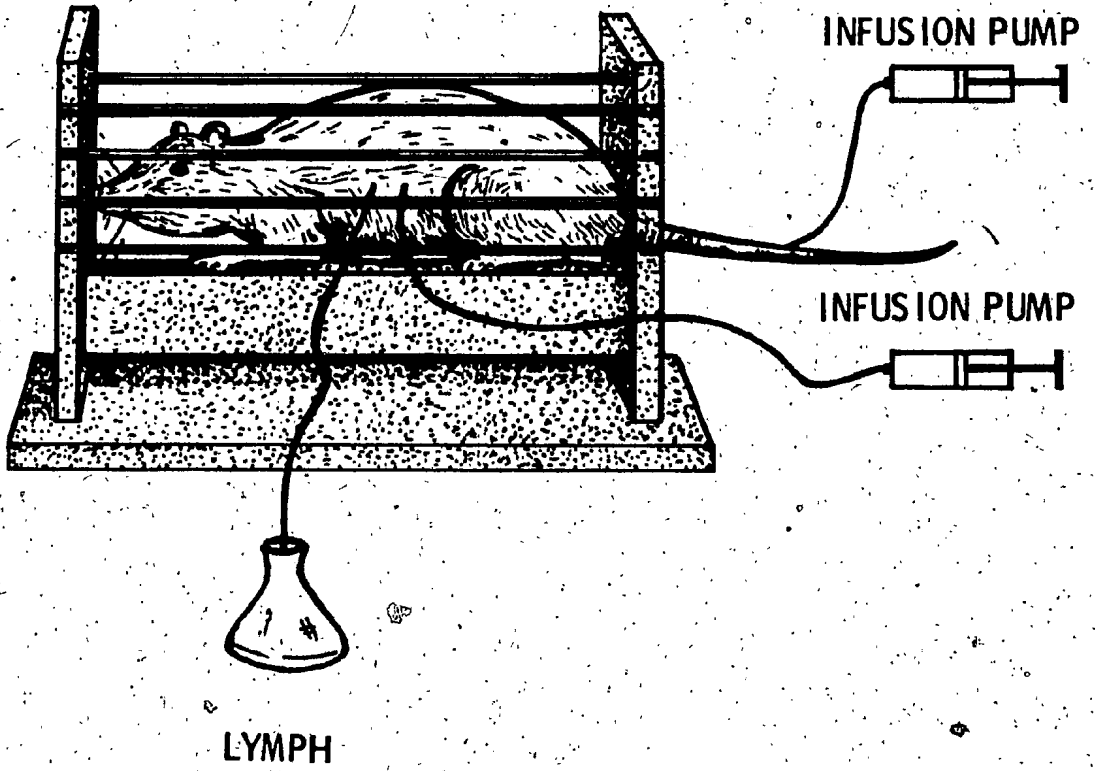
With the series 940, the 50cc syringe and 10cc syringe are attached to the infant feeding tube and tail-vein cannula, respectively. The pump is set at position 10 which gives 2.5 ml/hr

Figure 3

Bollman-type Restraint Cage

Schematic drawing of an animal in a Bollman-type restraint cage.

Note the lymphatic fistula, the intraduodenal fistula and the tail-vein cannula.



and 0.5 ml/hr to the infant feeding tube and tail vein, respectively.

With the series 975, the 50cc syringe and 5cc syringe are attached to the infant feeding tube and tail-vein cannula, respectively. This pump is set a position 22 which gives 2.5 ml/hr and 0.5 ml/hr to the duodenal fistula and tail vein, respectively.

HORMONE PREPARATIONS

Material

Pure natural secretin, 75 clinical units (U) per ampoule (cysteine-HCl (mg)), GIH Research Unit, Chemistry Department, Karolinska Institute, Stockholm, Sweden.

All solutions are made up with 0.85% sodium chloride. Six doses are used.

0.25 U	2.0 U
0.50 U	3.0 U
1.0 U	5.0 U

Synthetic secretin, pentaacetate, (white, lyophilized powder), Research Plus Laboratories Incorporated, P.O. Box 571, Denville, New Jersey, U.S.A.

Microgram amounts of the material are weighed out using the Cahn electrobalance. Research Plus quotes an approximation of 3.5 clinical units per microgram of synthetic secretin. Thus, using the appropriate amount of 0.85% sodium chloride, six doses are made up:

0.25 U	2.0 U
0.50 U	3.0 U
1.0 U	5.0 U

Vasoactive intestinal polypeptide, 1 mg of the lyophilized powder was a gift from Dr. V. Mutt, GIH Research Unit, Karolinska

Institute, Stockholm, Sweden.

Microgram amounts are weighed out using the Cahn electrobalance and diluted with 0.85% sodium chloride to obtain two doses:

0.1µg

0.5µg

Method

Each dose was freshly prepared prior to administration. The solutions were given intravenously via the tail-vein cannula as a 1 ml bolus or as a constant infusion.

BALANCES

- a) Top-pan precision balance 2250, Sartorius, Sartorius-Werke, Federal Republic of Germany.
- b) Top-loading electronic analytical balance, Mettler A30, Mettler Instruments AG, Greifensee, Switzerland.
- c) Cahn electrobalance, 4100, Cahn/Ventron Corp., Paramount, CA, U.S.A.

LYMPH COLLECTION

Material

sodium citrate, Fisher scientific

Method

All lymph is collected into pre-weighed, citrated vials. After each collection the vial is weighed and frozen. All weighings are done on the Mettler A30 balance. Lymph volume is determined by weight using a specific gravity of 1.000.

PROTEIN DETERMINATIONS

- 1) Biuret Assay

Material

bovine serum albumin, J.T. Baker Chemical Company.

Biuret Reagent

A stock solution of biuret reagent consisted of 45 grams of sodium potassium tartrate, 15 grams of copper sulfate, 5 grams of potassium iodide and 1 liter of 0.2N sodium hydroxide. A stock solution of alkaline iodide consisted of 5 grams of potassium iodide, 8 grams of sodium hydroxide, and 1 liter distilled water. A 1 liter working solution of Biuret is made by mixing 200 ml of the biuret stock and 800 ml of the alkaline iodide stock.

a) Centrifuge tubes

round bottom "oak ridge" type screw closure, polycarbonate, nalgene (16.1mm X 79.5mm), Canlab.

b) Ultracentrifuge

Beckman L2-65B ultracentrifuge, Beckman Instruments Inc.

65Ti rotor

c) Vortex

Genie, Scientific Industries Inc., Bohemia

d) Water bath

Thermolyne, Sybrow Corp.

e) Spectrophotometer

Unican SP 1800 ultraviolet spectrophotometer, Pye Unican

Method

Each vial of lymph, once thawed, is diluted to 5 grams with 0.85% sodium chloride. The sample is then transferred to a centrifuge tube and spun in the ultracentrifuge at an RPM of 40,000 and temperature range of 4 - 5°C for one hour. Then, the supernatant layer of

chylomicra is removed with an aspirator. One milliliter of each sample is placed in a tube to which 1 ml of biuret reagent is added. All samples are done in duplicate. Each tube is vortexed and placed in the water bath. The tubes remain here for 30 minutes at 37°C and are then left at room temperature, 28°C, for another 30 minutes.

To obtain a standard curve four concentrations of bovine serum albumin are used: 1-5 mg/ml. These are treated exactly like the prepared lymph samples.

After standing at room temperature each sample is read against a 0.85% sodium chloride blank in the spectrophotometer at a wavelength of 555nm. To find the protein concentration for each collection the optical density readings for the duplicates are averaged and the corresponding concentration read from the standard curve (Fig. 4).

2) "Bio-Rad" Microassay

Material

Dye reagent concentrate, Bio-Rad Laboratories, California
Protein Standard Solution (5.0g% Human Albumin, 3.0g% Human Globulin (gamma)), Sigma Chemical Company.

Method

One microliter samples are obtained from each lymph collection taken during the steady-state experiments. To obtain a standard curve four concentrations of standard human serum protein solution are used: 5 - 20µg/ml. Everything is done in duplicate.

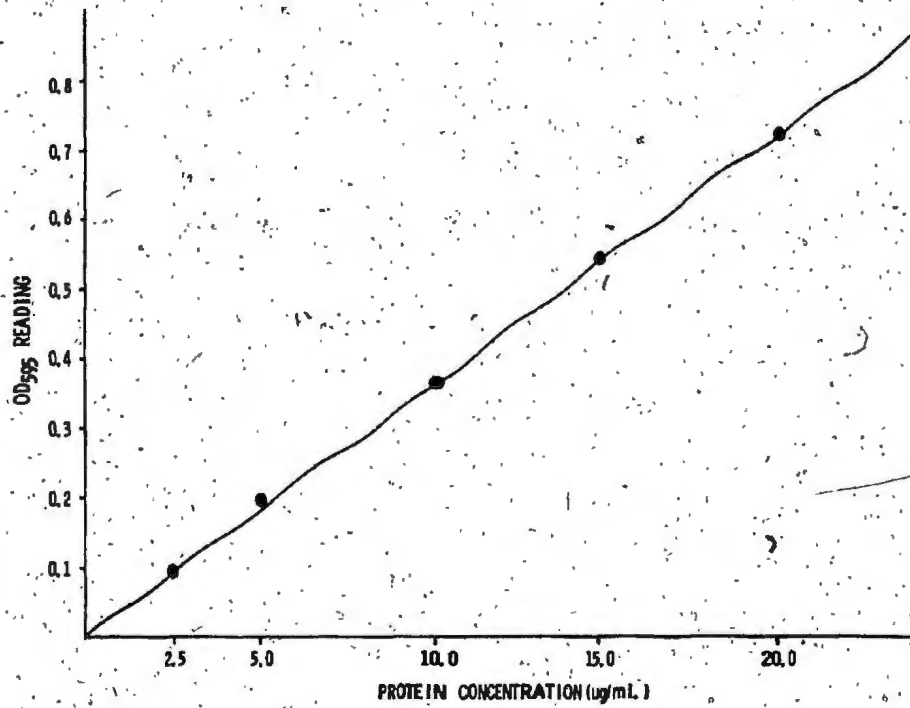
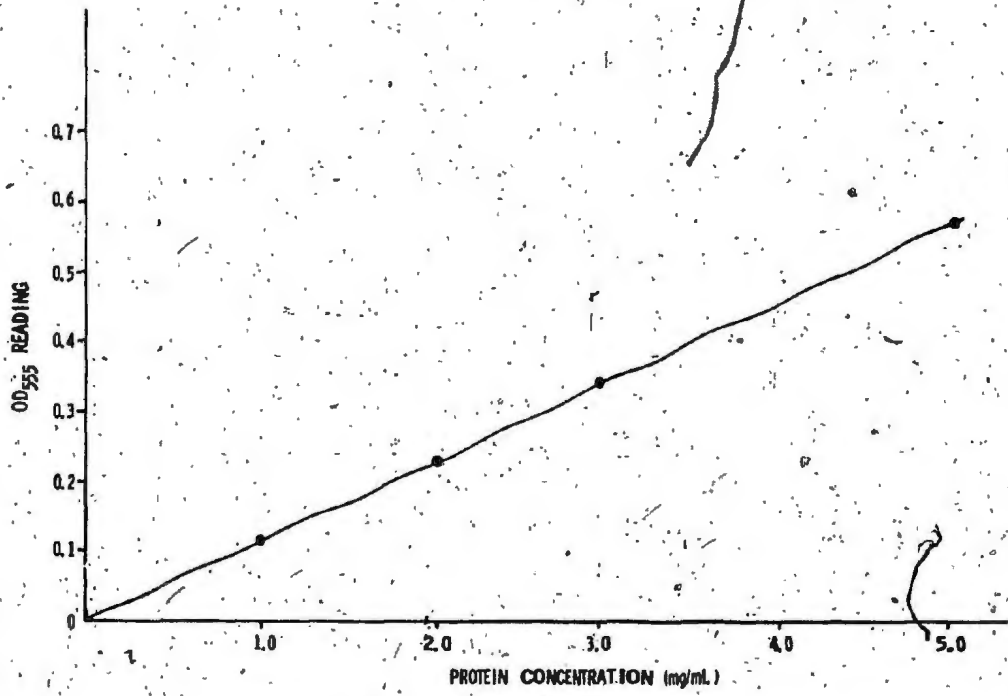
To each tube 0.2 ml of concentrated Bio-Rad reagent is added. The tubes are vortexed and left at room temperature for 20 minutes. Optical densities are obtained using the spectrophotometer at a wavelength of 595nm. To find the protein concentrations for each

Figure 4

Standard Curves for Protein Determination

upper: Biuret Protein Assay

lower: Bio-Rad Protein Microassay



collection the optical density readings for the duplicate are averaged and the corresponding concentration read from the standard curve (Fig. 4).

POLYACRYLAMIDE GRADIENT GEL ELECTROPHORESIS

Material

polyacrylamide gradient gels, PAA 4/30, Pharmacia Canada Ltd.,
Dorval, Quebec, Canada.

Pharmacia electrophoresis apparatus GE-4 II

Pharmacia electrophoresis power supply, EPS 500/400

fixative 9:3:1 distilled water: methanol: 7% acetic acid

0.1% Coomassie Brilliant Blue R-250, Eastman Chemical Company

Thermo-Shake bath, Forma Scientific

Method

The PAA 4/30 gels are placed into the gel electrophoresis apparatus GE-4 II. A pre-run is done at 125 volts for 20 minutes. The 9 μ l samples of each lymph collection are applied to the gels with the aid of an Eppendorf dispenser. Pre-electrophoresis is done for 20 minutes at 70 volts and electrophoresis is done for 16 hours at 125 volts.

Gels are removed from the electrophoresis apparatus and placed in a Petri dish with fresh fixative for 1 hour. The gels are stained with 0.1% Coomassie brilliant blue R-250. This is done by filling the Petri dishes with the stain and placing them on the Thermo-Shake bath at a slow speed for 4 hours. The gels are then destained with 7% acetic acid. The solution is changed three times: after 1 hour, after 19 hours, and after 20 hours when the gels are ready to be scanned.

A standard protein mixture (Pharmacia) was electrophoresed along with the lymph samples to check that the system was functioning satisfactorily. The standard was high molecular weight proteins including: thyroglobulin (669,000), ferritin (440,000), Catalase (232,000), lactate dehydrogenase (140,000) and albumin (67,000).

POLYACRYLAMIDE GEL ELECTROPHORESIS

Electrophoresis was performed with 7% polyacrylamide gels using the pH 8.6 buffer system of Ashton and Braden (1961).

Two lymph samples were run: a control sample from the pre-secretin collection and a sample from the post-secretin collection.

DENSITOMETRY

Material

Densitometer, model 750, Corning, Fisher Scientific.

Method

Each gel is placed in the scanner module and scanned, the results recorded graphically.

In order to find any changes in relative concentrations of various proteins that may have occurred quantitation is done as follows: on each graph six regularly identified peaks (Fig. 5) are cut out and weighed on the Mettler A30 balance. The largest peak albumin was assigned a value of unity. The area of each of the other five peaks is expressed as a ratio relative to albumin. These ratios are examined in each lymph sample and compared.

STATISTICS

The curves of figures 12, 13, 15 and 16 were calculated on the polynomial equation of $z = a + bx + cx^2$ where

z = change in lymph flow or protein output

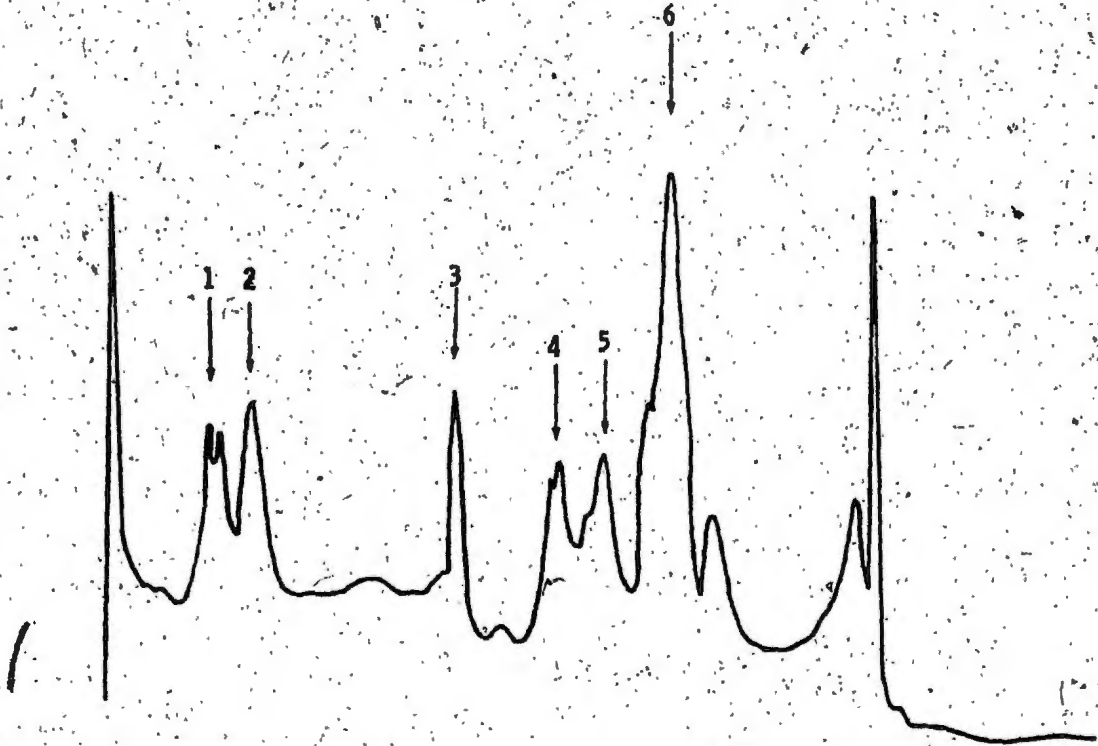
x = dose of hormone used

x^2 = square of the dose

The regression coefficients between equations are compared with Student's t-test at $P < 0.01$ for significance. This level was chosen to compensate for multiple testing among equations and provided at least 95% confidence for all tests in the experiments.

Figure 5

The six peaks identified and compared in each densitometry scan.



EXPERIMENTAL DESIGN

All the experiments were concerned with the effect of experimental manipulations upon the biological system, not upon each individual rat studied. The experimental manipulations are the treatments, either doses of vasoactive substances or 0.85% sodium chloride (control). The result of each treatment is the response which may or may not be a measurable change in intestinal lymph flow and protein output.

DESIGN 1

All collections are taken from the intestinal lymphatic fistula into pre-weighed citrated tubes. Each collection is twenty minutes in duration. All doses are 1 ml bolus injections given intravenously via the tail-vein cannula. For each animal a minimum of four collections establishes a baseline.

For both natural secretin and synthetic secretin the doses are administered in the following order: 1 U/ml, 0.25 U/ml, 0.50 U/ml, 2.0 U/ml, 3.0 U/ml and 5.0 U/ml. Not every animal received all six doses.

For vasoactive intestinal polypeptide, the 0.1 μ g/ml dose was administered followed by the 0.5 μ g/ml.

After each injection four collections are taken. After the control injection two collections are taken.

For each sample the volume is determined as described and the protein concentration is determined using the biuret assay.

DESIGN 2

A rat with its intestinal lymphatic fistula is placed at a position such that the cannula is hanging over, but not touching the Mettler A30 balance. A pre-weighed citrated tube is placed on the balance and the instrument set at zero. Lymph is then collected from the cannula for 10 minutes prior to and 20 minutes after a 2 U/ml dose of natural secretin is given as a bolus intravenously via the tail-vein. Throughout, this entire period, the tube is set to zero on the balance after each 30 second interval and the weight read.

DESIGN 3

A rat is set up as in design 2. During this procedure two minute collections are taken. After each collection period the weight is read and the citrated tube is exchanged for another whose weight is set to zero.

Three baseline collections are taken followed by a 15 minute infusion of 0.85% sodium chloride during which eight collections are taken. Post-infusion another five collections are taken. Next natural secretin is infused for 15 minutes at a rate of 0.25 U/min during which eight collections are taken. Post-secretin collections are taken until baseline values are reached, usually about sixteen collections.

Infusions are given with a Harvard pump, series 940, set at position 7 with a 30cc syringe.

All samples are diluted with an equal volume of 50% sucrose, run on polyacrylamide gel electrophoresis as described above, and scanned with the densitometer. Finally the protein concentration of each sample is determined using the "Bio-Rad" microassay.

RESULTS

Figure 9

An analysis of the effect of secretin on intestinal lymph flow in the rat. A 1U/ml bolus dose of secretin was administered intravenously. The solid line represents the weight of lymph recorded every 30 seconds. The broken line is a computer derived moving average of lymph mass.

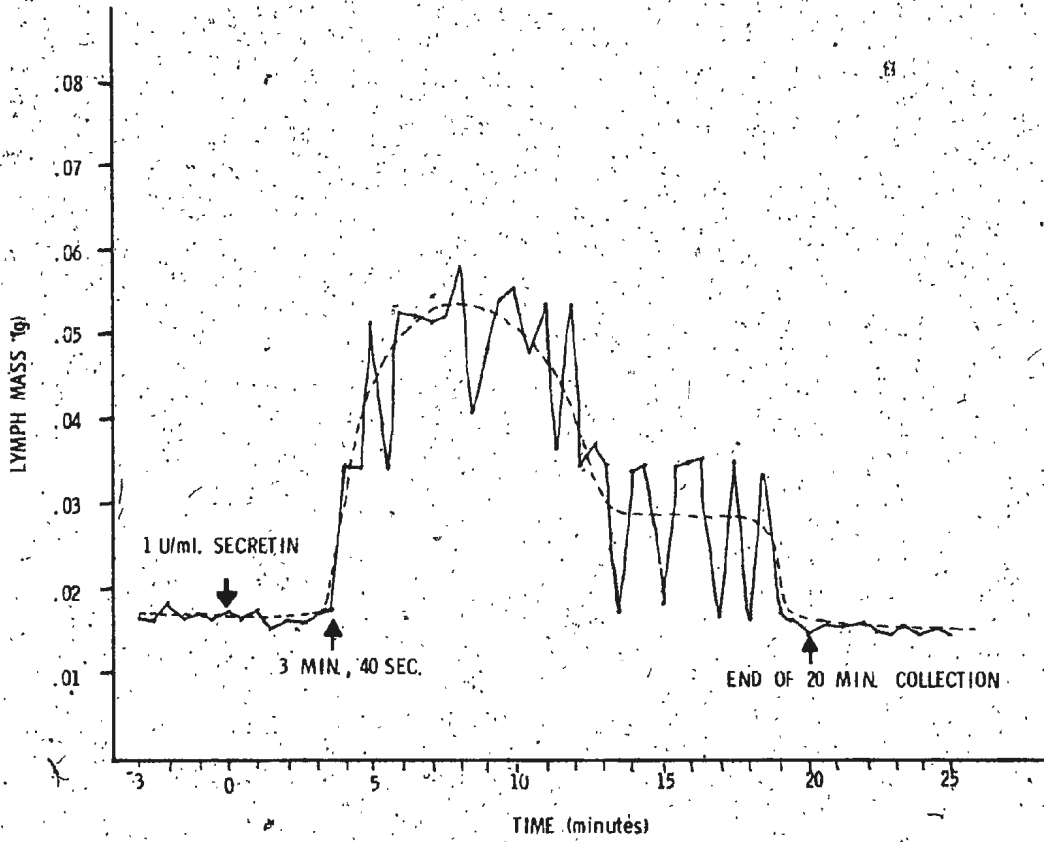


Figure 10

Natural Secretin - Intestinal Lymph Flow with Time

The response of an individual rat to graded secretin doses and a saline control. Each point represents a 20 minute collection period.

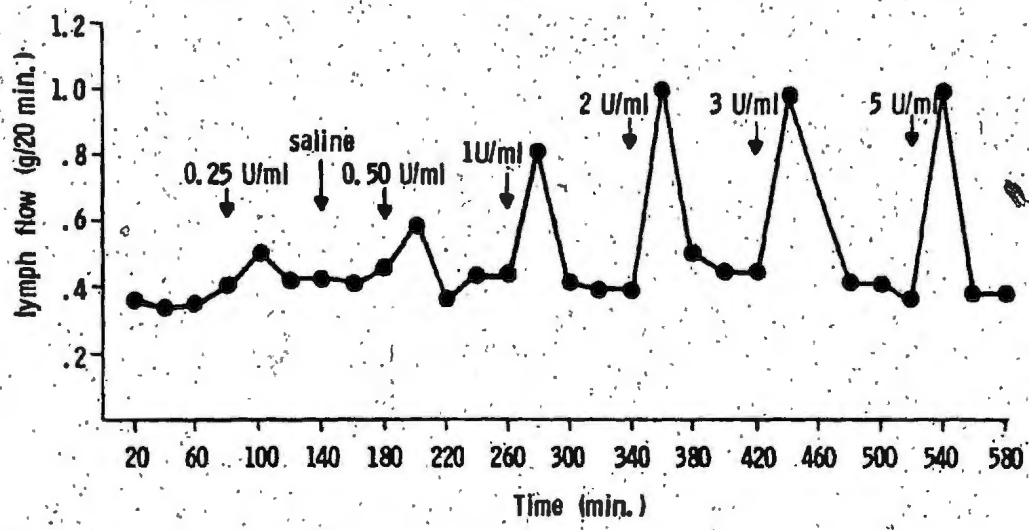


Figure 11

Natural Secretin - Intestinal Protein Output with Time

The response of an individual rat to graded secretin doses and a saline control. Each point represents a 20 minute collection period.

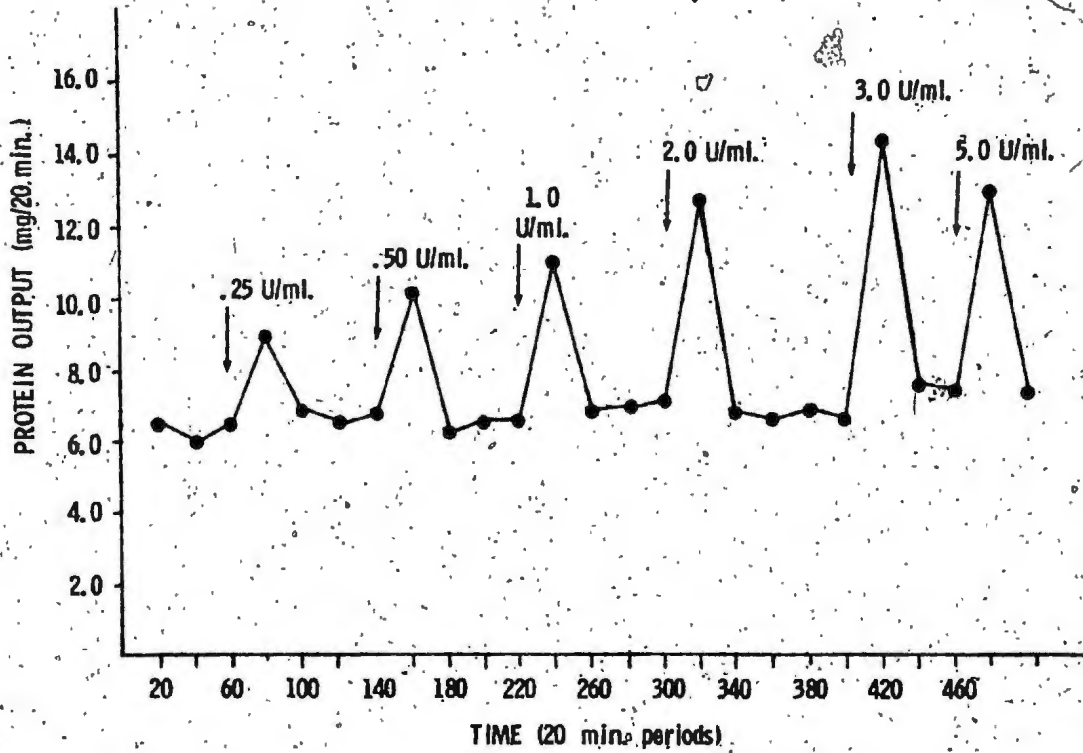


Figure 12

Natural Secretin - The change in Intestinal Lymph Flow with increasing secretin doses

An analysis of the responses from ten rats, studied with graded secretin doses, expressed as a polynomial dose-response curve with 95% confidence bands.

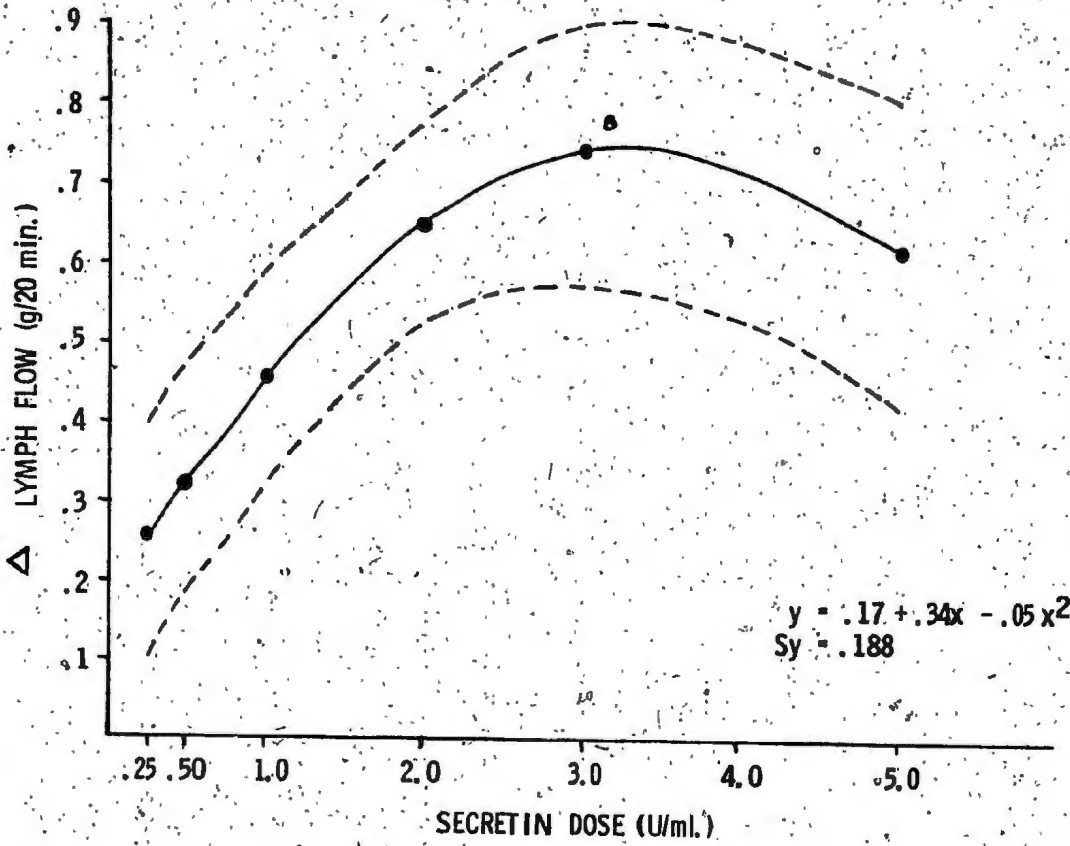


Figure 13

Natural Secretin - The change in Intestinal Protein Output with increasing secretin doses

An analysis of the responses from ten rats, studied with graded secretin doses, expressed as a polynomial dose-response curve with 95% confidence bands.

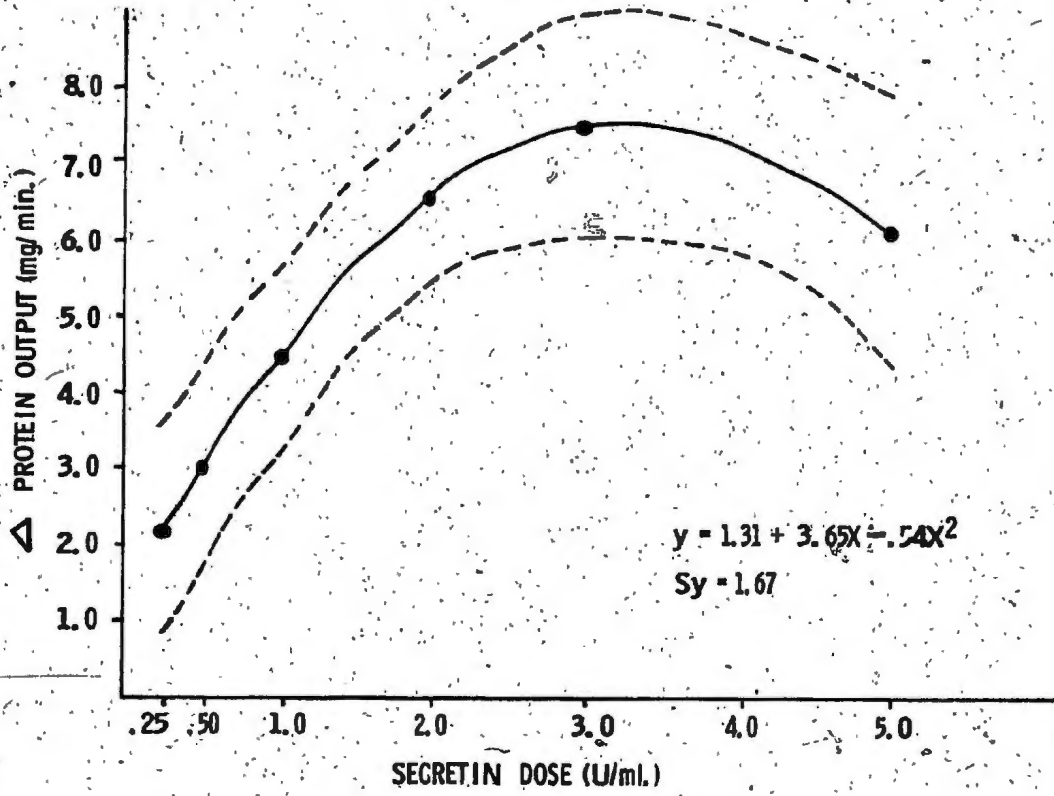


Figure 14

Natural Secretin versus Synthetic Secretin (Research Plus Laboratories)

The effect of a bolus dose of 1 U/ml natural secretin and 1 U/ml synthetic secretin on intestinal lymph flow in the rat.

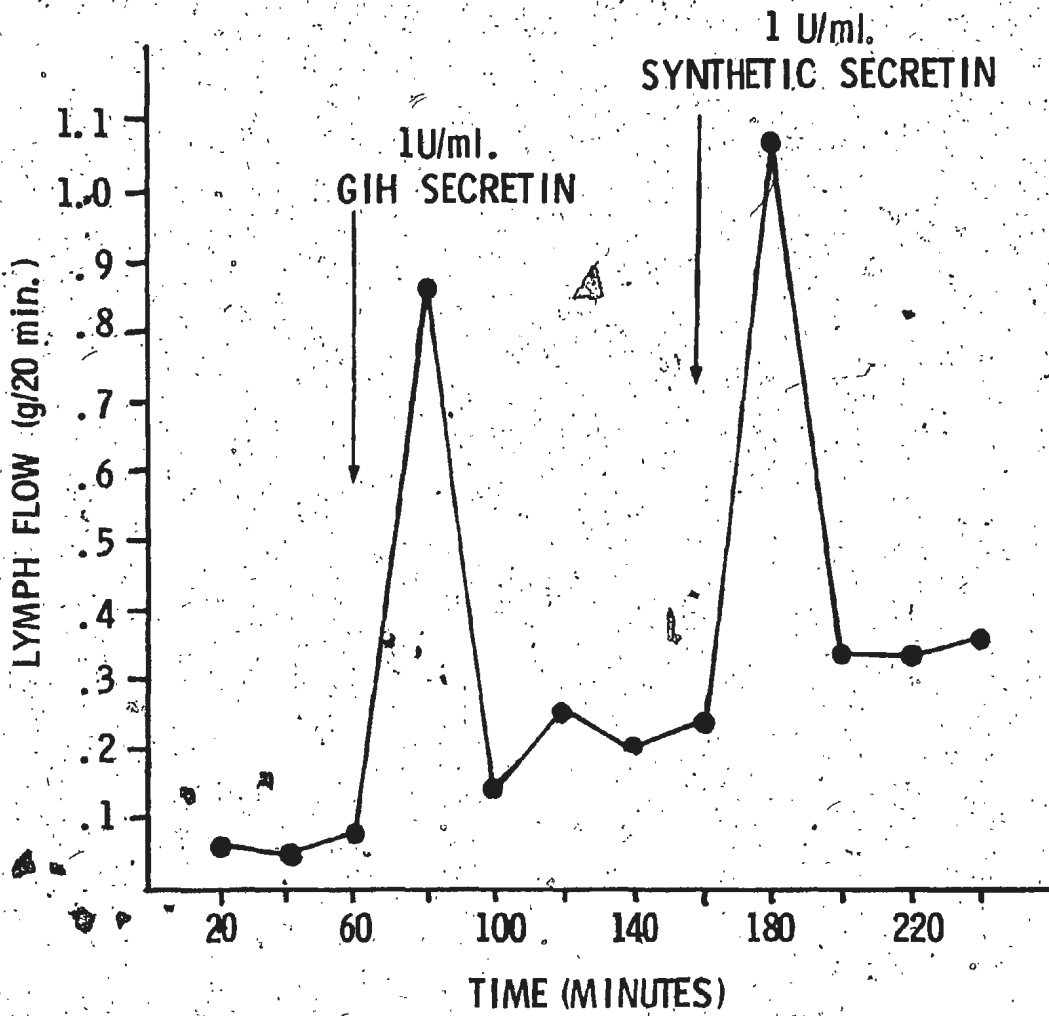


Figure 15

Synthetic Secretin - The change in Intestinal Lymph Flow with increasing secretin doses.

An analysis of the responses from three rats, studied with graded secretin doses, expressed as a polynomial dose-response curve with 95% confidence bands.

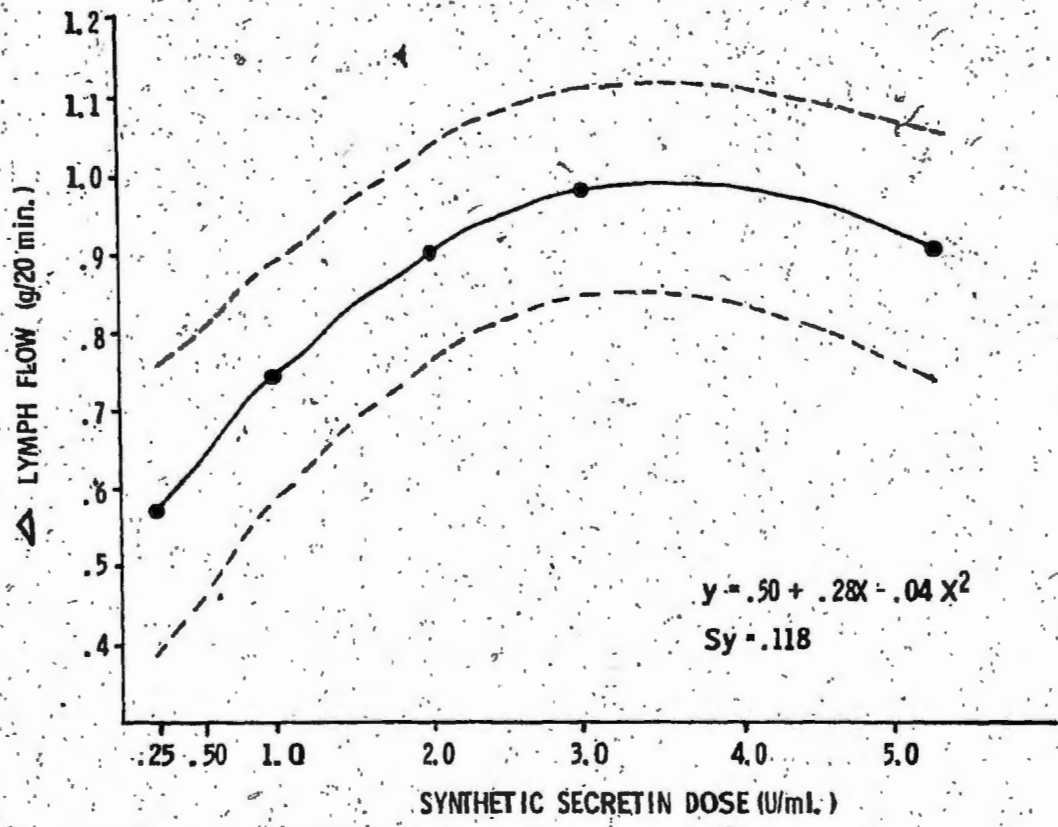


Figure 16

Synthetic Secretin - The change in Intestinal Protein Output
with increasing secretin doses

An analysis of the response from three rats, studied with graded
secretin doses, expressed as a polynomial dose-response curve with
95% confidence bands.

- 52 -

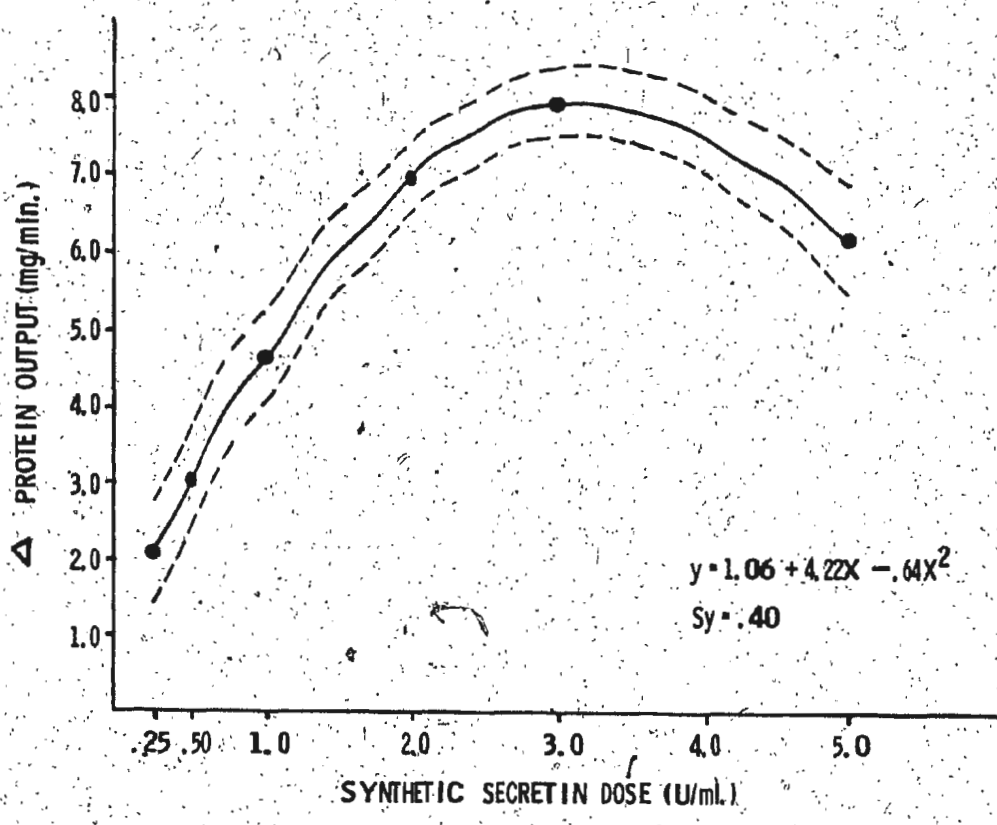


Figure 17

A Densitometry Scan of a Polyacrylamide Electrophoretic Gel

The dotted line represents the control pre-secretin lymph collection while the solid line represents the post-secretin lymph collection following a bolus dose of 1 U/ml of natural secretin.

Three peaks are identified as globulin, transferrin, and albumin. Their ratios are indicated.

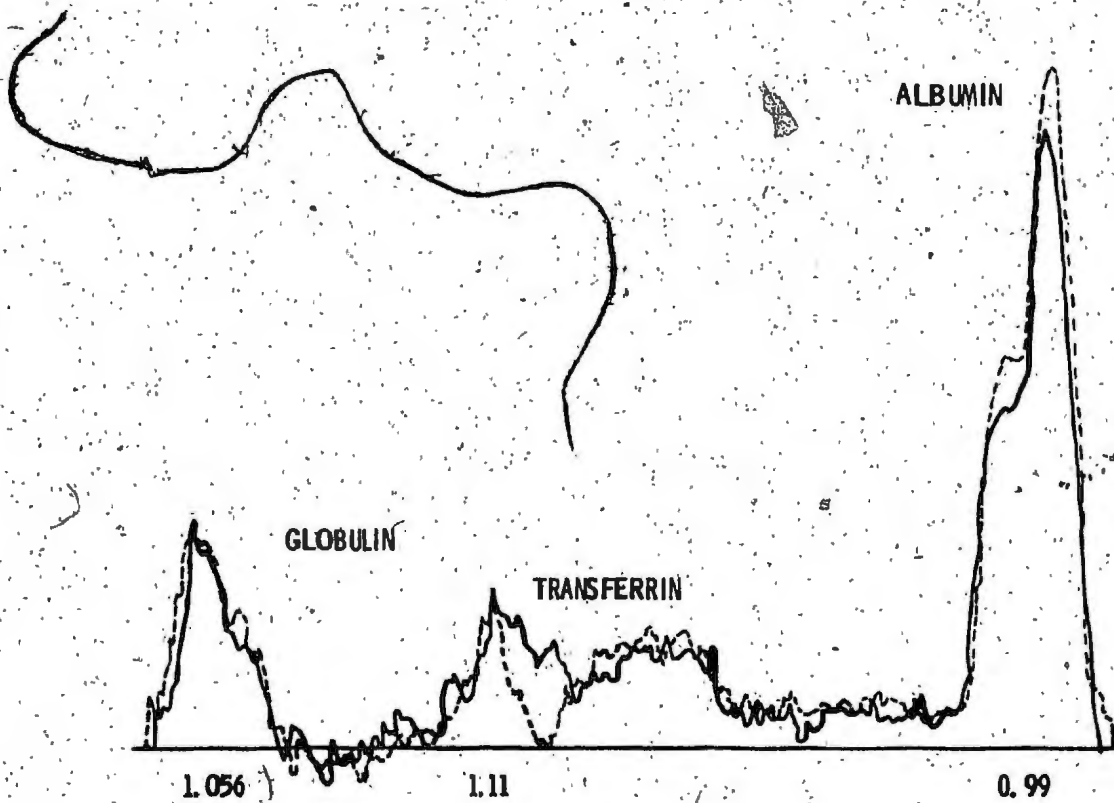


Figure 18

Establishment of a Steady-State with an Infusion of Secretin

A steady-state was established after a 15 minute infusion of 0.25 U/min of natural secretin. A saline control was also performed. Each point represents a 2 minute collection of lymph. This was repeated.

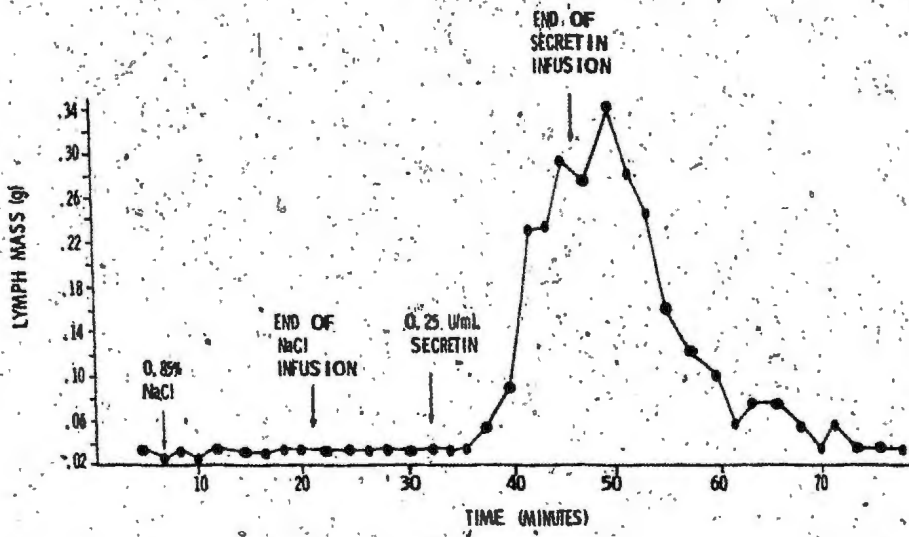


Figure 19^o (Rat A)

Effect of Natural Secretin on Intestinal Lymph Flow and
Composition

• The protein concentrations of each 2 minute lymph collection as
g% values is shown in the upper part of the figure.

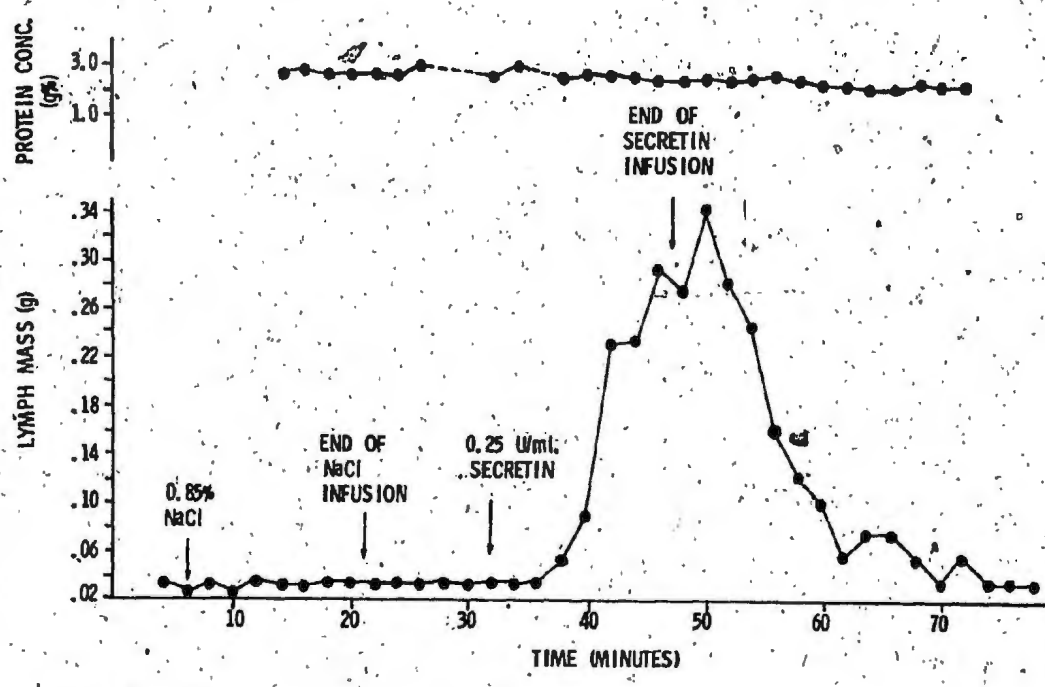


Figure 20 (Rat B)

Effect of Natural Secretin on Intestinal Lymph Flow and
Composition

The protein concentrations of each 2 minute lymph collection as
g% values is shown in the upper part of the figure.

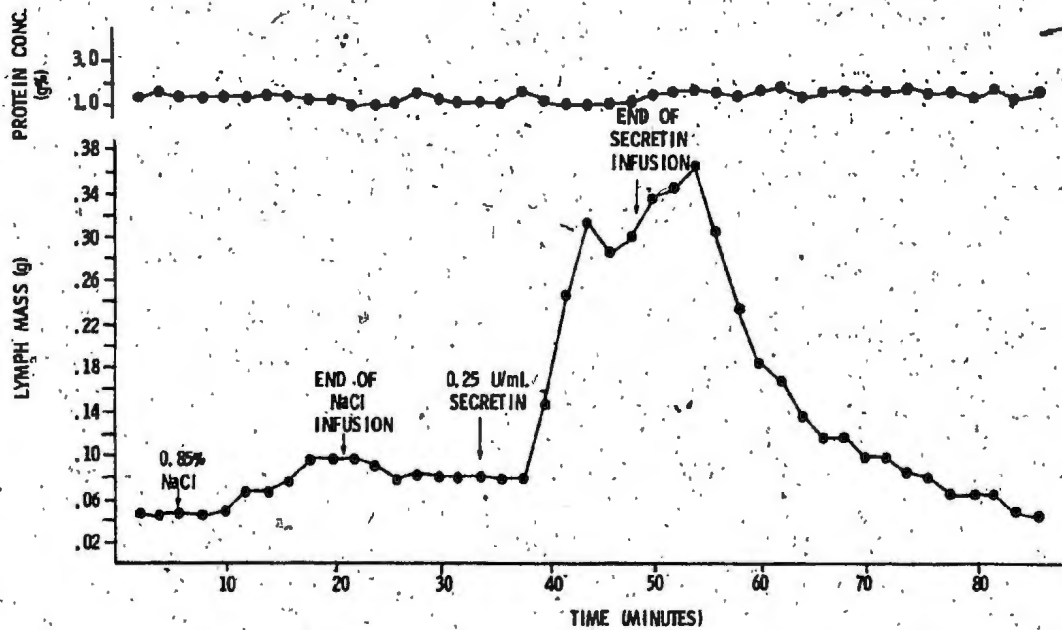


Figure 21 (Rat C)

Effect of Natural Secretin on Intestinal Lymph Flow and Composition

The protein concentrations of each 2 minute lymph collection as g% values is shown in the upper part of the figure

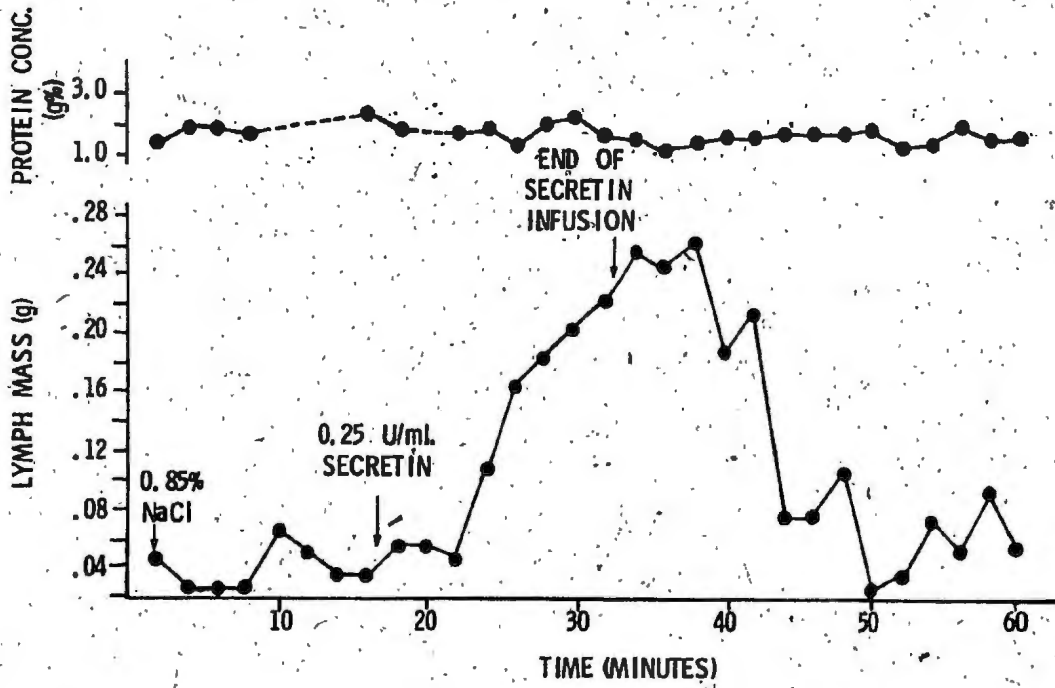


Figure 22 (Rat D)

Effect of Natural Secretin on Intestinal Lymph Flow and
Composition

The protein concentrations of each 2 minute lymph collection as
g% values is shown in the upper part of the figure

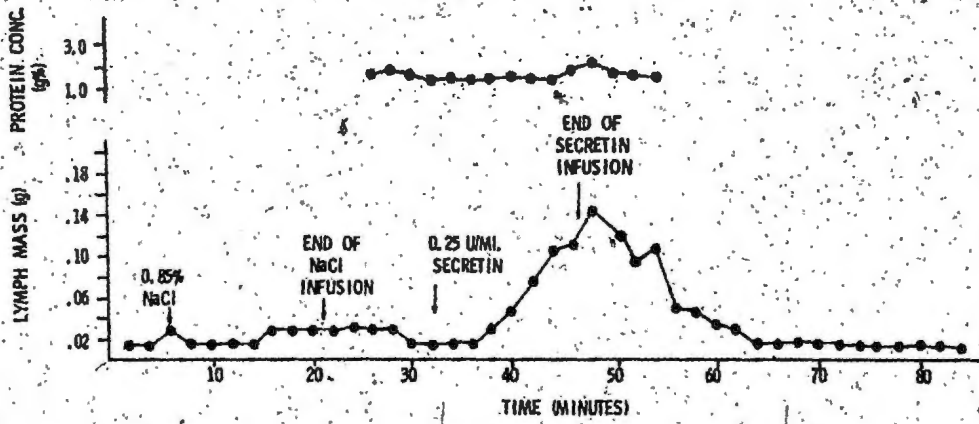
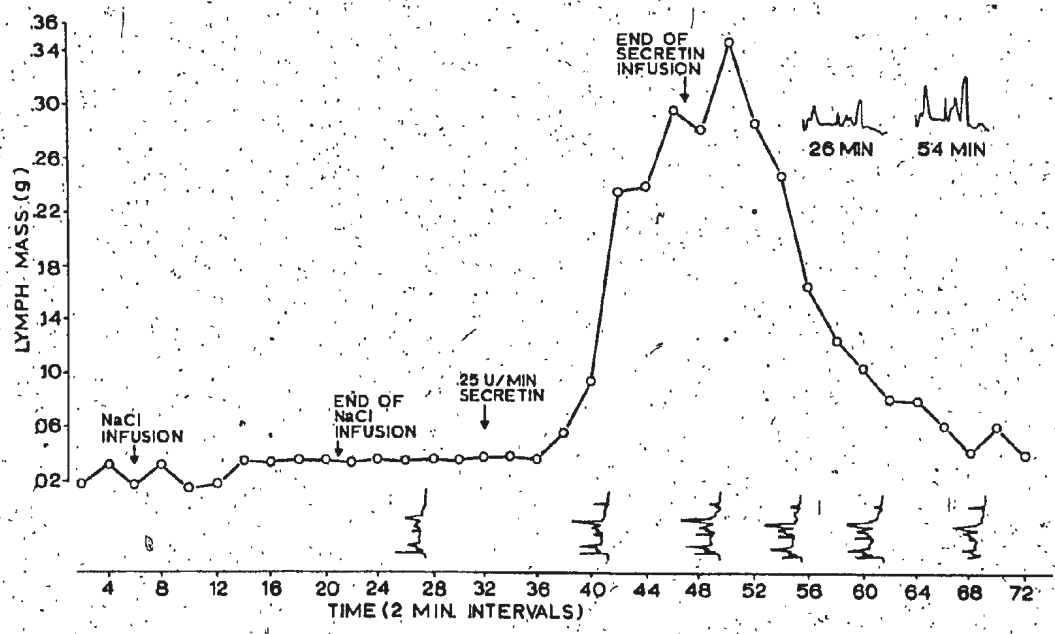


Figure 23 (Rat A)

Densitometry Scans of Polyacrylamide Gradient Gel Electrophoresis
Densitometry scans of samples from the 2 minute lymph collections
obtained throughout the steady-state infusion experiment is shown
along the bottom of the figure.

A control scan and a post-secretin scan are shown for
comparison in the upper right hand corner.



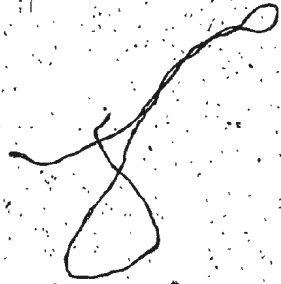


Figure 24 (Rat B)

Densitometry Scans of Polyacrylamide Gradient Gel Electrophoresis

Densitometry scans of samples from the 2 minute lymph collections obtained throughout the steady-state infusion experiment is shown along the bottom of the figure.

A control scan and a post-secretin scan are shown for comparison in the upper right hand corner.

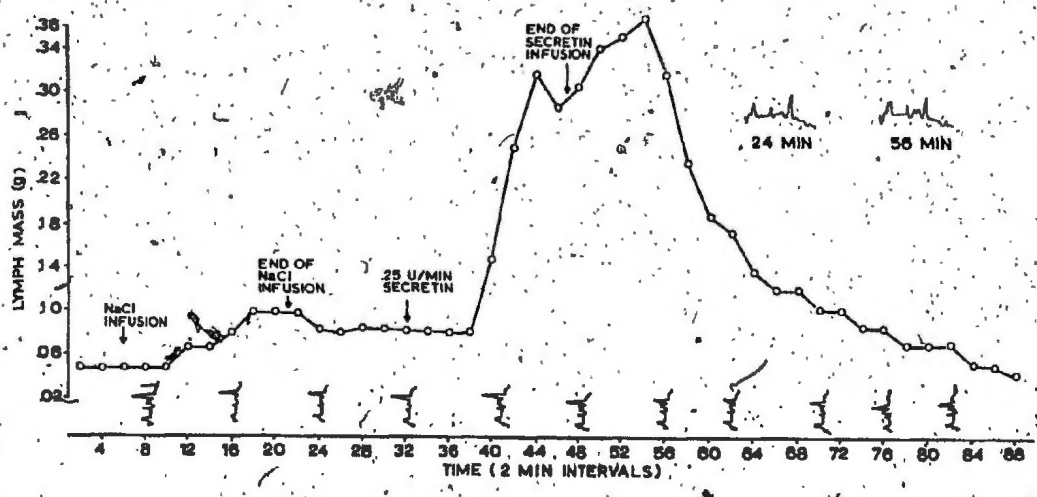


Figure 25 (Rat C)

Densitometry Scans of Polyacrylamide Gradient Gel Electrophoresis

Densitometry scans of samples from the 2 minute lymph collections obtained throughout the steady-state infusion experiment is shown along the bottom of the figure.

A control scan and a post-secretin scan are shown for comparison in the upper right hand corner.

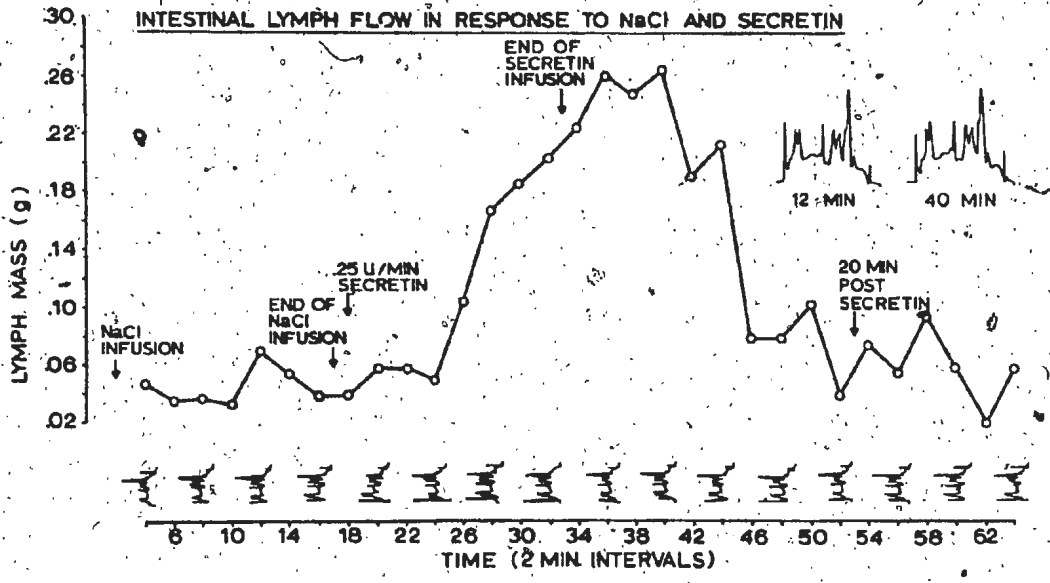


Figure 26 (Rat D)

Densitometry Scans of Polyacrylamide Gradient Gel Electrophoresis
Densitometry scans of samples from the 2 minute lymph collections
obtained throughout the steady-state infusion experiment is shown
along the bottom of the figure.

A control scan and a post-secretin scan are shown for
comparison on the upper right hand corner.

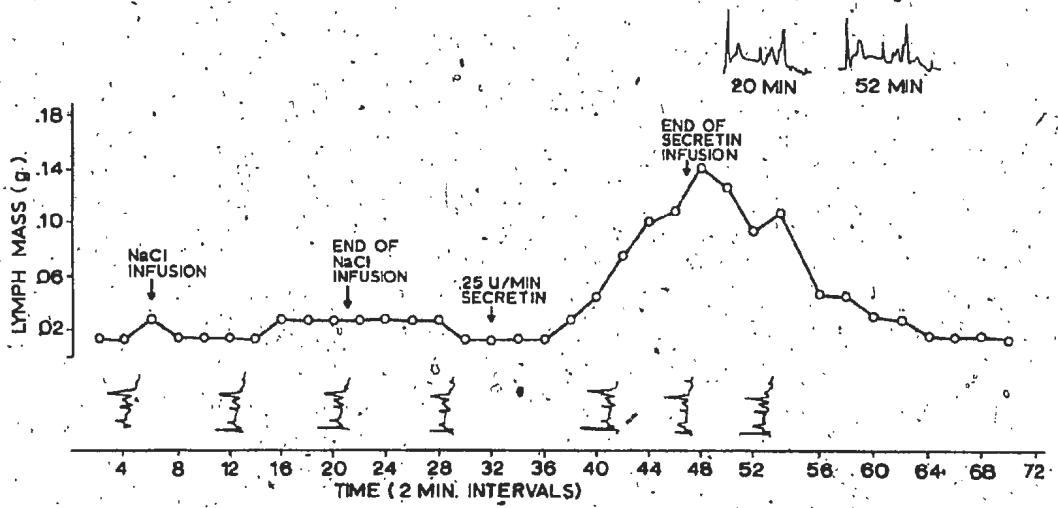


Figure 27

Natural Vasoactive Intestinal Polypeptide - Intestinal Lymph
Flow with Time

The response of an individual rat to two vasoactive intestinal polypeptide doses and a saline control. Each point represents a 20 minute collection period.

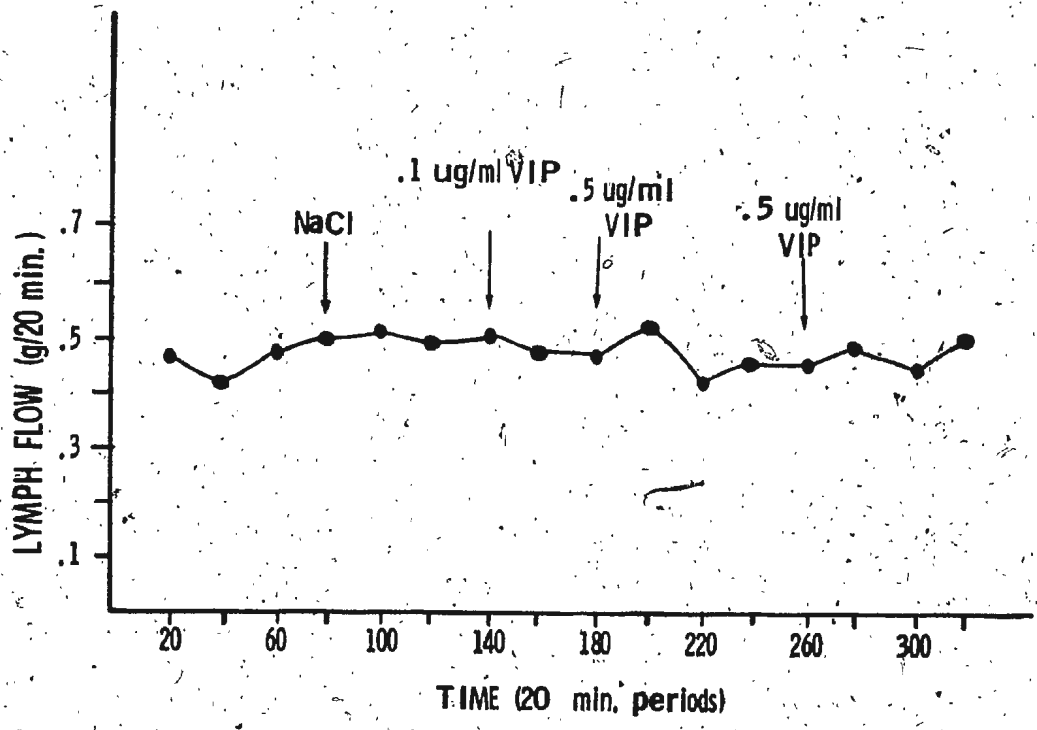
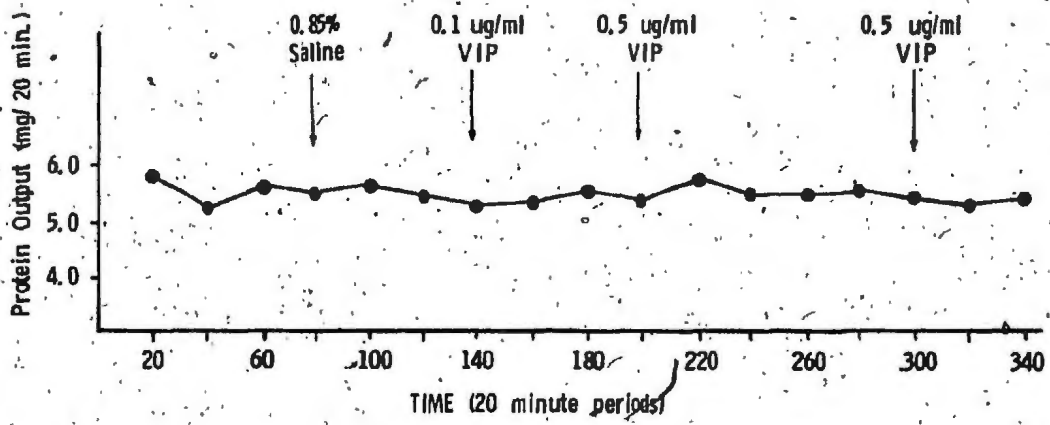


Figure 28

Natural Vasoactive Intestinal Polypeptide - Intestinal Protein Output with Time

The response of an individual rat to two vasoactive intestinal polypeptide doses and a saline control. Each point represents a 20 minute collection period.



DISCUSSION

Anatomical Background

The lymphatic system commences with the initial lymphatic capillaries which drain the interstitial fluid from almost every organ and tissue associated with blood capillaries. From these initial capillaries the lymph travels unidirectionally, due to the presence of valves, to either the thoracic duct or the right lymphatic duct which join to the blood vascular system via the left and right subclavian veins, respectively.

Generally, the lymphatic vessels of the lower extremities, pelvic and abdominal regions drain into the cisternae chyli which, along with lymph vessels draining the upper left side of the body, enter the thoracic duct. Lymph vessels draining the upper right side of the body enter the right lymphatic duct.

Of particular interest to this study are the intestinal lymphatic vessels which as part of the abdominal region drain into the cisternae chyli and ultimately into the thoracic duct. It is the main intestinal lymphatic vessel which is cannulated in the present study, the lymph collected originating only from the intestine (Fig. 6).

The intestine is attached to the posterior abdominal wall by a double layer of mesentery. It is within this mesentery that the intestinal blood vessels and intestinal lymphatic vessels are found.

Supplying blood to the small intestine is the superior mesenteric artery. This artery originates from the aorta and branches into smaller arteries distributed throughout the mesentery of the small intestine (Fig. 7, A). A cross-section of the small intestine reveals a unique anatomy (Fig. 7, Section A-A). The inner mucous membrane is made

Figure 6

The Main Intestinal Lymphatic

The main Intestinal Lymphatic is cannulated by inserting polyethylene tubing into the vessel about 3 mm and securing it with a drop of isobutyl-cyanoacrylate monomer..

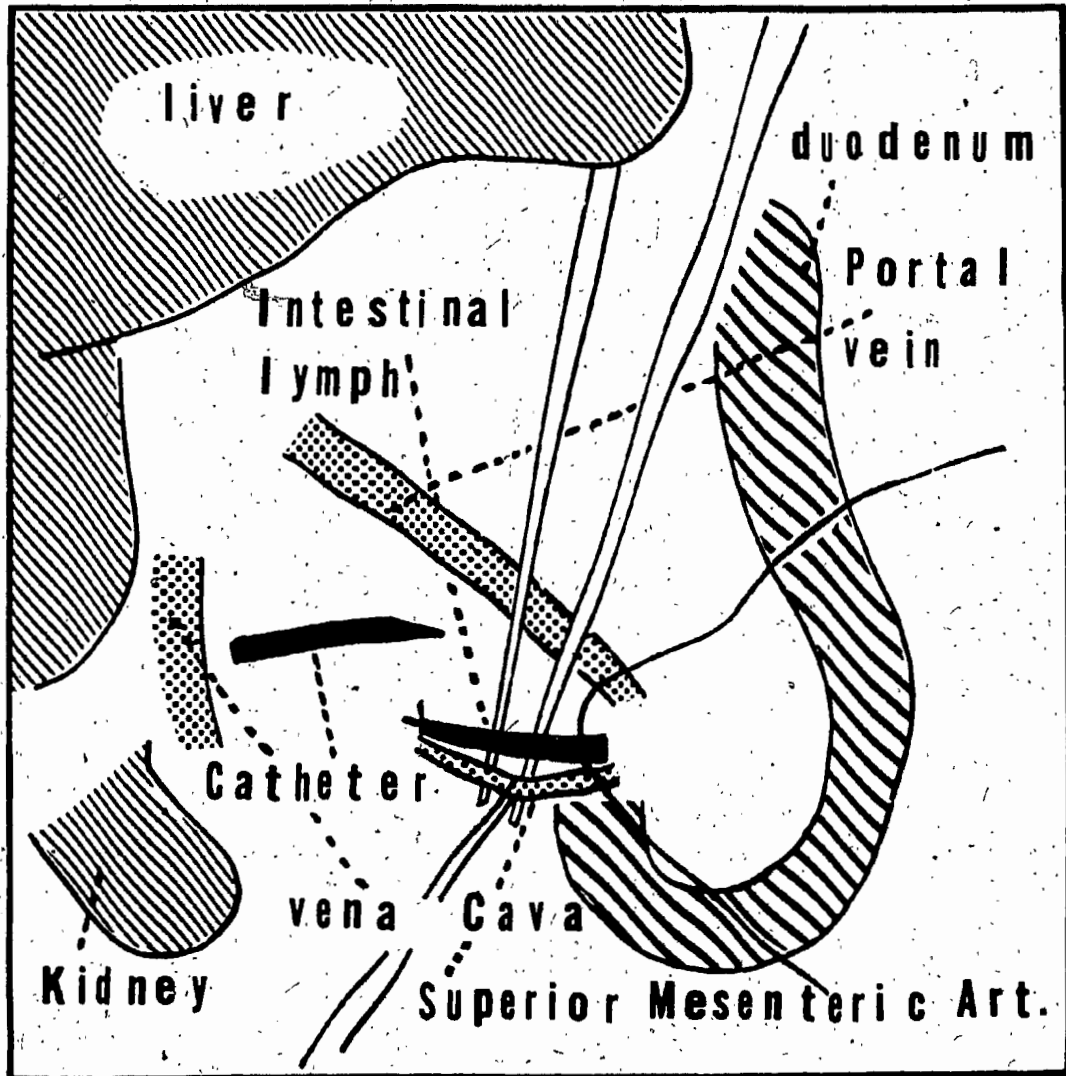
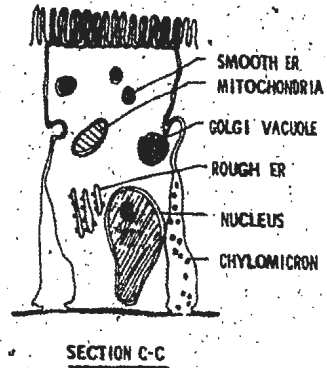
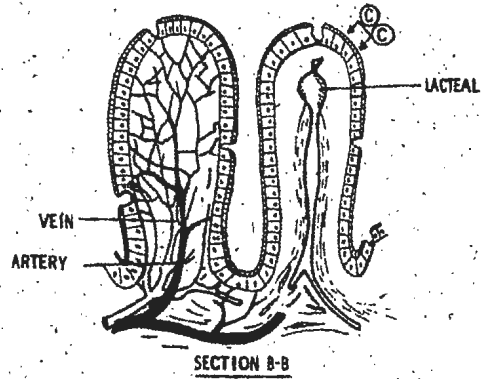
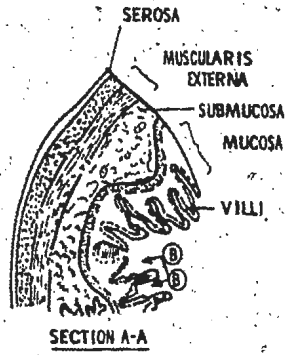
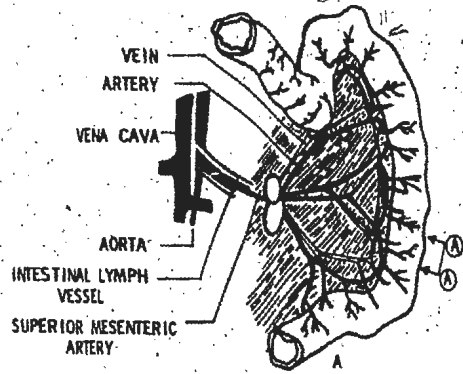
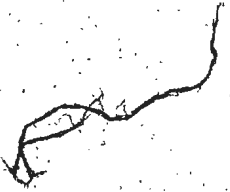


Figure 7.

The Intestinal Architecture

- A) arterial branches from the superior mesenteric artery supply the small intestine. the main intestinal lymphatic vessels drains the small intestine.
- A-A) cross-section of the small intestine: especially note the many inner folds called villi.
- B-B) the vasculature of the villi: In the left are the terminal branches of the arteries and veins while the right shows a central lacteal.
- C-C) An "enterocyte": The absorptive cell of the villi.



up of many folds known as villi (Fig. 7, Section B-B). These villi are outgrowths of the mucous membrane and are flattened and leaf-shaped in the rat. Functionally, the villi increase the surface area of absorption from the lumen. Histologically they are lined with a layer of simple columnar epithelium cells (Fig. 7, Section C-C). These "enterocytes" are the absorptive cells whose surface area is increased by many folds called microvilli. These microvilli project vertically into the lumen further increasing the absorptive surface. It is within the villi that the intestinal microcirculation is chiefly to be found (Fig. 7, Section B-B).

The network of arteries and veins within the villi are branches of the superior mesenteric artery and superior mesenteric vein, respectively. Much controversy exists concerning this blood supply. Two proposals have been put forth. One suggests that the blood vessels in the intestinal wall are in series (Ross, 1971; Greenway *et al.*, 1972) while the other suggests the blood vessels are in parallel circuits (Folkow, 1967; Lundgren, 1967). This question is not yet resolved, thus the nature of blood flow redistribution in the gut wall remains a matter of speculation.

Centrally within the villus is an intestinal lymphatic capillary which is called a lacteal. The lacteals were discovered by Gasparo Aselli (1581-1625). These initial lymphatic capillaries are blind-ended joining up with large lymphatic vessels to form eventually the main intestinal lymphatic vessel.

Functionally, the lymphatic system has three roles. The primary role is to return excess tissue fluid and protein derived from capillary filtration. It also have an immunological role producing and distri-

buting lymphocytes, and acts as a defense mechanism for the body against invading organisms. In addition, the intestinal lymphatics are responsible for the absorption of dietary fat along with fat-soluble vitamins and other lipophilic compounds.

It is the microanatomy of both the intestinal blood capillaries and the intestinal lymphatic capillaries that is important physiologically to the present study.

The intestinal blood capillary wall is a single endothelium layer. This endothelium is made up of continuous flattened cells. Within the cytoplasm of each cell are the usual cytoplasmic structures including a nucleus, mitochondria, and Golgi apparatus. The wall is surrounded by a basement membrane which also encloses an associated pericyte. Unlike some blood capillaries, the intestinal blood capillaries have only a few vesicles, which are spherical in shape. As well, the intestinal blood capillaries have fenestrations which presumably act as preferential channels for filtration of fluids and solutes from the blood to the interstitium.

The 'initial' lymphatic capillaries are lined by flattened polygonal endothelial cells, the only true component of their walls. They vary in thickness from $6\mu\text{m}$ in the nuclear region to as little as $0.1\mu\text{m}$ elsewhere (Yoffey & Courtice, 1970). Unlike the intestinal blood capillaries they have no fenestrations and a poorly defined basement membrane. However, each endothelial cell contains vesicles. Between the cells there are large intercellular gaps which are best seen when the vessel is distended. When the vessel is collapsed the lymphatic endothelial cells overlap or interdigitate with each other.

There are 'anchoring' filaments which connect the lymphatic capillaries to surrounding connective tissue. Leak et al., (1968) suggested that these filaments maintain the firm attachment of the lymphatic capillary wall to the adjoining collagen fibers and cells of the connective tissue area. Thus, an increase in interstitial fluid would pull the filaments causing them to pull the endothelial cells apart forming gaps and widening the lumen. The bulk of absorbed material is thought to enter the lymphatic capillaries by these gaps.

The basis of 'Starling's Principle' relies on the fact that blood capillaries are semi-permeable, that is although they are permeable to fluid and crystalloids they are relatively impermeable to plasma proteins. In contrast, the lymphatic capillaries which lack a basement membrane are very permeable to leaked plasma protein.

Besides the walls of the intestinal blood capillaries and the lacteal one has to consider how much of a barrier the interstitial matrix is. Guyton (1976) showed that the interstitial space of tissue is not merely an amorphous mucopolysaccharide gel but is permeated by channels through which fluid can move with little impedance.

Physiological Background

As discussed above fluid movement across a capillary wall is due to both Starling forces and the microanatomy of the capillary. From the previous discussion of the microanatomy of the villi it can be concluded that the blood capillary is relatively impermeable to plasma proteins which have leaked out. If these plasma proteins were not removed from the interstitial tissue Starling's forces would be unbalanced and fluid loss would not be opposed. These plasma proteins are removed by the

lymphatic capillaries which are more permeable than the blood capillaries. The nature of the forces determining uptake of fluid and solute by lymphatic capillaries is as yet unclear.

The basic concept of 'Starling's Principle' is a balance of opposing forces across capillary walls. That is, the difference between the hydrostatic pressure of blood and the hydrostatic pressure of the interstitial tissue fluid is opposed by the difference between the colloid osmotic pressure of blood and the colloid osmotic pressure of the interstitial fluid. When the difference in the hydrostatic pressure exceeds the difference in the colloid osmotic pressure, the net effect is an outward filtration from the blood to the interstitium. Generally, this occurs at the arterial end of a blood vessel. Conversely, if the difference in the hydrostatic pressure is less than the difference in the colloid osmotic pressure, the net effect is an inward filtration or absorption from the interstitium to the blood. Generally, this occurs at the venous end of the blood vessel. In addition to these forces one also has to consider the surface area available for filtration as well as the number of pores per unit membrane, along with their length and radius.

Although the colloid osmotic pressure in the blood capillary remains fairly constant, the hydrostatic pressure is regulated by arteriolar tone and by the state of the pre-capillary and post-capillary resistance vessels which control not only blood flow but the hydrostatic pressure within the vessel.

As for the forces responsible for lymph formation, they can only be speculated upon. The interstitial fluid pressure is thought to be

important in the formation of lymph but colloid osmotic forces within the lymphatic capillary have also been proposed as responsible for the uptake of fluid into these vessels. This question needs further investigation.

The control of gut blood flow

Two smooth muscle structures of great functional importance to the microcirculation of the gut are the arteriolar wall and the pre-capillary sphincter. It is the arteriolar wall which is the major regulator of resistance to blood flow. As the mesenteric vascular resistance decreases, superior mesenteric blood flow increases and vice versa. It is the pre-capillary sphincter which determines whether or not its capillary will be perfused with blood. The tone of the post-capillary venous vessels also influences hydrostatic pressures within the capillary as well as fluid movement across the vessel.

Postprandial hyperemia represents the increase in gut blood flow following a meal. The introduction of food orally, intragastrically, or intraduodenally causes an augmented gut blood flow, see, for example, Fara (1972). The mechanisms causing this postprandial hyperemia is not completely understood and has been postulated to be neural, humoral, or a combination of these. As discussed above, it seems more likely to be a combination.

Vasodilation can be defined as an increase in the cross-sectional area of blood vessels due to the relaxation of vascular smooth muscle. This relaxation is probably a result of an interaction between active dilation and inhibition of contraction.

The neurogenic control of gut blood flow involves the autonomic nervous system. Generally, the smooth muscles of all blood vessels except the capillaries receive a sympathetic innervation, these

adrenergic fibers being vasoconstrictors. Within the mesenteric blood vessels there is a dense supply of adrenergic fibers which cause vasoconstriction on stimulation. The innervation by cholinergic fibers from the parasympathetic division is very limited with respect to the gut blood vessels. Kewenter (1965) demonstrated that vagotomy followed by stimulation of the vagus nerve does not modify superior mesenteric blood flow. Further, he found no evidence that the vagal nerves carry any parasympathetic vasodilator fibers to the vessels of the small intestine. Overall, it seems that the autonomic nervous system has a minor role in gut blood flow except in "fight or flight" situations. A neurogenic mechanism solely responsible for postprandial hyperemia therefore seems unlikely.

As discussed previously, a number of vasoactive gastrointestinal hormones which have been shown to be released following a meal have been postulated as being responsible for the increased blood flow including secretin and vasoactive intestinal polypeptide.

In 1970 Vatner observed an augmented mesenteric blood flow in dogs following a meal which was blocked by anticholinergic agents administered preprandially but not by alpha or beta adrenergic blockade or bilateral thoracic vagotomy. Fara who showed, in 1972, that intraduodenal corn oil, L-phenylalanine or hydrochloric acid increased superior mesenteric blood flow, also demonstrated that vagotomy or atropine blocked this vascular response. He speculated that the blockade interfered with the release of the intestinal hormones. Subsequently, he observed that intravenous secretin and cholecystinin caused mesenteric vasodilation similar to that caused by the intraduodenal agents, this response not being affected by either vagotomy or the pharmacological blockers. Ross

(1970) had previously observed that the mesenteric vasodilation caused by exogenous secretin was unaffected by the prior administration of propranolol or atropine and by cutting all the visible nerves accompanying the mesenteric artery. Thus, it seems possible that feeding releases humoral vasodilator agents by local cholinergic mechanisms. However, the vasoactive effect on the gut blood vessels is a direct result of these intestinal hormones.

The idea of gastrointestinal hormones having a local or 'paracrine' effect has long been considered. A 'paracrine' hormone would be released from its cell of origin into the local tissues where it would exert its effect at comparatively high concentrations. Thus, local concentrations close to the vascular bed may be comparatively high during the stages of physiological stimulation in the postprandial state. Since there is no way of knowing the hormone's rate of secretion or concentration it is difficult to determine which may be a physiological effect. Currently, vasoactive intestinal polypeptide is thought to act in a paracrine fashion.

Influences on intestinal lymph flow

Augmented intestinal lymph flow can result from an increased intestinal capillary filtration or from absorbed fluids from the gut lumen. Both will increase the mucosal interstitial fluid available for absorption into the lacteals.

Physiologically a meal appears to affect the mechanisms controlling gut blood flow that cause vasodilation. It can be demonstrated that lymph flow increases following a meal, particularly if there is a substantial amount of fat present. Tasker (1951) observed this, demonstrating an enhanced lymph flow in rats following intragastric

administration of olive oil. This was confirmed by Simmonds (1955) and Wollin (1973) who concluded that since there is also an increase in the protein concentration of the lymph during fat absorption, the lymph must be derived from the plasma as a result of increased blood flow to the gut. Fara et al (1972) demonstrated that intravenous secretin and cholecystokinin produced a selective mesenteric vasodilation as did the introduction of fat or acid into the duodenum of the cat. This study suggested that hormones may be the mechanism by which fat feeding increases intestinal blood flow. A humoral role is supported further by experiments done by Turner and Barrowman (1978).

The other route causing augmented lymph flow is fluid absorption from the lumen. Simmonds (1954) observed that intragastric water gave an increased lymph flow with a fall in protein concentrations. Barrowman and Roberts (1967) demonstrated an increased lymph flow with diluted lymph following water administered either orally or intragastrically in unanaesthetized rats. Oral or intragastric administration of isotonic saline also increases lymph flow.

It is proposed that the lymphatics act as an overflow system once a certain amount of fluid accumulates in the interstitium regardless of its source.

The secretin family

Gastrointestinal hormones can be divided into two structurally homologous families: the gastrin family and the secretin family. The gastrin family includes gastrin, cholecystokinin, and related peptides such as caerulein while the secretin family includes secretin, vasoactive intestinal polypeptide (VIP), gastric inhibitory polypeptide (GIP), and glucagon. All of the above hormones have been proposed as

having a role in regulating gut blood flow. They are all vasodilators and with the exception of VIP selectively affect the splanchnic circulation.

The primary structure of secretin was not described until 1966 when Mutt and Jorpes showed its amino acid sequence. It has 27 amino acids (fig. 8) and a molecular weight of approximately 3055. Secretin is distributed in the S-cells of the mucosa of the gastrointestinal tract and is normally released by the acid pH of chyme entering the duodenum.

It was Wertheimer (1902 a&b) who showed that the secretion of secretin evoked by the presence of acid diminished as the acid was placed further down the small intestine and was absent when it was placed in the lowermost section of the ileum or in the small intestine. Konturek (1972) studied the release of secretin and cholecystokinin along various parts of the small intestine. His experiments with dogs showed that the amount of releasable intestinal hormones decreases with the distance from the pylorus, further supporting the work of Wertheimer and suggesting that the upper small intestine is the major source of secretin.

Since exogenous secretin rapidly disappears from the circulation it must be actively catabolized. Early reports on the degradation of secretin indicated that the liver was the site of inactivation. Experiments by Lehnert et al (1974) demonstrated that secretin inactivation was partly by the liver and partly by the kidneys in the dog. This was confirmed by Curtis et al (1976) who reported that the kidneys are the major organs for the removal of secretin from the circulation. Further, Rhodes et al (1975) had also shown that the kidney played a

Figure 8

The amino acid composition of secretin and vasoactive intestinal composition.

upper: secretin

lower: vasoactive intestinal polypeptide

Porcine Secretin (MW 3055; 27 amino acids)

1	2	3	4	5	6	7	8	9
HIS	SER	ASP	GLY	THR	PHE	THR	SER	GLU
10	11	12	13	14	15	16	17	18
LEU	SER	ARG	LEU	ARG	ASP	SER	ALA	ARG
19	20	21	22	23	24	25	26	27
LEU	GLN	ARG	LEU	LEU	GLN	GLY	LEU	VAL NH ₂

Porcine Vasoactive Intestinal Polypeptide (MW 3326, 28 amino acids)

1	2	3	4	5	6	7	8	9
HIS	SER	ASP	ALA	VAL	PHE	THR	ASP	ASN
10	11	12	13	14	15	16	17	18
TYR	THR	ARG	LEU	ARG	LYS	GLN	MET	ALA
19	20	21	22	23	24	25	26	27
VAL	LYS	LYS	TYR	LEU	ASN	SER	ILE	LEU
28								
ASN	NH ₂							

physiological role in the catabolism of secretin in man. In normal healthy subjects the $T_{1/2}$ of secretin was 2.57 minutes while in patients with chronic renal failure it was 6.14 minutes. Secretin, like most gastrointestinal hormones has a short plasma $T_{1/2}$ as can be seen in Table I. Finally, it would seem from a teleological standpoint inefficient for the liver to be the main site of inactivation since secretin passing by the portal vein would be mostly destroyed on its way to the pancreas, its target organ. Physiologically, the main action of secretin is to stimulate the secretion of pancreatic bicarbonate and water.

In 1974 Mutt and Said published the proposed amino acid sequence of VIP. It has a molecular weight of 3326 and has 28 amino acids of which nine are identical to secretin (fig. 8). VIP is distributed throughout the gastrointestinal tract in H-cells and is implicated as a peptidergic neurotransmitter. Experimentally VIP is released by calcium (Ebeid, et al, 1977), intraluminal osmotic changes (Ebeid, et al, 1977), and by electrical stimulation of the vagus (Schaffalitzky de Muckadell et al, 1977).

VIP is very rapidly cleared from the circulation as can be seen in Table I, its $T_{1/2}$ is approximately one minute. Such a rapid $T_{1/2}$ might indicate a widespread degradation. However, the liver has been suggested as the site of VIP degradation. Said et al (1974) demonstrated an increased plasma level of VIP in patients with hepatic cirrhosis and experiments by Kitamura et al (1975) and Konturek et al (1977) both indicated that the liver is the major site of VIP inactivation. To date no physiological role has been attributed to VIP. It has been speculated that VIP is a primitive hormone-like substance from which the more specialized peptides evolved (Johnson, 1977).

TABLE I: Plasma half-life for secretin & VIP

Secretin

<u>t_{1/2}</u>	<u>species</u>	<u>method</u>	<u>reference</u>
17 mins.	man	bioassay	Lagerlof <u>et al.</u> , 1962
2-5 mins.	dog	RIA	Boden <u>et al.</u> , 1974
2.57 mins.	man	RIA	Rhodes <u>et al.</u> , 1975
2-5 mins.	man	RIA	Kolts <u>et al.</u> , 1977

VIP

<u>t_{1/2}</u>	<u>species</u>	<u>method</u>	<u>reference</u>
0.85 ± 0.12 mins.	pig	RIA	Modlin <u>et al.</u> , 1978
1.03 mins.	man	RIA	Domschke <u>et al.</u> , 1978
1.22 mins.	man	RIA	Domschke <u>et al.</u> , 1978

RIA = radioimmunoassay

Why secretin and VIP were studied

Secretin and VIP were chosen for a number of reasons. There is no information on the effect of either hormone on the intestinal lymph flow in the rat and both have been considered as possibly responsible for postprandial intestinal hyperemia. They are structurally homologous so they might be expected to give similar responses in terms of intestinal blood flow and lymph flow. It is proposed that both secretin and VIP will increase superior mesenteric blood flow when injected intravenously, this change reflected by an increase in intestinal lymph flow. However, secretin as discussed above, appears to have a more selective splanchnic vasodilator effect than VIP, thus its action on intestinal lymph flow might be anticipated to be greater.

Discussion of the results

In order to analyze the effect of secretin in this system, design 2 was established. The lymph volume was determined after each 30 second interval throughout the experiment. The analysis is shown in figure 9 with lymph flow on the ordinate and the time in minutes on the abscissa. Following ten collections which established a baseline a 1 ml bolus dose containing one unit of secretin was administered intravenously. In all animals studied there appeared a highly reproducible lag phase of approximately 3 minutes following the injection (figure 9 shows 3 minutes, 40 seconds). Studies by Ross (1970) have shown that intra-arterial injections of secretin caused an increase in superior mesenteric blood flow within 3-7 seconds, the effect being a direct one. Thus, intravenous injections should only take the extra time needed to circulate to the artery, which is very rapid. Perhaps during the three minute lag phase some other mechanism is disposing of excess filtrate which is

eventually overwhelmed by continued transcapillary filtration.

After the delay, the lymph flow rapidly increases, reaching a maximum about seven minutes post-injection. Thereafter lymph flow decreases reaching a plateau for a few minutes and finally returns to baseline values.

Most importantly, this analysis shows that the response to secretin is well within the 20 minute collection time. Thus the subsequent experiments used 20 minute collection periods following bolus doses of secretin.

Figure 10 shows the typical lymph flow response of an animal to a series of secretin doses and a saline control. This figure demonstrates the results of design 1. Lymph flow is on the ordinate and time in 20 minute periods is on the abscissa. All doses gave an increased lymph flow with saline having virtually no effect. The amount of change over the baseline increased for each dose up to 3 units.

Although the sequence of doses varied from animal to animal, they were not administered entirely on a random basis. Two reasons exist for the choice. From pilot experiments with secretin it was established that the 1U dosage gave a recognizable positive response from the animal. Thus, the initial dose in subsequent experiments was usually the 1U secretin dosage. Occasionally problems arose with clots in the cannula obstructing lymph flow or with the tail-vein tubing not being directly in the vein, these animals not directly receiving the intravenous secretin. Therefore, the 1U dose was a test done to establish that the animal was responding as expected from previous studies.

The 3U and 5U doses are both considered pharmacological and could possibly cause some harm to the animal which could alter the response of

subsequent doses. To avoid this possibility, these doses were purposely administered at the end of the study.

The probability of a sequence effect, that is, one dose affecting the response of the following dose, was controlled for by the 4 post-injection collections.

Protein output from the fistula was determined for each collection. Figure 11 shows the typical response of an animal to natural secretin and saline. As with lymph flow the amount of change over the baseline increased for each dose up to 3U with saline having no effect.

Each dose of secretin resulted in a net excess of protein being recovered from the lymph fistula during that 20 minute period. Following that 'spike' of protein no inverse fall in protein output was observed. In fact, lymph protein concentration of the successive 20 minute samples did not change. Thus, there is no evidence that secretin was producing a dilute lymph or that the 'spike' was merely due to the displacement of concentrated dead-space lymph by a surge of dilute lymph.

Design 1 as discussed above was done with ten animals, each giving the typical responses as shown in figures 10 and 11. To see if any relationship existed between either lymph flow and the graded secretin doses or protein output and the graded secretin doses or both, statistical analysis of the results of the ten rats studied was performed. Multi-linear regression analysis gave polynomial equations for both sets of results, that is lymph flow and protein output.

For lymph flow the equation is $y = 0.17 + 0.34X - 0.05X^2$. This equation describes a polynomial dose-response curve which is the best fit for the data obtained. This is illustrated in figure 12. Within the system, as in any system, there exists an inherent variability. Hence,

to allow for this variability 95% confidence bands were calculated as shown in the figure. For protein output the equation is $y = 1.31 + 3.65x - 0.54x^2$ which also describes a polynomial dose-response curve which is the best fit for the data obtained. This is shown in figure 13. Again, to allow for inherent variability within the system, 95% confidence bands were calculated as shown in the figure. In both figures 12 and 13 there is an increase in response with increasing doses of secretin up to 3U which gives the maximum response. Thereafter, a diminished response occurs with the higher doses.

Although the porcine secretin was highly purified one could not exclude the possibility of a vasoactive contaminant. Thus, experiments with design 1 were repeated in three animals using synthetic secretin. The response of 1U of secretin had been well established by our initial experiments. Figure 14 illustrates that the effect of equivalent doses of natural and synthetic secretin are identical. The combined data of the 3 experiments was subjected to multi-linear regression analysis which again gave polynomial equations for both lymph flow and protein output.

For lymph flow the equation was $y = 0.50 + 0.28x - 0.04x^2$. The polynomial dose-response curve described by the equation is shown in figure 15 with its 95% confidence band. For protein output the equation is $y = 1.06 + 4.22x - 0.64x^2$. Figure 16 shows the polynomial dose-response curve this equation describes along with its 95% confidence band. Both figures 15 and 16 show an increase in response with increasing doses of secretin up to 3U, the maximum response, after which a diminished response occurs with higher doses.

The curves obtained for both natural and synthetic secretin was compared statistically to see if any difference existed between them.

The regression coefficients between equations were compared using Student's t-test at $P < 0.01$ for significance. Comparison showed that the equations for lymph flow from natural and synthetic secretin were not significantly different ($P > 0.01$). Also, the equations for protein output with natural and synthetic secretin were not significantly different ($P > 0.01$). These results strengthen the conclusion that the effects observed are due to secretin itself and not due to any possible contaminants. The 95% confidence bands for natural secretin were thought to better represent the inherent variability for protein output than those obtained with synthetic secretin.

These polynomial dose-response curves are not unlike the dose-response curves obtained by Domschke et al (1976). They studied the relationship between graded synthetic secretin doses and the cyclic AMP and bicarbonate concentrations in human pancreatic juice. Interestingly, they found a maximal effect after which there was a fall off with increasing doses of secretin.

Each polynomial curve has been drawn without including the origin. To do so one would be assuming that at 0 dose there was no change in lymph flow. This would be contradictory because there is an inherent variability in the system as is readily seen by the variation in the base levels. Even the saline control injection varies from no change, a slight negative change or slight positive change. Hence, the curves were drawn from actual data excluding theoretical assumptions.

The mechanism by which secretin produces an increase in lymph flow and protein output is unclear. As discussed previously it has been shown by a number of workers in species other than the rat that secretin is a selective splanchnic vasodilator which enhances superior mesenteric

blood flow. Two possibilities are considered as the possible mechanism by which secretin acted within this system. First, the observed effects could be due to an increased perfusion of the microcirculation of the gut, opening up non-perfused capillaries thus increasing the capillary surface area for exchange. Along with this a possible increase in intracapillary hydrostatic pressure may occur as a result of enhanced splanchnic blood flow or changes in pre- or post-capillary resistance vessels. Second, the observed effects could be due to a selective change in capillary permeability. In an attempt to gain some insight into the possible mechanism, further studies were done.

Initially, two lymph samples were electrophoresed on a polyacrylamide gel. The results are shown on figure 17 which is a densitometry scan of the gel. The dotted line represent the control sample, a pre-secretin collection. The solid line represents the sample taken after the bolus injection of secretin. Each sample was a 20 minute collection taken from an animal set up as described by design 1. Three peaks from each scan were identified, cut out, and weighed, with the ratio of post-secretin to control being determined from the weights. As illustrated no observable difference was obtained.

Subsequently with design 3 a steady state of enhanced lymph flow with an intravenous infusion of secretin was established (figure 18). Lymph mass is on the ordinate and time in minutes is on the abscissa. Each point represents a 2 minute collection. Saline was infused for 15 minutes as a control and gave little or no increase in lymph flow. An infusion of 0.25 U/min of natural secretin for 15 minutes followed the post-saline period.

At the initiation of the secretin infusion the lag phase of approximately 3 minutes can be noted, after which the rapid rise in lymph flow occurs. After the infusion of secretin is terminated there is a second lag phase during which the flow rate remains elevated for some time. This may be due to a combination of factors such as a delay in displacement of secretin from some receptor site and the time taken for the biological degradation of the hormone. Lymph flow finally falls back towards the baseline. Four rats were studied in this manner. Figures 19, 20, 21 and 22 show the protein concentration of successive lymph samples for each animal during the steady-state experiments. Each 2 minute lymph sample was submitted to the Bio-Rad assay method for protein determination. The protein for each sample is expressed as g/100 ml. Throughout the entire experiment no measurable change in the lymph concentration was observed in any of the four animals studied. All values for intestinal lymph protein concentration were between 2 and 3 g/100 ml as shown in the figures.

Each lymph sample from the steady-state experiments was electrophoresed on polyacrylamide gradient gels and scanned by densitometry. The results of the four rats studied are in figures 23, 24, 25 and 26. In these figures the scans obtained from the successive lymph samples are shown. No observable change in pattern occurred through the experiment. The enlarged scans in the right hand corner depict this. They represent a control sample as well as a sample obtained during enhanced lymph flow as a result of the secretin infusion.

As explained above six peaks were identified in each scan, cut out, and weighed. The area of each peak is expressed as a ratio relative to albumin, the largest peak, which was assigned a value of unity. Table

II shows the ratios obtained. For each animal there are five sets of ratios from the following sections of the experiment.

- 1) control
- 2) Saline infusion
- 3) post-saline infusion
- 4) secretin
- 5) post-secretin infusion

Each series of ratios shown in table II is the average of all the ratios for that section of the experiment. Comparing the sets of ratios for each animal one cannot conclude that any difference in pattern exists. Thus, we have no evidence that secretin caused a change in capillary permeability.

Vasoactive intestinal polypeptide a close relative of secretin was anticipated to have a similar effect upon intestinal lymph flow and protein output in the rat due to its potent vasodilator properties even though it has a very strong intestinal secretagogue property. Experiments done in three rats failed to show a lymphagogue role for vasoactive intestinal polypeptide at the doses chosen.

These results as obtained in one of the 3 animals are shown in figures 27 and 28. In figure 27 the effect of VIP on intestinal lymph flow is shown along with a saline control. The collections after both VIP and saline were within the baseline range suggesting no effect by either. Figure 28 shows the effect of VIP on protein output. Each sample collected from the three rats was assayed by the biuret method. Neither VIP nor saline had any effect on protein output. The experiments with VIP did not indicate a lymphagogue effect.

	Control	Saline	Post Saline	Secretin	Post Secretin
Rat 1	1.0 0.402 0.298 0.240 0.314 0.268	1.0 0.323 0.277 0.189 0.290 0.288	N.E.	1.0 0.405 0.306 0.236 0.413 0.301	1.0 0.381 0.298 0.210 0.370 0.266
Rat 2	1.0 0.425 0.392 0.228 0.454 0.344	1.0 0.521 0.393 0.259 0.491 0.339	1.0 0.430 0.293 0.237 0.419 0.240	1.0 0.439 0.314 0.216 0.400 0.290	1.0 0.516 0.305 0.274 0.503 0.317
Rat 3	1.0 0.455 0.315 0.298 0.555 0.372	1.0 0.471 0.257 0.230 0.328 0.255	1.0 0.426 0.237 0.229 0.295 0.178	1.0 0.443 0.201 0.220 0.355 0.174	1.0 0.466 0.236 0.262 0.532 0.258
Rat 4	N.E.	N.E.	1.0 0.427 0.292 0.245 0.612 0.235	1.0 0.460 0.196 0.194 0.659 0.223	1.0 0.533 0.228 0.188 0.610 0.185

Table II: Ratios relative to albumin of concentrations of 5 selected proteins in lymph samples fractionated by polyacrylamide gradient gel electrophoresis. (For details, see text p.28). Samples were obtained at basal lymph flow (control), during saline infusion, after saline infusion, during secretin infusion and after secretin infusion.

N.E. = not estimated.

Restraint Effects

It is important to consider the extent to which intervention has altered the physiological state of the animal in the present study.

Animals were kept in a Bollman-type restraint cage for a maximum of 30 hours (Fig. 3). While the Bollman cage restrains the rat sufficiently to prevent access to the cannula, the animal has a limited range of movement and appears comfortable throughout the experiment.

Restraint constitutes a form of stress (Pfeiffer, 1967). One important stress effect is gastrointestinal ulceration. Under the conditions described in the present experiments, however, such ulceration does not occur (Turner, 1978). Further, stress may cause gastric acid release which would in turn release endogenous secretin. Any effects observed in the present study are therefore superimposed on this background.

Lymphatic fistulae losses

An intestinal lymphatic fistula in the rat causes a considerable loss of fluid, electrolytes and protein. Intestinal lymph flows at approximately 2 ml/hr. This loss is offset by the intravenous and intraduodenal infusions of the isotonic saline which also replaced the sodium chloride. The protein loss is considerable. Over the 30 hours of the experiment about 60 ml of lymph having a protein concentration of approximately 2g% is collected. The total protein deficit during this period is therefore around 1.2 grams. It is unlikely that the animal could continue to sustain this rate of protein loss without serious depletion of its nitrogen stores. Thus, in the first post-operative day the disturbance of protein metabolism is probably not great enough to influence the results of the experimental manipulation.

Control-isotonic saline

In the present study 0.85% sodium chloride (saline) served as the control either as a 1 ml bolus or as a 15 minute infusion, both given intravenously. Intravenous saline will increase the extracellular fluid volume. In the present study although saline increased the extracellular fluid, at the volumes used it did not measurably increase the intestinal lymph flow. This was also the vehicle for the administration of hormones.

Explanation for the observed effects

The potent vasodilator action of secretin within the splanchnic circulation has been well established. The lymphagogic action of secretin has been demonstrated in the present study. However, the mechanism by which secretin produced these effect can still only be speculated upon. As discussed, secretin could possibly increase the permeability of the intestinal capillaries or could cause perfusion of previously non-perfused intestinal capillaries. Possibly an increased hydrostatic pressure within capillaries already perfused, brought about by alterations in pre- or post-capillary resistances could also be responsible. In any case, capillary filtration would be enhanced causing an increased fluid volume in the intestinal interstitium with a resultant increased intestinal lymph flow.

The idea of an increased capillary permeability is not new. Studies with fat feeding (Wollin, 1973) and fluid absorption from the lumen (Granger, 1978) suggested that the increased plasma proteins must be due to an increased capillary permeability. Further, Granger observed that the increase in intestinal permeability is related to the rate of volume absorbed from the lumen. In the present study examination of the spectra of plasma protein separated by gradient gel electrophoresis and of the

ratios obtained from densitometry scans of the gels (Table II) showed no evidence of alterations in the pattern of protein species as a result of lymph flow changes. Thus, these experiments suggest that secretin does not alter intestinal capillary permeability to macromolecules.

At any one time approximately 1/5 of the intestinal capillaries are being perfused with blood, according to Jacobson and Lanciault, (1979). Therefore, it is not unreasonable to assume that secretin may open up the non-perfused capillaries thus increasing the surface area available for filtration. This along with a possible increase in intracapillary hydrostatic pressure as a result of enhanced splanchnic blood flow seem, at present, the most probable explanations for these results. However, further experiments will have to be done in order to elucidate the mechanism by which secretin causes the augmented intestinal blood flow and hence augmented intestinal lymph flow.

For the fluid which accumulates in the intestinal interstitium as a result of the vasodilator actions of the gut hormones, three avenues of exit are available: the lymphatics, the blood capillaries and the intestinal lumen.

Nasset (1934) demonstrated a humoral control for fluid secretion into the lumen. Subsequently, this humoral control was attributed to many of the gut hormones. Studies show that one or the other routes of exit predominate depending upon the hormone in question.

As discussed, the present study concluded that secretin is a potent lymphagogue. Hubel (1967) with the rat and Bynum et al (1971) both showed that secretin in physiological doses that cause maximum pancreatic secretion, did not alter the movement of salt and water in the small intestine. Thus, if secretin does have a secretagogue role,

it is very minor.

In comparison, VIP did not have a lymphagogue effect in the present study. Since VIP has some vasodilator activity, an increase in superior mesenteric blood flow might occur with a subsequent increased filtration into the surrounding interstitial tissue. It is proposed that the present results may be explained by the fact that VIP has a very potent intestinal secretagogue effect. This has been demonstrated both in vivo and in vitro. Barbezat et al, (1971) studied the effect of several gastrointestinal hormones upon intestinal secretion in the dog. They found that while VIP, GIP, and glucagon all caused secretions in the ileum and jejunum, secretin and CCK did not. Again in 1973 Barbezat showed that VIP, GIP and glucagon were all stimulants of gut mucosal secretion while neither physiological nor pharmacological doses of secretin stimulated gut secretion. Further, he suggested that these secretagogue hormones may be involved in the watery diarrhea syndrome. Bloom et al (1973) demonstrated that elevated plasma levels and/or a high tumor content of VIP was associated with the watery diarrhea syndrome in six cases studied. Coupar (1976) observed that VIP infusions into the superior mesenteric artery caused a large net secretion of water and electrolytes into the lumen of the jejunum in rats. In 1977, Mailman measured the changes in mucosal blood flow in response to intravenous VIP in the dog. Again, VIP caused a secretion of sodium and water into the ileum.

The observations by Granger et al (1979) that VIP causes a fall in ileal lymph flow while causing a marked fluid and electrolyte secretion into the intestinal lumen are in keeping with the present results where no detectable alterations in lymph flow were obtained after intravenous

injections of VIP. Any excess mucosal interstitial fluid formed under the influence of the hormone is presumably channelled to the intestinal lumen.

A component of post-prandial hyperemia

In conclusion, gastrointestinal hormones are released postprandially and it is most probable that they have a role in the digestive process. In the present study, secretin both as bolus doses and as an infusion is a powerful intestinal lymphagogue in the conscious rat. VIP does not possess this action. Secretin already has a physiological role during digestion, viz its effect on the pancreas. Thus, the changes induced by secretin may be a component of postprandial intestinal hyperemia and enhanced gut lymph flow as demonstrated in a number of species.

SUMMARY

To examine the effects of secretin and vasoactive intestinal polypeptide on intestinal lymph flow and composition, adult, male Sprague-Dawley rats with intestinal lymphatic fistulae, duodenal cannulae, and tail-vein cannulae were studied. The study showed:

1) Secretin both as a bolus and as an infusion is a powerful intestinal lymphagogue in the rat.

2) Polynomial dose-response curves can be constructed for both lymph flow and lymph protein output with graded doses of secretin.

3) There is an increase in response with increasing secretin doses up to 3 U/ml, the maximum response, after which diminished responses occur.

4) The lymph flow change in response to secretin is transient. Following either a bolus injection or the initiation of an infusion there is a highly reproducible lag of approximately three minutes before the flow increases. After the bolus or the discontinuation of the infusion the lymph flow rate returns to basal values in a characteristic pattern over several minutes.

5) The lymph flow response to 1 U/ml of natural secretin and 1 U/ml of synthetic secretin is equivalent.

6) An excess amount of protein was recovered after each secretin dose. This resulted from enhanced lymph flow with a constant concentration of lymph protein.

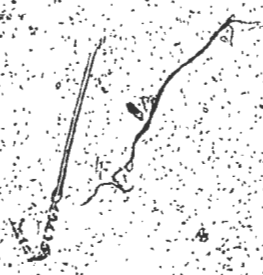
7) Plasma protein species and their relative proportions were not measurably altered during enhanced lymph flow caused by secretin as judged by polyacrylamide gel electrophoresis and polyacrylamide gradient gel electrophoresis.

8) Vasoactive intestinal polypeptide had no effect on intestinal

lymph flow or protein output at the doses studied.

9) These results are discussed in the context of gut blood flow regulation during digestive activity.

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