ABSTRACT

COMPARTMENTAL BLOOD FLOW IN THE CANINE JEJUNUM WITH FOOD OR 50% GLUCOSE IN THE LUMEN

By

Ya-Mei Tung Yu

The blood flow to the small intestine increases after meals or after ingestion of hypertonic glucose solution. It is not clear however, whether this increased flow is distributed equally to the three tissue layers of the intestine. The present study was designed to investigate the effects of luminal placement of digested dog food and 50% glucose on blood flow and its distribution in the three layers of the canine jejunum. The three layers were the mucosa, submucosa, and muscle-serosa of the jejunal wall. The radioactive microsphere method was used for this purpose. The validity of the method was also evaluated by using two types of microspheres, one labeled with cerium-141 and the other with strontium-85.

Isotonic polyethylene glycol (PEG), digested dog food, or 50% glucose was placed into the lumen of naturally perfused in situ jejunal segments in anesthetized dogs. PEG was used as the control of food and glucose. Twenty minutes after the luminal placement of these substances, two types of microspheres, 15 ± 5 μ in size, were injected into the left ventricle. These two types of microspheres were injected alternately at 3 minute intervals to each dog. In another group of dogs, 0.4%

dibucaine was placed into the lumen for 20 minutes before the placement of 50% glucose. The purpose was to see if local mucosal anesthesia would alter the response of blood flow to luminal placement of 50% glucose.

The segments containing food or 50% glucose had higher radioactivities in the whole wall and the mucosa than the control segments. The radioactivities in the submucosa and muscle-serosa of the segments containing food were not significantly different from those in the control segments. But in the segments containing 50% glucose, the radioactivity in the submucosa was slightly higher than or similar to that in the control segments and the radioactivity in the muscle-serosa was not different from that in the control segments. On the basis of percent distribution of total jejunal blood flow, the mucosa of the segments containing food or 50% glucose received the greater while the submucosa and muscle-serosa received the smaller shares of the total flow as compared to those in the control segments. The segments which were treated with dibucaine prior to placement of 50% glucose still had higher radioactivities in the whole wall and the mucosa than the segments containing PEG. But the segments had lower radioactivities in the whole wall and the mucosa than the segments containing 50% glucose.

The results obtained from microspheres labeled with Ce-141 correlated well with those labeled with Sr-85. This indicates that one type of microspheres may be used as a control for the other type.

Ya-Mei Tung Yu

It is concluded that luminal placement of food or 50% glucose in the jejunum increased total blood flow to the jejunum and caused redistribution of blood flow in the jejunal wall. Luminal placement of food caused an increase in mucosal flow, but no change in flow to the submucosa or muscle-serosa. Luminal placement of 50% glucose caused an increase in mucosal flow, a slight increase or no change in submucosal flow and no change in flow to the muscle-serosa. The increased blood flow caused by luminal placement of 50% glucose could be partly blocked by exposing the mucosa to a local anesthetic, dibucaine.

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Ya-Mei Tung Yu

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CHAPTER I

INTRODUCTION

The relation between intestinal blood flow and function is not only of interest to physiologists, but also of clinical importance in conditions such as the dumping syndrome. The majority of evidence indicates that splanchnic or the superior mesenteric arterial blood flow increases after meals (2, 3, 15, 20, 54). Some investigators proposed that the hyperemia results from increased cardiac output and involves all organs of the body (20). Recent studies (2, 3, 15, 54), however, indicate that following a meal the blood flow increases in the superior mesenteric artery and decreases in brachiocephalic and iliac arteries. The cardiac output is not changed. Thus, there is a redistribution of cardiac output favoring the vascular bed supplied by superior mesenteric artery at the expense of blood flow to other areas.

It has been also shown that the increased splanchnic blood flow following a meal is localized in the area exposed to chyme or in the area performing digestive and/or absorptive functions (10). Furthermore, the increased flow appears to occur only in the segment containing food or glucose (7). These studies thus appear to indicate that the increased flow following meals is a local phenomenon.

A review of the literature reveals that the information in regard to the effects of feeding on the compartmental blood flows of the jejunal wall is scarce. The present study was, therefore, designed to investigate the effects of luminal placement of digested dog food and 50% glucose on blood flow and its distribution in the wall of canine jejunum.

CHAPTER II

REVIEW OF LITERATURE

A. Feeding and Blood Flow

The majority of evidence indicates that mesenteric blood flow increases after meals. Herrick et al., in 1934 (20), used the thermo-stromuhr to measure blood flow in intact dogs and found that the superior mesenteric blood flow began to increase within 10 minutes after ingestion of a meal consisting of a milk-egg-glucose mixture and a dog food composed of meat and cereals. It reached a maximum (60% above the fasting level) 90 minutes after ingestion and then gradually declined and returned to nearly the original level four and one half hours after ingestion. They also found that the increase in the superior mesenteric arterial flow occurred concurrently with an increased flow in the femoral and carotid arteries and they surmised that an increase in cardiac output occurred postprandially.

Using electromagnetic flowmeters, Huse and Hinshaw in 1960 (22) showed, in dogs, an average increase of 45% of mesenteric blood flow when 50% glucose was injected into the proximal jejunum. The maximal effect was seen between 20 and 35 minutes after the glucose injection. In contradistinction, there was an average decrease of 34% in renal flow, 36% in carotid flow, and 32% in femoral flow. Cardiac outputs showed an average decrease of 20%. From these results, they suggested that there was a redistribution of the cardiac output favoring the expanded splanchnic vascular bed at the expense of blood flow to other areas.

By means of chronically implanted electromagnetic flow probes, Fronek and Stahlgren, in 1968 (15), showed in dogs that at 1 and 3 hours after ingestion of standard canned dog food there were no significant changes in cardiac output, heart rate, and mean arterial blood pressure. The flow in the superior mesenteric artery, however, increased to 133% and mesenteric regional resistance decreased to 82% of the control. Contrarily, the flows in the brachiocephalic and iliac arteries decreased to 86.5% and 74.6% of their controls, respectively. The ratio of flow in the superior mesenteric artery to cardiac output increased from 8.9% to 12.5% in the third hour. They concluded that there was a redistribution of blood flow during digestion with a preference for the vascular bed of the superior mesenteric artery.

Using the same method, Burns et al., in 1960 (2), found that the mesenteric blood flow in conscious dogs began to increase within 5 minutes after feeding of horsemeat and reached a maximum around 50 minutes. Although the flow gradually declined thereafter, the mean flow was still 50% above the fasting level 3 hours after feeding. There was no detectable rise in cardiac output in response to feeding except for occasional transient changes during ingestion. These results were similar to those reported by the same investigator in 1967 (3).

With the use of flowmeters, Vatner, Franklin, and VanCitters, in 1970 (54), found that in conscious dogs following an initial decrease of

10% during the anticipation of food, the mesenteric blood flow began to increase within 5-15 minutes and reached a maximum (from 115 to 300% of control) within 30-90 minutes after eating. The flow gradually returned to control levels within 3-7 hours. Transient increases in cardiac output, heart rate, and aortic blood pressure during anticipation and ingestion of food were followed by a return to control levels after 10-30 minutes and remained there throughout the experiments.

In 1972, by collecting local venous outflow, Chou and Dabney (7) showed an increase of 6%, 8%, 11% and 23% in blood flow of canine jejunum when 2.5%, 5.4%, 20% and 50% glucose solutions were placed into the jejunal lumen, respectively. Following the luminal placement of any glucose solution, venous outflow of the jejunal segment usually increased in the first three minutes and remained at the same level or increased slightly in the next eight minutes.

Brandt et al., in 1955 (1), studied the effect of oral protein and glucose feeding on splanchnic blood flow in normal and cirrhotic subjects by means of bromsulphalein (BSP) clearance method. They found that protein feeding induced an increase in splanchnic blood flow in both normal and cirrhotic subjects. The increased flow occurred sooner and was of greater magnitude in the normal subjects than the cirrhotic patients. They also found that glucose feeding resulted in minimal change in splanchnic blood flow in both normal and cirrhotic subjects. Using the same BSP method, Castenfors, Eliasch, and Hultman, in 1961 (4), found a 25% increase in splanchnic blood flow in normal subjects and a 70% increase in patients who were subjected to partial gastrectomy

19 minutes after the ingestion of hyperosmotic glucose solution. This appears to indicate that gastric emptying and thus the amount and/or duration of the presence of food in the small intestine is a significant factor influencing increased splanchnic flow.

From these studies, it can be seen that blood flow to the splanchnic viscera or small intestine increases after meals in dogs as well as in men. In general, the flow increases within 5-15 minutes and reaches a maximum within 20-90 minutes after ingestion of food. The flow then gradually returns to control within 4-7 hours. Although Herrick in 1934 suggested that no redistribution of cardiac output occurred post-prandially, recent studies, using electromagnetic flow-meters, has shown that there is redistribution of cardiac output in favor of the vascular bed of superior mesenteric artery.

In the above studies, only total splanchnic blood flow, superior mesenteric arterial flow, or blood flow to an intestinal segment was measured. The effects of digestion and absorption of food or glucose solution on blood flow distribution in the different compartments of the intestinal wall, however, have not been studied. Although Grim and Lindseth (16) made attempts to estimate the blood flow distribution in the intestinal wall during fasting and digestive states, they only showed the flow rate per 100 grams of each compartment. Percentage distribution of blood flow in the intestinal wall was not calculated. In order to determine the blood flow in the small intestine during digestive phase, the dogs were fed twice, 6 hours and 2 hours before the experiments. Whether the digested food was actually present in the intestinal loop at the time of sampling was not mentioned in their paper. In addition, they used glass microspheres labeled with Na-24 for measurement of blood flow. The specific gravity of these glass microspheres differs greatly from that of blood components. Also, they injected microspheres into the local artery of the isolated loop of intestine, instead of the left ventricle. These two factors could reduce the possibility of even distribution of microspheres with blood components and minimize the accuracy of the technique.

B. Methods for the Measurement of Compartmental Blood Flow in the Intestine

Three methods, namely, radioactive inert gas wash out technique, K-42 clearance technique, and radioactive microsphere technique, have been used to measure the compartmental blood flow in the intestine.

1. Radioactive Inert Gas (Kr-85 or Xe-133) Wash out Technique

The estimation of blood flow from clearance of radioactive tracers injected into the region of interest was first described by Kety in 1949 (28), and has been applied chiefly to the determination of cerebral (23) or myocardial (40) blood flow. Kampp et al. in 1966 (25) and Selkurt et al. in 1967 (45) applied the same technique to the small intestine. They measured and analyzed the multiple exponential curves obtained following intra-arterial injection of Kr-85 or Xe-133 to estimate total blood flow and flow distribution in the small intestine.

Theoretical considerations (26, 27, 29, 30)

After intra-arterial injection of radioactive inert gas, Kr-85 or Xe-133, multiple exponential curve was registered by a scintillation detector placed 4-6 cm from the intestine. The multiple exponential curve is composed of several components representing different elimination rates of injected substance. The relative amount of blood flow distributed to each tissue compartment which corresponds to each elimination rate can be estimated.

The elimination of an inert gas from a constantly and uniformly perfused tissue can be described by the following equation provided that the arterial concentration of the gas is zero or negligibly low during the wash out period:

$$C_t = C_o \cdot e^{-kt} \qquad 1$$

where C_t and C_o denote the tissue concentration of the gas at times t and o, and k denotes the clearance constant. The constant, k, which is closely related to the blood flow can be determined from the following equation:

$$k = \frac{\ln 2}{t_{\frac{1}{2}}} \qquad 2$$

where t ½ is the half time of decay in min. and is calculated from the straight line when plotting equation 1 on semilogarithmic paper.

If the disappearance of gas from two or more parallel-coupled, homogenously perfused tissues with different rates of clearance is simultaneously registered, the resulting elimination curve is the sum of the single exponentials according to the general formula

$$A_t = A1_o \cdot e^{-k1t} + A2_o \cdot e^{-k2t} + A3_o \cdot e^{-k3t} + \ldots + An_o \cdot e^{-knt} \qquad 3$$

Where A_t is total counts per min. at time t; $A1_o$, $A2_o$, $A3_o$, ... , An_o

are the number of counts per min. initially present in each component; and k1, k2, k3, ..., kn are the clearance constants of the different components. An arched curve is obtained when A_t is plotted semilogarithemically against time. Provided n (the number of components) is low and the k values sufficiently differ from each other, this composite curve can be resolved into its different components by means of the method originally described by Dobson and Warner in 1957 (14). Briefly, a straight line is drawn through the terminal straight portion of the curve and extrapolated to time zero. A new curve is constructed by subtracting this straight line from the original curve. A straight line is again drawn through the terminal straight portion of the constructed curve. The procedure is repeated until a final straight line is obtained. These straight lines represent the rates of clearance in different compartments.

In the small intestine, Kampp et al. resolved their curves into 4 and Selkurt et al. resolved into 3 components. The k values are obtained from equation 2. Correct estimations of the respective k values of the different components can be obtained only if the diffusion of the tracer between the different compartments is negligible.

The calculation of the blood flow is based on the assumption that the gas leaves the tissue compartment exclusively via the blood and the equilibrium of gas between blood and tissue is reached in a fraction of a second. The blood flow (f) in different compartments can be calculated in ml/min/100 gm from the following formula:

$$f = k \,.\, s \,.\, 100 \qquad 4$$

where s denotes the tissue blood partition coefficient of the gas divided by the specific weight of the tissue.

Kampp et al. (25) studied the blood flow and flow distribution within the denervated jejunum of the cat by injecting Kr-85 intraarterially and monitoring its clearance with a scintillation detector placed 4-6 cm from the intestine. They analyzed the multiexponential curve they obtained into four components based on the theoretical considerations described above. They proposed that component I (half time value 0.05 - 0.20 min; percent initial activity 35-50) reflects short circuit of Kr-85 via countercurrent exchange in villi, component II (half time value 1-2 min; percent initial activity 30-40) reflects blood flow within the mucosa, component III (half time value 4-9 min; percent initial activity 25) reflects blood flow of the muscularis, and component IV (half time value 20-60 min) is located outside the intestinal wall, probably in the perivascular fat of the mesentery. Flow values in ml/min/100 gm of musoca and muscularis were 30-70 and 10-20 respectively. They have used four independent methods to prove their impression of four different components in their registered curves. These methods are registration of the elimination of β-activity, local injections of Kr-85 into different parts of the intestinal segment, autoradiography and comparisons of weights of the compartments.

Based on the same principles, Selkurt et al. (45) estimated the compartmental blood flow in canine small intestine with the use of the Xe-133 wash out technique. They resolved the wash out curve into three components which reflected the blood flow to the epithelial glandular tissue, to smooth muscle, and to connective and supportive tissue. The average values per minute per 100 gm of these tissues were, respectively, 148.8, 35.7, and 3.4 ml. Applying these values to the anatomically distinct compartments gave a total flow in the wall of 64.2 ml/min/100 gm. The total blood flow measured simultaneously with the electromagnetic flow probe was 63.1 ml/min/100 gm.

2. K-42 Clearance Technique

In 1958, Sapirstein (43) used the clearance of K-42 or Rb-86 to estimate blood flow in various organs. Delaney and Grim, in 1964 (12) and 1965 (13), used the same technique to estimate the canine gastric blood flow and its distribution.

Theoretical consideration (12, 43)

This method is based upon the assumption that the extraction ratio of tracers in any organ in the body is the same and also all tissues in an organ have the same extraction ratio. It is also assumed that the K-42 contained in an organ or tissue 30-60 sec. after intravenous injection of a small bolus of the isotope is proportional to the blood flow perfusing that organ or tissue. With this method, the cardiac output can be estimated from the isotope dilution curve by the conventional Stewart-Hamilton equation:

$$\text{Cardiac output} = \frac{I}{\int_0^t c \, dt} \qquad 5$$

where I is the amount of isotope injected, c is the arterial radioactivity and t is time. Blood flow to any tissue or compartment, or to the whole organ, can be calculated as follows:

$$\text{Regional flow} = \frac{I_R}{I} \cdot \text{cardiac output} \qquad 6$$

where I_R is the amount of isotope recovered in the tissue at sacrifice. By combining equation 5 and 6, the formula for the calculation of regional flow becomes:

$$\text{Regional flow} = \frac{I_R}{\int_0^t c\,dt} \qquad 7$$

In other words, regional flow equals the K-42 content in the tissue divided by the area under the arterial dilution curve. Furthermore, the compartmental blood flow distribution, e.g., that in the intestinal wall, can be calculated simply by dividing the amount of isotope in each tissue layer by the amount of isotope in the whole intestinal wall.

Delaney and Grim (12) used this technique and found the distribution of blood flow among tissue layers of gastric corpus in intact anesthetized dogs to be mucosa 72%, submucosa 13%, and muscularis 15%, and that in intact unanesthetized dogs mucosa 74%, submucosa 14%, and muscularis 12%.

3. Radioactive Microsphere Method

The first application of this method to measure gastrointestinal blood flow was reported by Grim and Lindseth in 1958 (16). They used glass microspheres labeled with Na-24 to study the distribution of blood flow in different layers of the small intestinal wall of dogs. In 1964, Delaney and Grim (12) used the same method to determine the blood flow distribution in the wall of canine stomach.

Historical development of microspheres

Prinzmetal (38), the first investigator to use microspheres in the study of the circulation, used glass spheres to detect arterio-arterial and arteriovenous anastomoses in human hearts in 1947.

Subsequently, he and several other investigators (34, 37, 47, 51) injected glass microspheres of various sizes into the inflow of a number of organs of several species, and recovered the outflowing glass spheres. Although most of the studies were interpreted as demonstrating arteriovenous shunting, their conclusions have not been generally accepted (19). The problems with glass microspheres are their high density, the necessity of using large amounts and non-quantitative. Ryan prepared radioactive microspheres by neutron bombardment of glass microspheres to convert some of the sodium in the glass to Na-24 (55). This greatly improved the previous technique and was adopted by others. Ceramic microspheres labeled with different isotopes were developed subsequently by Lahy and Ryan. By 1966, ceramic microspheres with density of 3.0 gm/cc and plastic microspheres with density of 1.3 gm/cc were available labeled with any of 27 different radionuclides (55).

The plastic microspheres, supplied by the Nuclear Product Division of the Minnesota Mining and Manufacturing Company, are referred to as "carbonized microspheres". They are black in appearance and consist of carbon, hydrogen, oxygen and a trace amount of the nuclide of interest. The nuclide is incorporated in the microspheres and is not merely a coating on the surface. The microspheres resist temperatures up to $400^{\circ}C$, above which they begin to disintegrate. They are quite insoluble in all common organic or inorganic solvents at room temperature but can be dissolved by boiling in concentrated acids or bases.

<u>Theoretical consideration</u> (55)

The application of microspheres to the study of the circulation rests on four basic assumptions: 1) the microspheres are adequately mixed with the blood and have essentially the same rheology as red blood cells; 2) the microspheres themselves do not alter the circulation; 3) the radioactivity remains bound to the microspheres during the period of observation; and 4) the microspheres are sufficiently large to be removed from the circulation by lodging in the capillary beds. If the above assumptions are all true, the microspheres are mixed with the inflowing blood and distributed in the same manner as blood. The microspheres, however, are entrapped in the capillaries of the perfused tissues and are completely cleared from the blood in a single passage. The number of microspheres in the organ or tissue thus can be calculated from the following equation:

$$q = f \int_0^t c(t)dt \qquad 8$$

where q is the number of microspheres in the organ or tissue of interest, f is blood flow (ml/min) to the organ or tissue and c is the concentration of microspheres in the blood (microspheres/ml). Since the radioactivity remains bound to the microspheres during the period of observation, the distribution of the radioactivity is a measure of the relative distribution of blood perfusing the region at the time of injection of the microspheres. Therefore, the blood flow distribution in the intestinal wall can be estimated from the distribution of radioactivity among its three compartments, i.e., the mucosa, submucosa, and muscle-serosa.

Experiments which support the assumptions

1. The adequacy of mixing of the microspheres:

Shibata and MacLean, in 1966 (55), demonstrated uniform distribution of microspheres in the lung by obtaining multiple small tissue samples after an intravenous injection of radioactive microspheres. Phibbs et al. in 1967 (36) and 1970 (35) and Neutze et al. in 1968 (33) studied the distribution of radioactive microspheres within flowing blood by ultra-rapid freezing of femoral arteries in rabbits. Using this technique, blood flowing through a segment of the artery was immobilized in less than 0.05 seconds. The serial cross sections were examined microscopically. They found that the majority of the microspheres were in the more central three-fourths of the arterial lumen, where flow is faster. The finding suggests that the distribution of microspheres is at least similar, if not identical to that of the flowing blood cells.

If axial streaming were important, there could be a difference in the concentration of microspheres in carotid and femoral arterial blood samples after left ventricular injection. Neutze et al. (33) compared the amount of radioactivity in carotid and femoral arterial blood withdrawn simultaneously during left ventricular injection of microspheres. They found that there was no difference between the radioactivities in blood samples collected from these two arteries. Therefore, significant preferential streaming to either artery is excluded.

Rudolph and Haymann, in 1967 (42), measured the flows in the two umbilical veins of fetus of sheep and goats with electromagnetic flowmeters and simultaneously measured the radioactivity in the placenta following injection of radioactive microspheres. They found that the ratio of flows in the two umbilical veins as determined by flowmeters was similar to the ratio of radioactivities in each part of the placenta drained by each vein. In addition, they used a physical model to prove this principle. The physical model was composed of a rotary pump and a system of four branching tubes, the flow through each of these tubes could be regulated. They also found satisfactory agreement between the measured flow and the distribution of radioactive microspheres. Neutze et al., in 1968 (33), simultaneously collected the blood flow from the right and left femoral arteries during an injection of radioactive microspheres into the left ventricle. The volume and radioactivity of the blood from each artery were measured. They found that the ratio of the flows of the two femoral arteries determined by direct collection was in close agreement with the ratio of the radioactivities in the collected blood. These studies indicate that the microspheres are distributed in direct proportion to blood flow.

Kaihara et al., in 1968 (24), injected microspheres labeled with two different radionuclides, Sc-46 or Yb-169, successively into the left atrium, left ventricle, or at the origin of the aorta to test the completeness of mixing of the microspheres after injection. The interval between the two injections was 5 to 10 minutes. If mixing of microspheres was complete, the distribution of the two differently labeled microspheres would be the same. They found that when microspheres were injected into the left atrium, there was no difference in the distribution of the two radionuclides. When microspheres were injected into the left ventricle or at the origin of the aorta, all organs showed the same distribution except the heart. These results suggested that when microspheres were injected into the left ventricle or aorta, mixing was not complete at the origin of the coronary artery. On the other hand, when they were injected into the left atrium, mixing was complete before microspheres left the heart. Therefore, they concluded that the microspheres should be injected into the left atrium if the distribution of flow in the cardiac tissues is to be studied. If the relative blood flow to areas other than the heart is of interest, injection at the origin of the aorta or into the left ventricle is satisfactory.

2. The effect of microspheres on the circulation:

The experiments described above, done by Kaihara et al. in 1968 (24), also demonstrated that injection of microspheres per se has no effect on the circulation. They injected one type of radioactive micropheres into the canine left heart soon after another type of microspheres labeled with a different nuclide. The two injections produced the same distribution of cardiac output suggesting that the first injection did not alter the distribution of blood flow in the body.

Hoffbrand and Forsyth, in 1969 (21), made duplicate determinations at an interval of 24 hours. They showed that the presence of microspheres in the tissues does not disturb the circulation at rest since the monkey had a satisfactorily constant distribution of cardiac output, organ flow and resistance. The more acute effects of microsphere infusions were also studied in conscious monkeys. Four separate batches of radioactive microspheres were given over a two hour period. The changes in the distribution of cardiac output as compared to the results obtained from the first batch of microspheres were generally not significant. There was, however, a small but statistically significant increase in total peripheral resistance with the third batch.

3. The fate of radioactive carbonized microspheres following injections:

If the radioactivity remains bound to the microspheres, the distribution of the radioactivity in the body will not be altered during the period of observation. To test this, Kaihara et al. (24) injected radioactive carbonized microspheres, 50 μ in diameter, labeled with Sc-46, into the left atrium of dogs. The concentration of radioactivity in various organs was then measured over a two-week period by means of an external detector. In the first 5 days, the radioactivity in the urine and feces was measured. They found there was no change in the organ content of radioactivity over the two-week period. The radioactivity found in the urine and feces during the first 5 days was negligible. They concluded that the carbonized microspheres were not metabolized and stayed in the capillaries almost indefinitely.

4. The extraction efficiency of microspheres by capillary beds:

Kaihara et al. (24) found that no recirculation of microspheres of either 15 μ or 50 μ in diameter following intravenous injections. All of the microspheres, i.e., 100% of radioactivity, injected were recovered in the lungs and no radioactivity was detected in the liver, kidneys and spleen. When microspheres with 15 μ in diameter were injected into the internal carotid artery, the common carotid artery, or the abdominal aorta, about 5-10% of microspheres passed through the systemic vascular beds and appeared in the lungs. But no detectable radioactivity was found in the lungs when the microspheres of 50 μ in diameter were injected into a systemic artery. Thus, small microspheres

(15 μ) are not completely removed by some peripheral vascular beds. Since the lungs can completely remove the microspheres of 15 μ diameter (24), recirculation will not be a problem if the microspheres are injected into the systemic arterial system.

Applications of radioactive microsphere method to the study of gastrointestinal blood flow and its distribution

Grim and Lindseth, in 1958 (16), used radioactive microsphere method to study the distribution of blood flow in different tissue layers of the small intestine of dogs. They injected 12 μ glass microspheres, labeled with Na-24, into the local artery of an isolated loop of small intestine to estimate capillary blood flow. Venous outflow of the loop was also simultaneously measured by collection of venous outflow. The loop was then removed from the animal and separated into four tissue fractions, i.e., the mucosa, submucosa, muscle, and mesentery. The radioactivity of each tissue sample and venous blood was determined. They found that the capillary flows per unit weight of the mucosa, submucosa, muscle, and mesentery in fasted jejunum and ileum were about the same (40-50 ml/min/100 gm). A possible exception was flow to the ileal muscle which was slightly higher (55 ml/min/100 gm). By using different sizes of microspheres, they also showed that the arteriovenous anastomotic flow was very low in the intestine (2-4%).

Delaney and Grim, in 1964 (12), estimated the canine gastric blood flow distribution by using two different methods, K-42 clearance and microsphere methods. Radioactive microspheres, 16 μ or 20 μ in diameter, labeled with Na-24, were injected into the celiac artery. The distribution of capillary blood flow among tissue layers of gastric body determined by the radioactive microsphere technique was mucosa 68%, submucosa 11%, and muscle 21%. These data were in close agreement with those determined by K-42 method which showed mucosa 72%, submucosa 13%, and muscle 15%. They concluded that both methods provide reasonably accurate measures of blood flow distribution in the stomach.

CHAPTER III

MATERIALS AND METHODS

1. Preparation of Radioactive Microspheres

Carbonized microspheres labeled with either cerium-141 or strontium-85 were obtained from the Nuclear Product Division of the Minnesota Mining and Manufacturing Company (3M Center, St. Paul, Minnesota). The size of the microspheres was 15 μ in diameter with a variation of ± 5 μ. The specific activity was 10 millicuries/gm. One milligram of the microspheres contained about 440,000 microspheres. The stock microspheres were suspended in a solution of 10% dextran (1 millicurie/10 ml). A drop of Tween 80 (polyoxyethylene sorbitan mono-oleate) was added to the stock solution to prevent aggregation of the microspheres. Microsphere suspensions to be used for experiments were prepared from the stock solution a few minutes before use. Four-tenth ml of the stock microspheres was added to 2 ml of 20% dextran. The suspension was then treated with an ultrasonic sonifier cell disruptor (Branson Instrument Co., Long Island, N.Y.) to achieve uniform dispersion of the microspheres.

2. Preparation of Digested Dog Food

One can of dog food (Alpo liver or beef, Allen Products Co., Allentown, Pa.) was mixed in an electric blender until its consistency was that of a thick, smooth milk shake. To the blended food was added

0.75 grams of pancreatin (whole porcine pancreas containing protease, amylase, lipase, esterases, peptidases, nucleases, elastase, Viokase^{R}, Viobin Co., Monticello, Ill.). The pH was adjusted to about 7.0 by adding sodium bicarbonate. Then, the mixture was stirred for 5 hours with a magnetic stirrer (Thomas stirrer, Model 15, Arthur H. Thomas Co., Philadelphia, Pa.) at room temperature.

3. Surgical Procedures

A. Group A

Ten mongrel dogs of either sex, fasted for 24 hours, weighing between 13 and 15 kilograms were used. They were anesthetized with sodium pentobarbital (30 mg/kg body weight) and ventilated with a positive pressure respiration pump (Harvard, Model No. 607, Dover, Mass.) via an endotrachael tube. A polyethylene tube, filled with heparinized saline, was inserted into a femoral artery and connected to a Statham pressure transducer (Model No. P 23 Gb) for monitoring the systemic arterial pressure. The abdominal cavity was opened through a midline incision. A loop of the jejunum about 20 cm aboral to the ligament of Treitz was exteriorized and divided into four segments according to the natural vascular pattern. A rubber tube, with an outer diameter of 0.5 cm, was placed into the lumen of each segment for introduction of the substances to be tested. Both ends of each segment were tired and the mesentery was cut to exclude collateral flow. Thus, four separate and naturally perfused in situ segments were formed. The segments were kept moist and warm during the experiment by covering them with a sheet of plastic film and by a heating lamp.

B. Group B

In another eight dogs, the same surgical procedures were performed as described above except that 3 segments of jejunum were prepared.

Following the above procedures, in the group A or B, a special catheter used in angiography (Bardic deseret Angiocath, catheter 16 G x 2¼", needle 19 G x 2 3/4") was inserted into the left ventricle of the heart through the chest wall. The needle inside the catheter was then removed. The presence of the catheter in the left ventricle was confirmed by connecting the catheter to a Statham pressure transducer (Model No. P 23 Gb) to record the pressure. The catheter was then fixed to the chest wall with a suture to prevent it from moving out of the left ventricle. This catheter was used for injecting radioactive microspheres.

4. Experimental Procedures

A. Group A

One segment of the four prepared jejunal segments was left empty but each of the other three segments received 10 ml of either isotonic nonabsorbable polyethylene glycol (PEG), digested dog food or 50% glucose. PEG was used as the control of food and 50% glucose. The test solutions were placed into the four segments in random sequence.

B. Group B

The experiment in this group was designed to see if local mucosal anesthesia will alter the response of blood flow to luminal placement of 50% glucose. One jejunal segment was pretreated by luminal placement of 10 ml of 0.4% dibucaine for 20 minutes. Dibucaine was then withdrawn and 10 ml of 50% glucose was introduced into the lumen. At the same time, each of the other two segments received 10 ml of either PEG or 50% glucose. PEG was again used as a control.

In both groups, the test solutions remained in the lumen for 20 minutes. Two types of the prepared microspheres (Ce-141 and Sr-85) were injected into the left ventricle at 3 minute intervals through the preinserted catheter in each dog. The purpose of injecting two types of microspheres in the same dog was to see if they would produce similar results. The order of the injections was thus alternated in the experiments. The dog was then sacrificed by an intracardiac injection of the saturated potassium chloride solution. The segments were removed and the mesentery was trimmed off from the segments. Each segment was separated into three portions, i.e., the mucosa, submucosa, and muscle plus serosa. This dissection was accomplished easily, the mucosa and muscle were simply scraped off the submucosa with a blunt instrument. Each tissue sample, in duplicate, was placed into the preweighed plastic counting tube. The actual weight of each tissue sample was calculated after reweighing the counting tube. In order to get an accurate counting, each tissue sample was placed in the bottom of the counting tube not exceeding 2 cm in height and care was taken to avoid sticking the tissue on the wall of the tube. The amount of radioactivity of each nuclide was measured with a scintillation gamma spectrometer (Parkard Instrument Co., Model 3002 Tri-carb scintillation spectrometer).

5. Measurement of Radioactivity

Separation of two isotopes in each tissue sample was performed according to the following steps. The windows of the spectrometer were set so that the window A included the main energy peaks of gamma spectrum of Ce-141 and the window B included that of Sr-85. The energy peaks were determined by counting a small amount of the stock microspheres labeled with Ce-141 or Sr-85 with the spectrometer. The determinations were done for each batch of the microspheres. The channels 1 and 2 of the spectrometer recorded the radioactivities counted in the windows A and B respectively. Since the window A would count not only the radioactivity of Ce-141, but also some radioactivity of Sr-85, the latter should be excluded to obtain the real radioactivity of Ce-141 counted in window A. The same phenomenon would also occur in window B. The real radioactivity of each isotope was calculated as follows:

$$\text{Ce-141 (cpm)} = \frac{b\ (\text{Total cmp} - \text{BG. cpm})_{\text{Ch.1}} - (\text{Total cpm} - \text{BG. cpm})_{\text{Ch.2}}}{b - a} \qquad 9$$

$$\text{Sr-85 (cpm)} = \frac{b\ [(\text{Total cpm} - \text{BG. cpm})_{\text{Ch.2}} - a\ (\text{Total cpm} - \text{BG. cpm})_{\text{Ch. 1}}]}{b - a} \qquad 10$$

where: BG. = background, Ch. 1 = channel 1, Ch. 2 = channel 2.

$a = (\text{Ce cpm})_{\text{Ch.2}}/(\text{Ce cpm})_{\text{Ch. 1}}$, $b = (\text{Sr cpm})_{\text{Ch. 2}}/(\text{Sr cpm})_{\text{Ch. 1}}$

The cpm of Ce-141 or Sr-85 in each channel was obtained by counting the blood sampled from a femoral artery immediately following the injection of each type of microspheres. Since there was no recirculation of microspheres in the systemic arterial blood, the sampled blood contained only one type of microspheres. The tissue samples, however, contained both types of isotopes. Therefore, the data obtained from blood samples as calculated as a and b were used to obtain and real radioactivity of Ce-141 and Sr-85 in the tissues.

Formulas 9 and 10 shown above were derived from the following steps:

In both channel 1 and channel 2,

$$\text{Total cpm} = \text{Ce cpm} + \text{Sr cpm} + \text{BG. cpm}$$

$$(\text{Ce cpm})_{\text{Ch. 1}} = (\text{Total cpm})_{\text{Ch. 1}} - (\text{Sr cpm})_{\text{Ch. 1}} - (\text{BG. cpm})_{\text{Ch. 1}}$$

since $b = (\text{Sr cpm})_{\text{Ch. 2}}/(\text{Sr cpm})_{\text{Ch. 1}}$

$$(\text{Ce cpm})_{\text{Ch. 1}} = (\text{Total cpm})_{\text{Ch. 1}} - \frac{(\text{Sr cpm})_{\text{Ch. 2}}}{b} - (\text{BG. cpm})_{\text{Ch. 1}}$$

$$= (\text{Total cpm})_{\text{Ch. 1}} - \frac{(\text{Total cpm})_{\text{Ch. 2}} - (\text{Ce cpm})_{\text{Ch. 2}} - (\text{BG. cpm})_{\text{Ch. 2}}}{b} - (\text{BG. cpm})_{\text{Ch. 1}}$$

since $a = (\text{Ce cpm})_{\text{Ch. 2}}/(\text{Ce cpm})_{\text{Ch. 1}}$

$$(\text{Ce cpm})_{\text{Ch. 1}} = (\text{Total cpm})_{\text{Ch. 1}} - \frac{(\text{Total cpm})_{\text{Ch. 2}} - a(\text{Ce cpm})_{\text{Ch. 1}} - (\text{BG. cpm})_{\text{Ch.2}}}{b} - (\text{BG. cpm})_{\text{Ch. 1}}$$

$$(b - a)(\text{Ce cpm})_{\text{Ch. 1}} = b\,(\text{Total cpm} - \text{BG. cpm})_{\text{Ch. 1}} - (\text{Total cpm} - \text{BG. cpm})_{\text{Ch. 2}}$$

by moving (b - a) to the right side of the equation, the equation becomes formula 9.

$$(\text{Sr cpm})_{\text{Ch. 2}} = (\text{Total cpm})_{\text{Ch. 2}} - (\text{Ce cpm})_{\text{Ch. 2}} - (\text{BG. cpm})_{\text{Ch. 2}}$$

since $a = (\text{Ce cpm})_{\text{Ch. 2}}/(\text{Ce cpm})_{\text{Ch. 1}}$

$$(\text{Sr cpm})_{\text{Ch. 2}} = (\text{Total cpm})_{\text{Ch. 2}} - a\,(\text{Ce cpm})_{\text{Ch. 1}} - (\text{BG. cpm})_{\text{Ch. 2}}$$

$$= (\text{Total cpm})_{\text{Ch. 2}} - a\,[(\text{Total cpm})_{\text{Ch. 1}} - (\text{Sr cpm})_{\text{Ch. 1}} - (\text{BG. cpm})_{\text{Ch. 1}}] - (\text{BG. cpm})_{\text{Ch. 2}}$$

since $b = (\text{Sr cpm})_{\text{Ch. 2}}/(\text{Sr cpm})_{\text{Ch. 1}}$

$$(\text{Sr cpm})_{\text{Ch. 2}} = (\text{Total cpm})_{\text{Ch. 2}} - a\,[(\text{Total cpm})_{\text{Ch. 1}} - \frac{(\text{Sr cpm})_{\text{Ch. 2}}}{b} - (\text{BG. cpm})_{\text{Ch. 1}}] - (\text{BG. cpm})_{\text{Ch. 2}}$$

$$(b - a)\,(\text{Sr cpm})_{\text{Ch. 2}} = b\,[(\text{Total cpm} - \text{BG. cpm})_{\text{Ch. 2}} - a\,(\text{Total cpm} - \text{BG. cpm})_{\text{Ch. 1}}]$$

by moving (b - a) to the right side of the equation, the equation becomes formula 10.

6. Calculation and Expression of the Results

A. Total radioactivity in the whole wall of the jejunum

Since total radioactivity in the whole wall of the jejunum was technically difficult to measure, the total radioactivity in the whole wall was calculated from the radioactivity of each tissue layer (cpm/gm) and the weight distribution among the three tissue layers (as % of the whole wall). The weight distribution in the jejunal wall was obtained from randomly selected 12 dogs (see below). The formula for calculation of the total radioactivity in the whole wall was:

Total radioactivity in the whole wall (cpm/gm) =

(cpm/gm mucosa x mucosa weight % + cpm/gm submucosa x submucosa weight % + cpm/gm muscle-serosa x muscle-serosa weight %) x 10^{-2} 11

B. Flow distribution among three tissue layers of the jejunum

Since the microspheres were well mixed with the inflowing blood and distributed in the same manner as blood and entrapped in the capillaries of the perfused tissues, the distribution of the radioactivity in the tissues is a measure of the distribution of blood flow. The flow distributed to each layer in % of the total blood flow of the jejunum was determined from the following formula:

$$\begin{aligned}&\text{Radioactivity in one compartment (\% of total radioactivity)}\\&\quad = \text{Blood flow to the compartment (\% of total blood flow)}\\&\quad = \frac{\text{radioactivity of the compartment (cpm/gm)} \times \text{weight percentage of the compartment (\%)}}{\text{radioactivity of the whole wall (cpm/gm)}}\end{aligned} \qquad (12)$$

7. Weight Distribution of the Canine Jejunal Wall

Twelve mongrel dogs of either sex, weighing between 13 to 15 kilograms were anesthetized with sodium pentobarbital (30 mg/kg body weight). The abdominal cavity was opened through a midline incision and the jejunum was exposed. One segment of the jejunum was randomly selected and removed. After the mesentery was trimmed off, the jejunal wall was separated into the three portions, i.e., the mucosa, submucosa, and muscle plus serosa in the same way as described above. The dissected three layers were checked microscopically to make sure that the separation was complete. All of the tissue samples obtained from each layer were weighed. The weight distribution in the jejunal wall in each dog was calculated by dividing the weight of each tissue layer of a segment by the total weight of the segment.

8. Statistical Analysis of the Results

Student's t test for paired comparison, correlation coefficient, and regression analysis were employed in statistical analysis of the results (49).

CHAPTER IV

RESULTS

In all experiments, the systemic arterial pressure remained constant throughout the experiments. The average systemic arterial pressure was 120 mm Hg. The systemic arterial pressure was not altered following injections of radioactive microspheres or following placement of test solutions into the lumens of the jejunal segments.

Since good separation of the three tissue layers of the jejunal wall is essential for reliable results, many preliminary studies were performed to refine the technique of the separation. During these studies the dissected three layers were examined microscopically. As can be seen in Figures 1, 2, and 3, separation of the three tissue layers was perfected by these studies.

1. Mean Weight Distribution in the Jejunal Wall

Table 1 shows the mean weight distribution in the jejunal wall determined in 12 randomly selected mongrel dogs. The standard errors for the means were all small. This indicates that the relative weights in the jejunal wall varies little from animal to animal. In this study, these means were used to calculate the radioactivity, in cpm per gram tissue weight, of the whole wall as shown in the following formula (see Formula 11 on page 27).

Figure 1. Photomicrograph of the mucosa of the canine jejunum after separating it from the submucosa and muscle-serosa.

Figure 2. Photomicrograph of the submucosa of the canine jejunum after separating it from the mucosa and muscle-serosa.

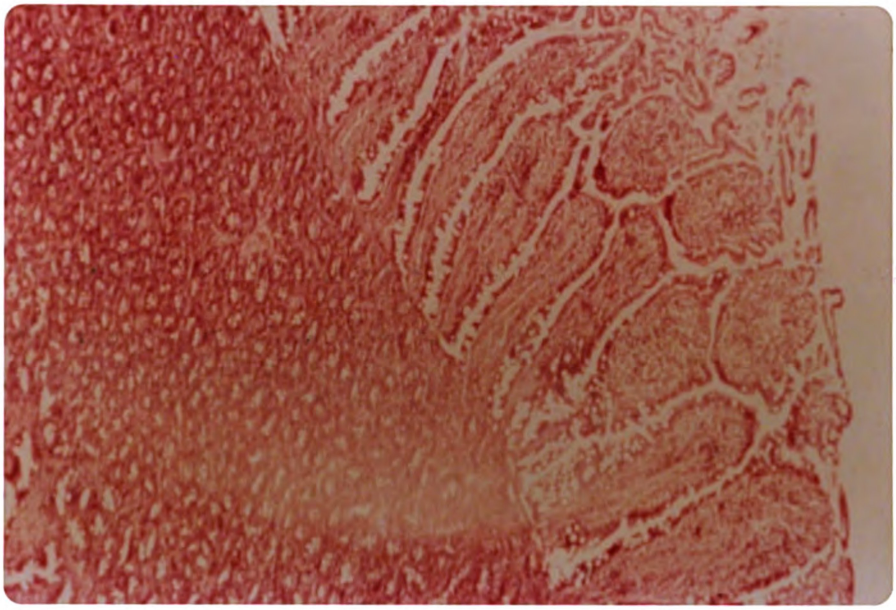

Figure 1

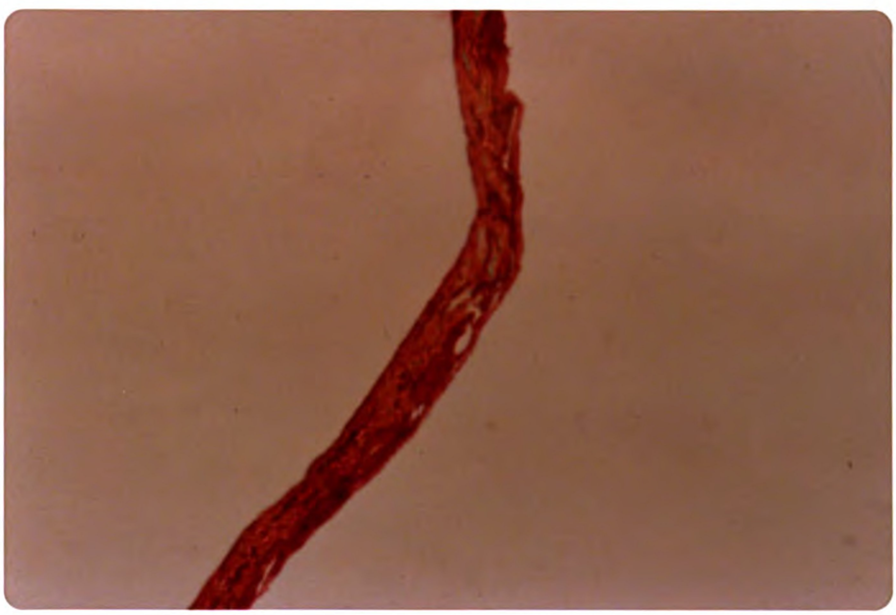

Figure 2

Figure 3. Photomicrograph of the muscle-serosa of the canine jejunum after separating it from the mucosa and submucosa.

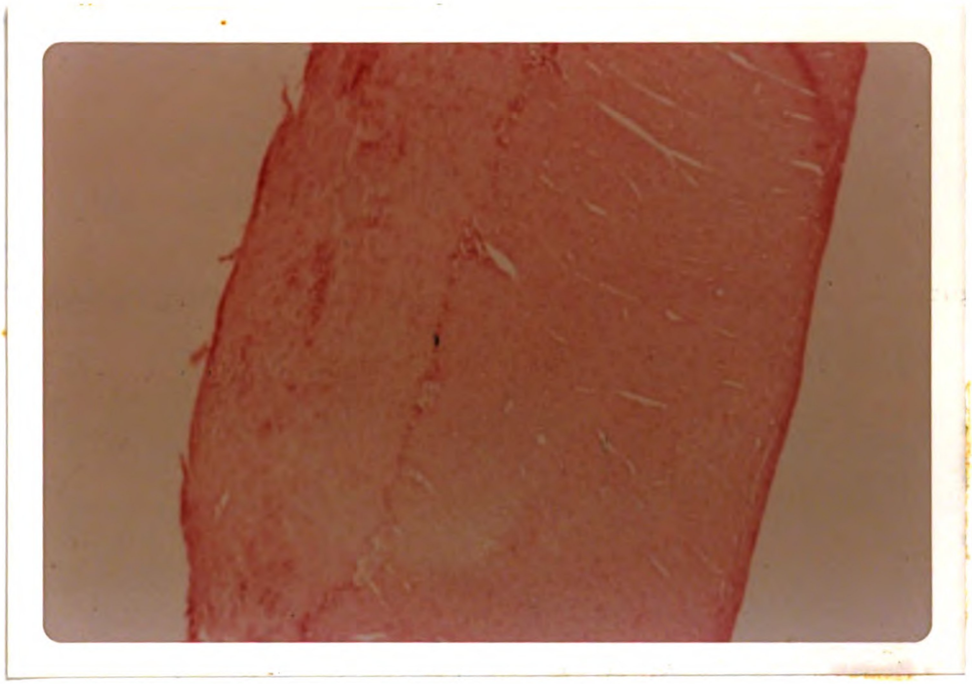

Figure 3

Table 1.--Weight distribution in the jejunal wall (%).
(mean ± S.E.) (N = 12)

Tissue layer	Weight, %
Mucosa	63.1 ± 0.9
Submucosa	11.9 ± 0.5
Muscle-Serosa	25.0 ± 0.7

Total radioactivity in the whole wall (cpm/gm) =

$$\text{cpm/gm mucosa} \times 0.631 + \text{cpm/gm submucosa} \times 0.119 + \text{cpm/gm muscle-serosa} \times 0.250 \qquad 13$$

These means were also used to calculate the percentage distribution of the radioactivity in the wall and thus the percentage distribution of the total blood flow among the three tissue layers of the jejunal wall as shown in the following formulas (see Formula 12 on page 28).

Radioactivity in mucosa (% of total radioactivity)

= Blood flow to mucosa (% of total blood flow)

$$= \frac{\text{cpm/gm mucosa} \times 0.631}{\text{cpm/gm whole wall}} \times 100\% \qquad 14$$

Radioactivity in submucosa (% of total radioactivity)

= Blood flow to submucosa (% of total blood flow)

$$= \frac{\text{cpm/gm submucosa} \times 0.119}{\text{cpm/gm whole wall}} \times 100\% \qquad 15$$

Radioactivity in muscle-serosa (% of total radioactivity)

= Blood flow to muscle-serosa (% of total blood flow)

$$= \frac{\text{cpm/gm muscle-serosa} \times 0.250}{\text{cpm/gm whole wall}} \times 100\% \qquad 16$$

2. <u>Effects of Luminal Placement of PEG, Food, and 50% Glucose</u>

A. <u>Results obtained from the microspheres labeled with Ce-141</u>:

The average radioactivities of Ce-141 of the whole wall, the mucosa, submucosa, and muscle-serosa of the empty segments and the segments containing PEG, food, and 50% glucose are shown in Table 2. As can be seen in Table 2, the radioactivities of the whole wall, the mucosa, submucosa, and muscle-serosa of the segments containing PEG were

Table 2.--The average radioactivity (cpm/gm tissue) of Ce-141 of the whole wall, the mucosa, submucosa, and muscle-serosa of the segments which were empty or containing PEG, food, or 50% glucose. (mean $\pm$ S.E.) (N = 10)

	PEG	Food	50% Glucose	Empty
Whole Wall	4015 $\pm$ 355	6004 $\pm$ 816*	9430 $\pm$ 1317**	4734 $\pm$ 546
Mucosa	4303 $\pm$ 414	6770 $\pm$ 759**	11693 $\pm$ 1591**	5240 $\pm$ 628
Submucosa	1647 $\pm$ 230	2101 $\pm$ 370	2939 $\pm$ 719*	1966 $\pm$ 345
Muscle-Serosa	4412 $\pm$ 547	5930 $\pm$ 1574	6808 $\pm$ 1521	4774 $\pm$ 1137

*Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.05$.

**Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.01$.

not significantly different from those of the empty segments. These results thus indicate that luminal placement of 10 ml of PEG for 20 minutes did not significantly alter the total blood flow and compartmental blood flow of the jejunum. The segments containing food, on the other hand, had significantly higher radioactivities in the whole wall and the mucosa as compared to the segments containing PEG. The radioactivity in the submucosa or muscle-serosa of the segments containing food however was not significantly different from that of the segments containing PEG. The segments containing 50% glucose had significantly higher radioactivities in the whole wall, the mucosa, and submucosa as compared to the segments containing PEG. The radioactivity in the muscle-serosa of the segments containing 50% glucose was not significantly different from that of the segments containing PEG.

Table 3 shows the percentage distribution of the blood flow among the three tissue layers of the jejunal wall following luminal placement of various solutions. The percentage distribution in the jejunal segments containing PEG was similar to that in the empty segments. In the segments containing food, the mucosa had, on the average, a greater share of the total blood flow than that in the segments containing PEG. Although the difference between these two segments was not statistically significant at p values less than 0.05, it was statistically significant at p values less than 0.1. In the segments containing 50% glucose, the mucosa had significantly greater and the submucosa or muscle-serosa had significantly smaller shares of the total blood flow than those in the segments containing PEG.

Table 3.--Average percentage distribution of blood flow in the wall of the jejunal segments which were empty or containing PEG, food, or 50% glucose as determined by Ce-141 labeled microspheres. (mean ± S.E.) (N = 10)

	PEG	Food	50% Glucose	Empty
Mucosa	67.3 ± 2.7	72.7 ± 3.5@	78.7 ± 2.3**	70.1 ± 3.5
Submucosa	5.0 ± 0.6	4.7 ± 0.9	3.9 ± 0.8*	5.3 ± 0.8
Muscle-Serosa	27.7 ± 2.7	22.6 ± 3.5	17.4 ± 2.3**	24.6 ± 3.3

@Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.1$.

*Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.05$.

**Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.01$.

B. <u>Results obtained from the microspheres labeled with Sr-85</u>:

The average radioactivities of Sr-85 of the whole wall, the mucosa, submucosa, and muscle-serosa of the empty segments and the segments containing PEG, food, and 50% glucose are shown in Table 4. The radioactivities of the whole wall and three tissue layers of the segments containing PEG were not significantly different from those of the empty segments. These results again indicate that luminal placement of 10 ml of PEG for 20 minutes did not alter the total blood flow and compartmental blood flow of the jejunum. The segments containing food or 50% glucose had significantly higher radioactivities in the whole wall and the mucosa as compared to the segments containing PEG. The radioactivities in the submucosa and muscle-serosa of the segments containing food or 50% glucose, on the other hand, were not significantly different from those of the segments containing PEG.

Table 5 shows the percentage distribution of the total blood flow among the three tissue layers of the jejunum following luminal placement of various solutions. The percentage distribution of the total blood flow among the three layers in the jejunal segments containing PEG was not significantly different from that in the empty segments. In the segments containing food, the mucosa, on the average, had greater share of the total blood flow than that in the segments containing PEG. The difference was not statistically significant at p values less than 0.05, but it was statistically significant at p values less than 0.1. In the segments containing 50% glucose, the mucosa had significantly greater and the submucosa or muscle-serosa had significantly smaller

Table 4.--The average radioactivity (cpm/gm tissue) of Sr-85 of the whole wall, the mucosa, submucosa, and muscle-serosa of the segments which were empty or containing PEG, food, or 50% glucose. (mean $\pm$ S.E.) (N = 10)

	PEG	Food	50% Glucose	Empty
Whole Wall	1204 $\pm$ 126	1627 $\pm$ 209*	2817 $\pm$ 386**	1359 $\pm$ 158
Mucosa	1294 $\pm$ 152	1857 $\pm$ 224**	3589 $\pm$ 503**	1497 $\pm$ 206
Submucosa	597 $\pm$ 106	696 $\pm$ 129	776 $\pm$ 152	631 $\pm$ 116
Muscle-Serosa	1267 $\pm$ 157	1485 $\pm$ 317	1840 $\pm$ 390	1357 $\pm$ 254

*Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.05$.

**Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.01$.

Table 5.--Average percentage distribution of blood flow in the wall of the jejunal segments which were empty or containing PEG, food, or 50% glucose as determined by Sr-85 labeled microspheres. (mean $\pm$ S.E.) (N = 10)

	PEG	Food	50% Glucose	Empty
Mucosa	67.6 $\pm$ 3.1	73.0 $\pm$ 5.4@	80.3 $\pm$ 2.2**	67.6 $\pm$ 4.8
Submucosa	6.0 $\pm$ 0.8	5.4 $\pm$ 0.9	3.4 $\pm$ 0.5**	7.2 $\pm$ 2.4
Muscle-Serosa	26.4 $\pm$ 2.8	21.6 $\pm$ 3.0	16.3 $\pm$ 2.1**	25.2 $\pm$ 3.3

[@]Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.1$.

[**]Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.01$.

shares of the total blood flow than those in the segments containing PEG.

3. <u>Effects of Mucosal Anesthesia on Blood Flow Responses to 50% Glucose</u>

A. <u>Results obtained from the microspheres labeled with Ce-141</u>:

As can be seen in Table 6, the segments which were treated with luminal placement of 0.4% dibucaine prior to placement of 50% glucose still had significantly higher radioactivities in the whole wall and the mucosa as compared to the segments containing PEG. But the segments had significantly lower radioactivities in the whole wall and the mucosa as compared to the segments containing 50% glucose without prior exposure to dibucaine. The radioactivities in the submucosa and muscle-serosa were not significantly different from those of the segments containing PEG.

B. <u>Results obtained from the microspheres labeled with Sr-85</u>:

The results (Table 7) were similar to those obtained from the microspheres labeled with Ce-141. The segments containing 50% glucose with prior exposure to 0.4% dibucaine had significantly higher radioactivities in the whole wall and the mucosa than the segments containing PEG. But the segments had significantly lower radioactivities in the whole wall and the mucosa than the segments containing 50% glucose without prior exposure to 0.4% dibucaine. The radioactivities in the submucosa and muscle-serosa were not significantly different from those of the segments containing PEG.

Table 6.--The average radioactivity (cpm/gm tissue) of Ce-141 of the whole wall, the mucosa, submucosa, and muscle-serosa of the segments containing PEG, 50% glucose, or dibucaine-50% glucose.[1] (mean ± S.E.) (N = 8)

	PEG	50% Glucose	Dibucaine-50% Glucose
Whole Wall	4301 ± 380	10171 ± 1541**	7105 ± 957** ##
Mucosa	4682 ± 411	12496 ± 1892**	8586 ± 1190** ##
Submucosa	1825 ± 250	3361 ± 826	2054 ± 329
Muscle-Serosa	4514 ± 681	7544 ± 1824	5769 ± 981

[1]The lumen of these segments were exposed to 10 ml of 0.4% dibucaine for 20 min before placement of 50% glucose.

**Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.01$.

##Denotes that the value is statistically different from the corresponding value of 50% glucose at $p < 0.01$.

Table 7.--The average radioactivity (cpm/gm tissue) of Sr-85 of the whole wall, the mucosa, submucosa, and muscle-serosa of the segments containing PEG, 50% glucose, or dibucaine-50% glucose.[1] (mean ± S.E.) (N = 8)

	PEG	50% Glucose	Dibucaine-50% Glucose
Whole Wall	1222 ± 139	2899 ± 480**	1927 ± 250** ##
Mucosa	1336 ± 164	3674 ± 627**	2350 ± 319** ##
Submucosa	598 ± 134	774 ± 188	634 ± 165
Muscle-Serosa	1231 ± 184	1956 ± 481	1474 ± 219

[1]The lumen of these segments were exposed to 10 ml of 0.4% dibucaine for 20 min before placement of 50% glucose.

**Denotes that the value is statistically different from the corresponding value of PEG at $p < 0.01$.

##Denotes that the value is statistically different from the corresponding value of 50% glucose at $p < 0.01$.

4. Comparison of the Results Obtained from the Microspheres Labeled with Ce-141 and Those Labeled with Sr-85

To test if the results obtained from the microspheres labeled with Ce-141 and those obtained from the microspheres labeled with Sr-85 were similar, the results from these two types of microspheres were statistically compared. Both correlation and paired comparison methods were used for this purpose. All data from group A, i.e., four segments of 10 dogs, were included in the comparison.

In Figure 4, the mucosal blood flows, as percent of total jejunal blood flow, obtained from Sr-85 were plotted against those obtained from Ce-141. There was a highly significant ($P < 0.001$) correlation between the results obtained from the two types of the microspheres. The Pearson product moment correlation coefficient was 0.912. The slope of the linear regression line ($Y = -4.806 + 1.065\ X$) was 1.065.

Figure 5 shows the comparison of the submucosal blood flows estimated by Sr-85 and Ce-141. Although the Pearson product moment correlation (correlation coefficient, $r = 0.608$) was not as high as that of the mucosal flow, the results obtained from the two isotopes were still highly significantly related ($P < 0.001$). The slope of the regression line ($Y = 0.525 + 1.052\ X$) was 1.052.

The linear relationship between the blood flows to the muscle-serosal layer obtained from the two isotopes is shown in Figure 6. Similar to the mucosal layer, the Pearson product moment correlation coefficient was high ($r = 0.935$). The correlation between the results from the two isotopes was highly significant ($P < 0.001$). The slope of the regression line ($Y = 1.393 + 0.911\ X$) was 0.911.

Figure 4. Comparison of the mucosal blood flows, expressed as percent of total blood flow in the jejunal wall, estimated by two types of the microspheres, one labeled with Ce-141 and the other with Sr-85. The linear regression line was $Y = -4.806 + 1.065 X$.

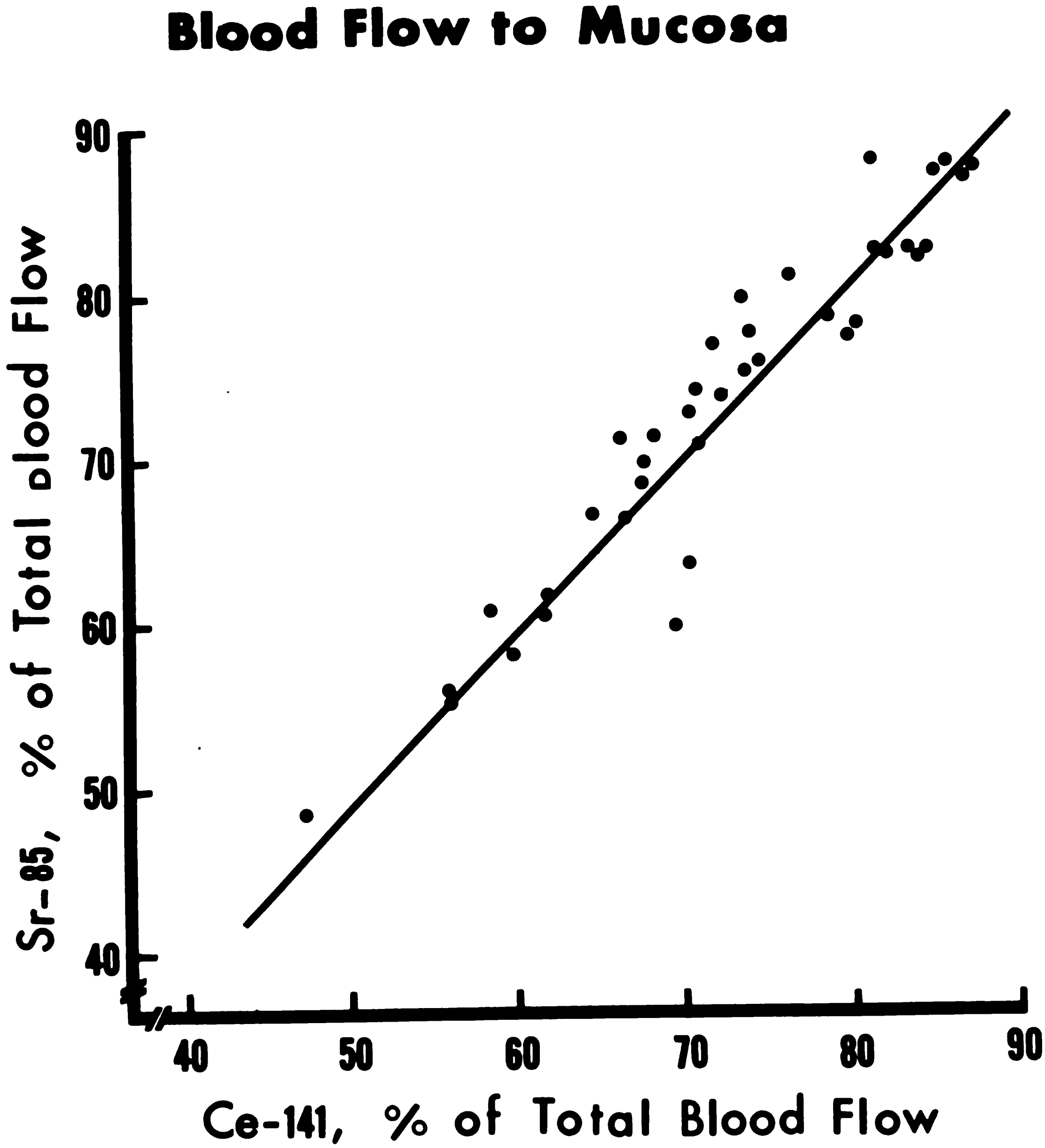
Blood Flow to Mucosa
Sr-85, % of Total Blood Flow
90
80
70
60
50
40
40
50
60
70
80
90
Ce-141, % of Total Blood Flow

Figure 4

Figure 5. Comparison of the submucosal blood flows, expressed as percent of total blood flow in the jejunal wall, estimated by two types of the microspheres, one labeled with Ce-141 and the other with Sr-85. The linear regression line was $Y = 0.525 + 1.052\ X$.

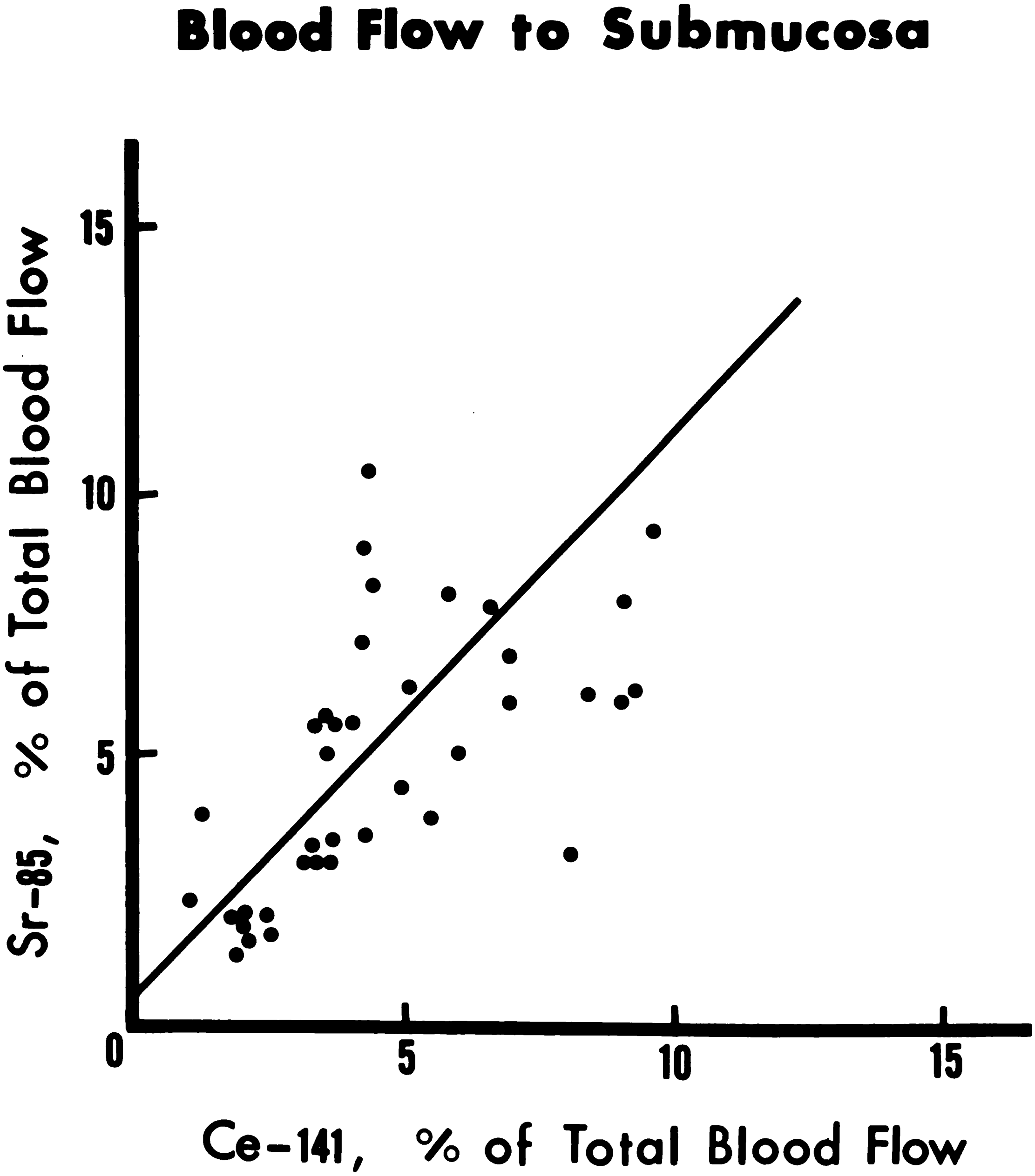
Blood Flow to Submucosa
Sr-85, % of Total Blood Flow
15
10
5
0
5
10
15
Ce-141, % of Total Blood Flow

Figure 5

Figure 6. Comparison of the muscle-serosal blood flows, expressed as percent of total blood flow in the jejunal wall, estimated by two types of the microspheres, one labeled with Ce-141 and the other with Sr-85. The linear regression line was $Y = 1.393 + 0.911\ X$.

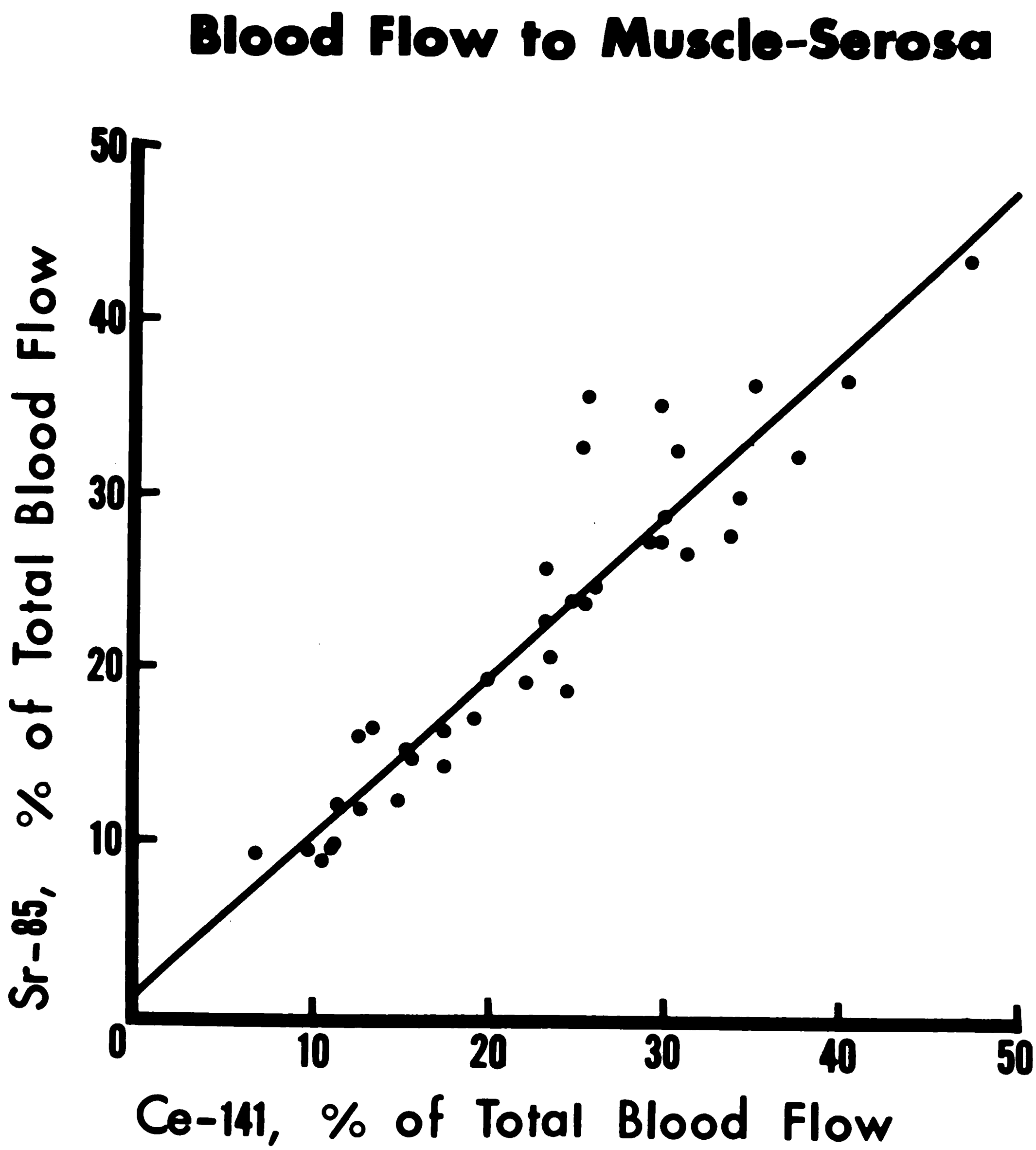
Blood Flow to Muscle-Serosa
Sr-85, % of Total Blood Flow
Ce-141, % of Total Blood Flow
0
10
20
30
40
50
10
20
30
40
50

Figure 6

Statistical analysis by using paired comparison showed that the blood flows to each tissue layer, as percent of total jejunal blood flow, obtained from Ce-141 were not significantly different from those obtained from Sr-85. This again indicated that the results obtained from the two types of microspheres were similar.

CHAPTER V

DISCUSSION

The present study was designed to investigate the effects of luminal placement of digested dog food and 50% glucose on blood flow and its distribution in the wall of the canine jejunum. The radioactive microsphere method was used for this purpose. Because the method has not been widely used in the study of compartmental blood flows in the intestine, the validity of the method was evaluated in this study by using two types of microspheres, one labeled with Ce-141 and the other with Sr-85.

The microsphere method is based on the assumptions that the microspheres are mixed well in the inflowing blood and distributed to the tissues in the same manner as blood. They are entrapped in the capillaries of the perfused tissues and are completely cleared from the blood in a single passage (55). Thus, the concentration of the radioactivity in the tissue is an indicator of the capillary blood flow. Furthermore, the distribution of the radioactivity in an organ is a measure of the relative distribution of the blood perfusing the organ at the time of injection of the microspheres (55).

The validity of the microsphere method has been proved by several investigators (21, 24, 33, 35, 36, 42). That the microspheres are adequately mixed with arterial blood (21, 24, 33, 35, 36, 42) and that the microspheres themselves do not alter the circulation (21, 24) have been demonstrated. Although 5-10% of the microspheres of 15 $\pm$ 5 μ diameter can pass through systemic capillary beds, all of these microspheres are completely removed by the lungs (24). Therefore, there is no recirculation of the microspheres of this size. It has been also shown that only a very small fraction of the mesenteric blood flow passes through the arteriovenous anastomoses (11, 16) and the extraction of the microspheres of 15 $\pm$ 5 μ in diameter by the mesenteric capillary beds is nearly 100%. The distribution of the blood flow among the three tissue layers of the jejunum thus can be estimated from the distribution of the radioactivity in the jejunal wall.

In this present study, two types of the radioactive microspheres labeled with two different isotopes, Ce-141 and Sr-85, were injected into the same dog three minutes apart. The purpose was to see if they would produce similar results. As shown in Figures 4, 5, and 6, there was a good correlation between the results obtained from Ce-141 and Sr-85. The slopes of the three regression lines obtained from the data of the three tissue layers were 1.065 for the mucosa, 1.052 for the submucosa, and 0.911 for the muscle-serosa. They were all not significantly different from 1.000. Comparison with student's t test modified for paired comparison also showed that results from the two types of microspheres were identical. These findings thus support the assumptions that the microspheres are adequately mixed with the blood and the microspheres themselves do not alter the circulation. These findings also indicate that one type of the radioactive microspheres may be used as the control for the other type. The microspheres labeled with one isotope can be injected during the control state and the microspheres labeled with another isotope injected during an experimental procedure. The changes in blood flow distribution induced by the experimental procedure can be obtained by comparing the results from two different isotopes. The similarity in the distribution of the microspheres labeled with different isotopes in the body has also been shown by other investigators by injecting different types of the radioactive microspheres successively into the left heart (21, 24).

Comprehensive reviews on the regional circulation of gastrointestinal tract indicate that the information in regard to the blood flow distribution among different tissue layers of gastrointestinal tract is scarce. Delancy and Grim (12) used two different methods, K-42 clearance and microsphere methods, to estimate the gastric blood flow distribution in dogs. They found that the distribution of blood flow among tissue layers of gastric body determined by the K-42 clearance technique was mucosa 72%, submucosa 13%, and muscle 15%, and that determined by the microsphere method was mucosa 68%, submucosa 11%, and muscle 21%. The results from these two different techniques are very similar.

Grim and Lindseth (16) used radioactive microsphere method to measure the compartmental blood flow of the small intestine of dogs. They found that the capillary flows to the mucosa, submucosa, muscle, and mesentery in fasted jejunum and ileum were about the same (40-50 ml/min/100 gm). A possible exception was the flow to the ileal muscle, which was slightly higher (55 ml/min/100 gm). Kampp et al. (25, 26, 27), using Kr-85 wash out technique showed in cats that about 80% of the intestinal blood flow was distributed to the mucosa-submucosa and the remainder, 20%, was distributed to the muscle. The flow rate to the mucosa-submucosa was 40-80 ml/min/100 gm and that to the muscle was 10-20 ml/min/100 gm.

As described above, the capillary flow values for the muscularis of the small intestine reported by Grim and Lindseth, 40-50 ml/min/100 gm, were considerably larger than those reported by Kampp et al., i.e., 10-20 ml/min/100 gm. However, the mean flows of the mucosa-submucosa determined by either group were similar.

In this present study, the average radioactivity per gram tissue of the mucosa and muscle in the empty segments were about the same, i.e., 5240 $\pm$ 628 and 4774 $\pm$ 1137 cpm/gm as determined by Ce-141, and 1497 $\pm$ 206 and 1357 $\pm$ 254 cpm/gm as determined by Sr-85 (Tables 2 and 4). This indicates that the flow per gram of the mucosa and that per gram of the muscle were about the same. These results are similar to those of Grim and Lindseth.

As shown in Tables 3 and 5, the percentage distribution of blood flow in the empty segments as determined by Ce-141 or Sr-85 was mucosa 70.1 $\pm$ 3.5% or 67.6 $\pm$ 4.8%, submucosa 5.3 $\pm$ 0.8% or 7.2 $\pm$ 2.4%, and muscle 24.6 $\pm$ 3.3% or 25.2 $\pm$ 3.3%. Our values for the muscle is slightly higher than that obtained by Kampp et al. (25, 26, 27). They found percent distribution to the muscle to be 20.

The comparison of the data from different laboratories is difficult. This is because the studies were not performed under the same conditions. Many factors, such as anesthesia, animal species, individual variations, and surgical manipulation, all contribute to produce different results. Manipulation of the intestine during surgical procedures may increase percentage distribution of total blood flow to the muscle and decrease that to the mucosa. We have found this phenomenon in this present study and Delaney and Grim have also found the same phenomenon (12). The manipulation of the intestine may be a factor that produces a higher muscular blood flow in our and Grim's studies than that in Kampp's study. Probably, the manipulation of the intestine increases intestinal motility whereby the muscular blood flow is increased.

The effect of manipulating the intestine on the muscular flow, however, is usually transient. In this present study, the test solutions were placed into the jejunal lumens 15 minutes after the surgical manipulation and the test solutions were remained in the lumens for 20 minutes before the injection of microspheres. Thus, the effect of surgical manipulation was probably minimal. Since all the jejunal segments, control as well as experimental, in the present study were subjected to similar amounts of surgical manipulation, and since we were more interested in whether luminal placement of food or 50% glucose alters blood flows and flow distribution than the absolute values of flows and flow distribution, the change in flows and flow distribution caused by manipulation is not so important in our study.

In this study, the mean weight distribution in the jejunal wall was used to calculate the percentage distribution of blood flow and total radioactivity in the jejunal wall. As can be seen in Table 1, the mean weight distribution in the jejunum was found to be mucosa 63.1%, submucosa 11.9%, and muscle-serosa 25.0%. These values are similar to those obtained by other investigators (39). We also found that the weight distributions in the wall of the stomach, duodenum, jejunum, ileum, and colon were different. From duodenum to colon, the relative weight of the mucosa decreases while the relative weights of the submucosa and muscle-serosa increase. The relative weights of the mucosa of duodenum and colon were 66.6% and 36.1% respectively. Since almost all digestion and absorption of food and water occur in the upper small intestine, especially in the duodenum and jejunum, it is not surprising to find that the relative weight of the mucosa is highest in the duodenum and jejunum.

Placement of 10 ml of test solutions might distend the segments thereby altering the local flows. To test this possibility, an isotonic solution of nonabsorbable polyethylene glycol (molecular weight 4,000, PEG) was also placed into the lumen of the jejunal segments. As can be seen in Tables 2, 3, 4, and 5, all the values obtained in the segments containing PEG were not significantly different from those in the empty segments. This indicates that presence of 10 ml of PEG in the lumen for 20 minutes has no effect on total blood flow and its distribution. Nevertheless, the values obtained from the segments containing PEG were used as the control for those containing food or 50% glucose. Since the same amount of food or 50% glucose was placed into the lumen, the difference in the results between food, glucose and PEG can be reasonably attributed to the nature and quality of the content rather than the volume. Also, since both control and experimental segments were subjected to the same systemic influences at any given time (e.g., blood pressure, blood constituents, and systemic nerve activity), differences in the effects between food, glucose and PEG can be reasonably attributed to differences in their local actions.

The effects of food intake on the blood flow to the splanchnic viscera or small intestine has been studied by several investigators (1, 2, 3, 15, 20, 54). The majority of evidence indicates that splanchnic or mesenteric blood flow increases after meals. Some investigators proposed that the hyperemia results from increased cardiac output and involves all organs of the body (20). Other studies (2, 3, 15, 54), however, indicate that following a meal the blood flow increases in the superior mesenteric artery and decreases in brachiocephalic and iliac arteries. The cardiac output is not changed. Thus, there is a redistribution of cardiac output favoring the vascular bed of the superior mesenteric artery at the expense of blood flow to other areas.

A recent study which involved the simultaneous measurement of celiac and superior mesenteric arterial blood flows with electromagnetic flowmeters, indicates that the increased splanchnic blood flow following a meal is localized in the area exposed to chyme or in the area performing digestive and/or absorptive functions (10). Another study, using two segments of the jejunum and simultaneously measuring their blood flows, shows that the increased flow occurs only in the segment containing food (7). These studies thus appear to indicate that the increased flow following a meal is a local phenomenon.

The effects of feeding on blood flow distribution in the intestinal wall has not been carefully studied. It is not known whether the increase in local blood flow involves all three tissue layers of the intestinal wall or is confined to a certain layer. The results obtained from this present study show that the segments containing digested dog food had significantly higher radioactivities in the whole wall and the mucosa than the control segments. The radioactivities in the submucosa and muscle-serosa, on the other hand, were not significantly different from those of the control segments (Tables 2 and 4). These findings indicate that luminal placement of food increased total blood flow to the jejunum and the increased flow was mainly in the mucosal layer.

It appears that placement of food also produces redistribution of blood flow in the jejunal wall. As shown in Tables 3 and 5, on the average, placement of food increased, as compared to control segments, the percentage distribution of flow to the mucosa while it decreased that to the muscle-serosa. An increase in the mucosal share of the total blood flow occurred in 7 of 10 dogs. In two dogs the percentage distribution of total blood flow to the mucosa was not altered and in one dog it was decreased.

Ingestion of hypertonic glucose solution is known to increase splanchnic blood flow or superior mesenteric blood flow (4, 22). Van Heerden et al. (53), using microspheres labeled with two different isotopes, found that the blood flow to the gastrointestinal tract increased only in the part of the jejunum perfused with hypertonic glucose solution. Chou and Dabney (7), using two segments of the jejunum and simultaneously measuring their blood flows, also showed that the increased flow was confined to the segment exposed to hypertonic glucose solution. Whether the increased flow is confined to the mucosal layer of the jejunum, however, is not known.

As shown in Tables 2 and 4, the segments containing 50% glucose had significantly higher radioactivities in the whole wall and the mucosa than the control segments. The radioactivity in the submucosa was slightly higher than the control value (Table 2) or similar to the control value (Table 4). The radioactivity in the muscle-serosa was not significantly different from the control value. These findings indicate that luminal placement of 50% glucose caused an increase in total blood flow which results from hyperemia in the mucosa and possibly also in the submucosa. There was no change in the blood flow to the muscle-serosa.

As can be seen in Tables 3 and 5, 50% glucose produced redistribution of blood flow in the jejunal wall in favor of the mucosa at the expense of the submucosa and muscle-serosa. The percentage distribution of flow to the mucosa in the segment containing 50% glucose was significantly greater than that in the segment containing PEG.

This present study thus shows that luminal placement of digested dog food or 50% glucose increased total blood flow to the jejunum and the increased flow was mainly in the mucosal layer. There was a redistribution of blood flow in the jejunal wall, in favor of the mucosa. The mechanism involved in the increase of total blood flow which appears to result from hyperemia only in the mucosal layer is not clear. There are several possibilities. The increased flow may result from 1) vasodilator effect of the absorbed substances; 2) increased mucosal metabolism as a result of active absorption; 3) increased local osmolarity; 4) local release of vasodilator substances; 5) increased motility and 6) stimulation of mucosal nerves.

Absorption of the substances into the mucosa can alter the chemical composition of the mucosal tissue fluids bathing the mucosal blood vessels and thereby increase the local blood flow. The digested dog food contains many substances which may be vasodilators. The 50% glucose solution contains only glucose. It has been shown that intra-arterial infusion of isotonic glucose solution raised plasma glucose concentration to the level similar or greater than that caused by luminal placement of 50% glucose but did not raise blood flow (7). This indicates that the increased blood flow caused by luminal placement of food which contains glucose or 50% glucose appears not to result from a direct vasodilator effect of increased local glucose concentration.

Luminal placement of food or 50% glucose may produce an increase in the mucosal metabolism as a result of active absorption of these nutrients. The increased metabolic activity can raise local concentration of vasodilator metabolites, pCO_2, and temperature while lowering pO_2 and pH (31, 41, 50). All these factors produce vasodilation. This phenomenon has been called active hyperemia. In the present study, PEG was used as a control which is presumably a nonabsorbable substance. Thus, it can be speculated that an increase in metabolic activity may be involved in the increase of local jejunal blood flow in the segments containing food or 50% glucose. Furthermore, since the mucosa is the only tissue involved in the absorption, it can be easily understood that placement of food or 50% glucose in the lumen produce an increased blood flow mainly in the mucosal layer.

Although the osmolarity of food was not measured, it might be expected that the osmolarity of food is higher than that of plasma or tissue (300 mOsm/kg). The osmolarity of the 50% glucose solution is about 3,000 mOsm/kg which is much higher than that of plasma or tissue. Thus, the hyperosmolarity of food or 50% glucose may be one factor contributed to the increased flow. The contribution of hyperosmolarity to the increased flow has been evaluated (7, 9). Chou and Dabney (7) found that luminal placement of hypertonic solutions of glucose and PEG of the same osmolarity (20% glucose = 34% PEG and 50% glucose = 85% PEG) increased the venous osmolarity to the same degree, but the 34% PEG solution did not increase flow, and the 85% PEG solution increased flow less than did the 50% glucose solution. Furthermore, they found that luminal placement of the 2.5% and 5.4% glucose solutions raised flow but did not significantly alter venous osmolarity. These findings indicate that the presence of glucose in the lumen may increase blood flow through other mechanisms in addition to a direct vascular effect of hyperosmolarity.

It is not known whether the change in tissue osmolarity is the same as the change in venous osmolarity. However, it might be speculated that at least the tissue osmolarity of the mucosa which was exposed to the high osmolarity of food or 50% glucose would be higher than the venous osmolarity. And an osmotic gradient might exist in the intestinal tissue, higher in the mucosal layer and lower in the muscle-serosal layer. The results of the present study showed that luminal placement of food or 50% glucose increased blood flow mainly in the mucosal layer (Tables 2 and 4). The increased mucosal flow without significant change in the outer layers of the jejunum may result from the higher tissue osmolarity in the musosal layer than in the other two layers.

A tonic contraction of the gut decrease local blood flow whereas rhythmic contractions without a change in gut wall tension increase local blood flow (6, 8, 18, 44, 46). Chou and Dabney (7) showed that placement of 50% glucose into the jejunal lumen produced a small change in motility during the first three minutes, but the increase in blood flow was already near a maximum. A further increase in motility which was apparent during the next eight minutes was not accompanied by a significant change in blood flow. Furthermore, this present study showed that the increased flow in the segments containing food or 50% glucose was mainly in the mucosal layer. The blood flow to the muscle layer should be altered if the increased flow results from altered motility. As can be seen in Tables 2 and 4, muscle blood flow was not significantly altered. Thus, the increased flow appears not to be caused by changes in motility. It has been shown that the presence of hypertonic glucose in the intestinal lumen released substances such as serotonin and bradykinin (32). These substances probably play a role in the increase of blood flow.

A neural mechanism may be involved in the increase of blood flow. It has been reported that mucosal chemoreceptors can react selectively to the intraluminal introduction of acid or glucose (52). Mucosal osmoreceptors have also been demonstrated to exist in the intestine (48). Thus, it is possible that luminal placement of food or 50% glucose might stimulate these chemoreceptors or osmoreceptors. This excitation could possibly bring about a local reflex and cause vasodilation.

Indeed, local vasodilation caused by placement of hypertonic solution of glucose, KCl, NaCl, or $CaCl_2$ into the lumen of the jejunum has been shown to be abolished or attenuated by anesthetizing the mucosa with a local anesthetic (5, 7). In these studies, the concentration of these chemicals in the local venous blood was increased to the same extent before and after mucosal anesthesia. These studies thus suggest that mucosal nerves may be involved in the local vasodilation.

The results obtained from this present study showed that the segments containing 50% glucose with prior exposure to 0.4% dibucaine had significantly higher radioactivities in the whole wall and the mucosa than the control segments, but had significantly lower radioactivities in the whole wall and the mucosa than the segments containing 50% glucose without prior exposure to dibucaine (Tables 6 and 7). These findings indicate that mucosal anesthetis could partly block the increase of blood flow caused by luminal placement of 50% glucose. Dibucaine may partly block the effect of 50% glucose on jejunal blood flow by anesthetizing the nerves involved in a local reflex. It is also possible that dibucaine blocks the release of vasodilator substances. This possibility has a precedent in the fact that release of gastrin from the gastric antrum has been shown to be mediated by mucosal nerves and can be blocked by local anesthetics (17).

The increased blood flow, mainly in the mucosa, caused by luminal placement of digested dog food or 50% glucose may result from one or more factors described above.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present study was designed to investigate whether or not the luminal placement of digested dog food or 50% glucose solution can affect the local blood flow and if so which compartment of the jejunal wall is involved in the change. This was accomplished by comparing the radioactivities of the whole wall and the three tissue layers of the jejunal segments containing food, 50% glucose or isotonic solution of a nonabsorbable substance, PEG, following the injections of radioactive microspheres. Two types of the microspheres, one labeled with Ce-141 and the other with Sr-85, were injected into the left ventricle 3 minutes apart in all experiments. The role of mucosal nerves as a mediator of changes in local blood flow was tested by comparing the blood flow responses to 50% glucose with and without prior exposure of the mucosa to a local anesthetic, dibucaine. The results are summarized as follows:

1. The results obtained from microspheres labeled with Ce-141 were similar to those labeled with Sr-85.
2. Total and compartmental flows in the segments containing PEG were not significantly different from those in the empty segments.

3. The segments containing food had significantly higher radioactivities in the whole wall and the mucosa than the segments containing PEG. The radioactivity in the submucosa or muscle-serosa, on the other hand, was not significantly different from that in the segments containing PEG.
4. The segments containing 50% glucose had significantly higher radioactivities in the whole wall and the mucosa than the segments containing PEG. The radioactivity in the submucosa was slightly higher than or not different from that in the segments containing PEG. The radioactivity in the muscle-serosa was not significantly different from that in the segments containing PEG.
5. In the segments containing food or 50% glucose, the mucosa had greater while the submucosa and muscle-serosa had smaller shares of the total jejunal blood flow as compared to those in the segments containing PEG.
6. The segments containing 50% glucose with prior exposure to 0.4% dibucaine had significantly higher radioactivities in the whole wall and the mucosa than the segments containing PEG. But, these segments had significantly lower radioactivities in the whole wall and the mucosa than the segments containing 50% glucose without prior exposure to dibucaine.

In conclusion, the present study indicates that:

1. Two types of the radioactive microspheres used in this study produced similar results. The finding supports the assumptions that the microspheres are adequately mixed with the

arterial blood after injecting into the left ventricle and the microspheres do not alter the circulation. One type of the radioactive microspheres can be used as a control of the other type.

2. Luminal placement of digested dog food or 50% glucose increases total blood flow to the jejunal segment and the increased flow is mainly in the mucosal layer.

3. Several factors may be involved in the change of blood flow following placement of food or 50% glucose in the lumen. The increased flow caused by luminal placement of 50% glucose appears to be mediated by the mechanisms that can be partly blocked by exposing the mucosa to a local anesthetic.

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