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Effects of vasopressin and isoprenaline infusions on the distribution of blood flow in the intestine; criteria for the validity of microsphere studies

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Summary

1. The distribution of superior mesenteric arterial flow was investigated by radioactive microspheres. The small intestine received 83% of the flow ((85 ml/min)/100 g intestine) and flow was uniform along the length of the small intestine.

2. The intestinal wall was separated into 3 layers—muscle, submucosa and mucosa. The muscle received (8 ml/min)/100 g intestine and the combined submucosa and mucosa (77 ml/min)/100 g intestine.

3. The distribution of microspheres between the mucosa and submucosa depended on the size of the microspheres; the smaller the spheres, the more were found in the mucosa. It also depended on the state of the vascular bed; if microspheres were given during an infusion of vasopressin, a subsequent infusion of isoprenaline resulted in movement of some of the spheres from the submucosa into the mucosa.

4. Histological studies after India ink injection showed few capillary-sized vessels but many large vessels in the submucosa. Capillary-sized vessels arose close to the junction with the mucosa and passed into the mucosa.

5. These and other data suggest that the intestine consists of two parallel-coupled sections, one to the muscle and the other through the submucosa to the mucosa. The vessels in the submucosa are in series with those in the mucosa and submucosal shunts do not exist. Redistributions of flow between mucosa and submucosa cannot therefore occur during stimulation of the sympathetic nerves or infusions of drugs such as noradrenaline or adrenaline.

6. When microspheres are used in pharmacological investigations on distribution of blood flow in organs, controls to validate the method for the particular areas being studied are essential. If the vessels in the areas studied are in series rather than in parallel, the method is invalid.

Introduction

This study was begun as an attempt to compare the effects of a variety of vasoactive agents on the distribution of blood flow within the wall of the small intestine. A variety of effects on total intestinal blood flow have been reported in the literature and some have been explained on the basis of complex changes in flow distribution within the intestinal wall (Dresel & Wallentin, 1966; Greenway & Lawson, 1966; Ross, 1971a; Shanbour & Jacobson, 1971; Shehadeh, Price & Jacobson, 1969; Swan & Reynolds, 1971a, b). The following is a brief outline of

the development of the concept of parallel-coupled vascular circuits in the intestinal wall.

In 1964, Folkow, Lewis, Lundgren, Mellander & Wallentin showed that stimulation of the sympathetic nerves to the intestinal vascular bed in cats produced an initial vasoconstriction followed by a recovery of the flow towards the control level in spite of maintained nerve stimulation. They termed this recovery ' autoregulatory escape '. The capillary filtration coefficient (a measure of capillary surface area) decreased for the duration of nerve stimulation, there was a post-stimulatory hyperaemia and India ink was not distributed to the mucosa to the same extent during stimulation as compared with the control period. These observations were interpreted on the hypothesis that there was a neurogenic redistribution of blood flow from the mucosa towards the submucosa (Folkow, Lewis, Lundgren, Mellander & Wallentin, 1964a, b ; Dresel & Wallentin, 1966). Further studies on the arterio-venous extraction of rubidium excluded the involvement of true arterio-venous shunts and led to the concept of a dense plexus of thin-walled, small vessels which allowed exchange across their walls in the submucosa (Dresel, Folkow & Wallentin, 1966). This concept was elaborated further by Lundgren (1967) from studies on the wash-out of intra-arterially injected ^{85}Kr. Three parallel-coupled circuits were postulated: the muscle layer with a flow of (10–15 ml/min)/100 g at rest, a well-vascularized area located in the submucosa and adjacent mucosa with a flow of (400–600 ml/min)/100 g and the mucosa with a flow of (40–60 ml/min)/100 g. During isoprenaline infusion, the flow in the submucosal area increased to 50–60% of the total flow—(800–1,000 ml/min)/100 g. The situation was further complicated by evidence for a countercurrent exchange of material between the ascending and descending limbs of mucosal vascular loops mainly located in the villi (Folkow, 1967 ; Lundgren, 1967).

Recently evidence has been accumulating against this concept of three parallel-coupled sections and a redistribution from mucosa to submucosa during autoregulatory escape. This evidence has been reviewed by Ross (1971b) who also presented the most convincing evidence against the hypothesis. He showed, from the uptake of ^{86}Rb, that the distribution of flow between mucosa, submucosa and muscle during autoregulatory escape was not significantly different from that in a control group of animals. This recent work has been interpreted to suggest that escape was due to relaxation of the same vascular elements which were originally constricted by noradrenaline (Ross, 1971c). In addition, ^{86}Rb uptake by the submucosa was only 12% of the total, suggesting that an extensive capillary network was not present in the submucosa.

The recent availability of radioactive microspheres suggested a new approach to this question of parallel-coupled circuits in the intestine and this work was begun before the studies of Ross (1971b, c) were published. Grim & Lindseth (1958) studied the distribution of radioactive microspheres in the layers of the intestinal wall and although the distribution between mucosa and submucosa was markedly different when different sized microspheres were used, they interpreted this distribution as a valid measure of flow in each layer and calculated mucosal, submucosal and muscle flows. We began to use this method to determine whether the distribution between the layers was altered during infusions of vasopressin, isoprenaline and noradrenaline but as the work progressed, it became clear that the distribution of the spheres between the mucosa and submucosa was not a measure

of the distribution of flow. The data and our interpretation of them are described in this paper.

Methods

Cats were anaesthetized by intraperitoneal injection of sodium pentobarbitone (Abbott Laboratories, 30 mg/kg body weight). Supplementary doses (2 mg/kg) were given through a cannula in a forelimb cutaneous vein when reflex ear, eye and swallowing movements returned. The trachea was cannulated and arterial pressure (1 mmHg≡1·333 mbar) was recorded from a femoral artery. The abdomen was opened by a mid-line incision and the anastomotic branch of the superior mesenteric artery was identified. This is the first branch of the superior mesenteric artery and it anastomoses with the inferior mesenteric artery. Its ligation does not deprive any area of flow and it was cannulated for injection of radioactive microspheres into the superior mesenteric artery. The total flow in the superior mesenteric artery was measured by a 2 mm diameter non-cannulating flow probe of an electromagnetic flowmeter (Nycotron, Oslo). The probe was set up, zero flow was determined and the probe was calibrated as previously described (Greenway & Lawson, 1966).

Microspheres (15 ± 5 μ diameter), labelled with ^{141}Ce or ^{51}Cr, were suspended in 10% dextran solution (3M Nuclear Products, Minnesota) and a dose of approximately 220,000 microspheres was given into the superior mesenteric artery in each experiment.

Isoprenaline HCl (B.D.H.) was dissolved in 0·9% w/v NaCl containing ascorbic acid (0·2 mg/ml) and vasopressin (Pitressin, Parke, Davis & Co.) was diluted in 0·9% NaCl. The isoprenaline was infused into the superior mesenteric artery through the cannula in the anastomotic branch while the vasopressin was infused intravenously in doses which produced a relatively specific intestinal vasoconstriction (Cohen, Sitar, McNeill & Greenway, 1970).

At the end of each experiment, the abdominal viscera, lungs and liver were removed and the following procedures were carried out. The pancreas, lymph nodes, mesentery, colon, mesocolon, lungs and liver were cut into small pieces and placed in plastic tubes. The intestine was opened along the mesenteric border and cut into 7 cm lengths. Each length was laid on a paper towel with the mucosal surface in contact with the paper. The muscular layer was then stripped from the submucosa and placed in a plastic tube. Care was taken not to press on the mucosa since this might have damaged it. The remaining tissue was then spread on another piece of paper with the mucosa uppermost and the mucosa was stripped from the submucosa. The two layers were then placed in separate plastic tubes. This was done for the whole length of the intestine. In three experiments, the papers on which this dissection was done were placed in plastic tubes to estimate losses of radioactivity during the dissection.

The tissue samples were weighed and the radioactivity was counted in a two-channel auto-gamma spectrometer (Packard Instrument Co.). Corrections for overlap of radioactivity between the two channels were made (Greenway & Oshiro, 1972). Fractional flow to each piece of tissue was calculated from the principle of Stewart-Hamilton as described by Wagner, Rhodes, Sasaki & Ryan (1969):

$$f = \frac{F.q}{\bar{Q}}$$

where f is the fractional blood flow to the tissue, F is the total blood flow in the superior mesenteric artery at the time the microspheres were given, q is the radioactivity in the piece of tissue and Q is the total injected radioactivity obtained by summation of the counts in all the samples. Since tissue weight was measured, flow/100 g tissue could also be calculated.

To study the architecture of the intestinal vascular bed, India ink was injected into the superior mesenteric artery in 3 experiments. Paraffin sections (15 and 30 μ) of the whole intestinal wall were stained with eosin and serial sections were examined. In addition, separated sheets of submucosa were cleared with glycerine and examined (Boulter & Parks, 1960; Reynolds, Brim & Sheehy, 1969).

Results

Distribution of superior mesenteric arterial flow

Thirty-five cats were used ($2{\cdot}8 \pm 0{\cdot}3$ kg body weight; mean $\pm$ S.E.). Mean arterial pressure was 134 ± 7 mmHg and superior mesenteric arterial flow was (25 ± 4 ml/min)/kg body weight or (67 ± 9 ml/min)/100 g tissue at the time the microspheres were injected.

In 17 cats, ^{141}Ce-microspheres were injected into the superior mesenteric artery during the control period. The organs in which significant radioactivity was found are shown in Table 1. The small intestine with the exception of the first 2–3 cm of the duodenum, the mesentery, the lymph nodes, the proximal half of the colon and its mesocolon and the head but not the body of the pancreas were supplied by the superior mesenteric artery. The relative weights, proportions of the superior mesenteric arterial flow and the calculated flows to the regions are shown in Table 1. The validity of the calculations is discussed later.

Distribution of microspheres in the layers of the intestine

The method used to separate the layers of the intestine is very similar to that subsequently published by Ross (1971c). Histological sections of the separated layers were made to determine the contamination between layers and examples are shown in Figure 1. The loss of radioactivity onto paper was $0{\cdot}35 \pm 0{\cdot}08\%$ (mean $\pm$ S.E.) during separation of the muscle layer and $1{\cdot}72 \pm 0{\cdot}31\%$ during removal of the mucosa. It is concluded that the separation procedure did cleanly separate the layers and that the loss of radioactivity during these procedures was small.

In 11 cats, the mean length of the small intestine was $90 \pm 6{\cdot}5$ cm and the weight was $0{\cdot}74 \pm 0{\cdot}05$ g/cm. The mucosa formed $37 \pm 2{\cdot}0\%$, the submucosa

TABLE 1. *The weights (drained of blood) and blood flows (means $\pm$ S.E.) of the splanchnic organs perfused by the superior mesenteric artery (SMA)*

	Weight of perfused tissue (g/kg body weight)	% of SMA flow	Flow (ml/min)/kg body weight	Flow (ml/min)/ 100 g tissue
Intestine	26 ±2·5	83 ±1·2	21 ±3·1	85 ±13
Mesenteric lymph nodes	1·6 ±0·17	3·3 ±0·19	0·87±0·15	59 ±12
Mesentery	5·2 ±0·70	1·4 ±0·06	0·32±0·05	8·9 ± 2·7
Head of pancreas	0·97±0·13	1·0 ±0·22	0·23±0·08	25 ±10
Proximal colon	3·2 ±0·50	11 ±1·2	2·7 ±0·51	92 ±22
Proximal mesocolon	1·2 ±0·18	0·56±0·42	0·13±0·04	12 ± 5·5

13 ± 0·4% and the muscle 50 ± 2·3% of the total intestinal weight. There was no significant difference in the weight/unit length or in the relative proportions of mucosa, submucosa and muscle between segments of duodenum, jejunum or ileum ($P > 0·1$, unpaired t test).

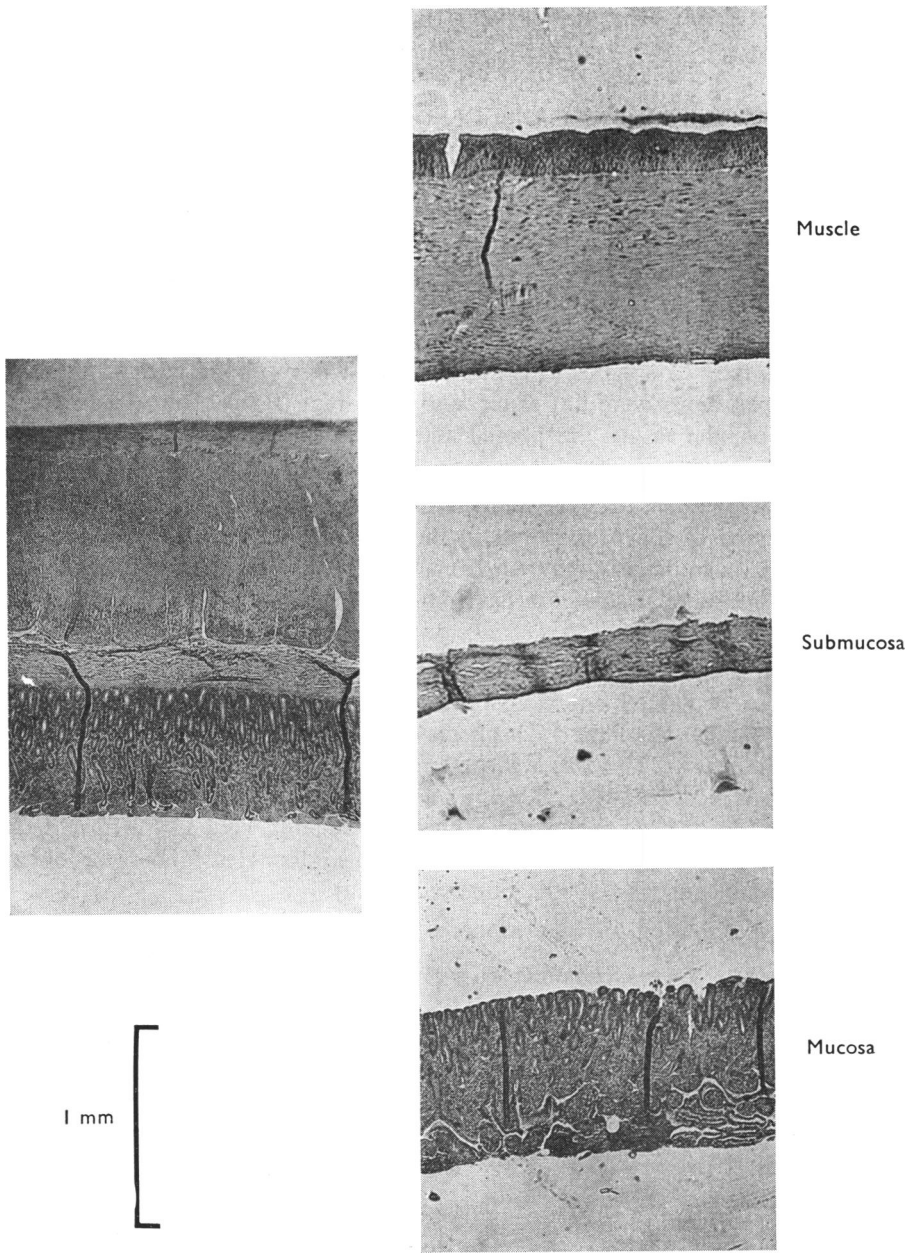

FIG. 1. Histological sections of the intestinal wall and of the separated layers of the wall. Note the clean separation of the layers.

The small intestine received $83 \pm 1{\cdot}2\%$ (mean $\pm$ S.E.) of the total injected radioactivity (Table 1). The mucosa received $47 \pm 4{\cdot}6\%$, the submucosa $28 \pm 1{\cdot}8\%$ and the muscle $7{\cdot}8 \pm 1{\cdot}4\%$ of the total radioactivity. This distribution cannot be interpreted in terms of flow for reasons to be discussed later. The proportion of the total injected in each 7 cm length of intestine and the proportion in each layer were not significantly different ($P > 0{\cdot}1$, unpaired t test) between duodenum, jejunum and ileum, except for the first 2–3 cm of duodenum which was not perfused by the superior mesenteric artery.

Injection of two types of microsphere

At this stage in the experiments, we began the use of two types of microsphere, labelled with ^{141}Ce and ^{51}Cr respectively, with the aim of obtaining the control distribution of flow and the distribution during infusion of a drug in each animal. To test the validity of this procedure and the effect of time on the flow distribution the following experiments were done. The ^{141}Ce- and ^{51}Cr-spheres were mixed and the mixture was injected (2 experiments), the ^{141}Ce-spheres were given 10 min before the ^{51}Cr-spheres (3 experiments), the ^{141}Ce-spheres were given 2 h before the ^{51}Cr-spheres (3 experiments) and a ten times larger dose of ^{141}Ce-spheres was given 10 min before the ^{51}Cr-spheres (3 experiments). The results in each of these series of control experiments were not significantly different ($P > 0{\cdot}2$, unpaired t test) and for presentation, the results are pooled and shown in Table 2.

The proportion of the ^{51}Cr-spheres in the mucosa was always lower and the proportion in the submucosa higher than that for the ^{141}Ce-spheres ($P < 0{\cdot}0001$, paired t test). The sum of the proportions in the mucosa and submucosa for the ^{141}Ce-spheres was not significantly different from that for the ^{51}Cr-spheres. In the muscle layer and in the other tissues, the distribution of the two types of sphere was not significantly different.

Since Grim & Lindseth (1958) had shown that the distribution between mucosa and submucosa depended on microsphere size, it seemed possible that the ^{141}Ce-spheres were smaller than the ^{51}Cr-spheres even though they were both purchased as 15 ± 5 μ diameter. The size was determined in two ways: by direct measurement under a microscope with an eyepiece graticule and by measurement of photomicrographs of known magnification. The ^{141}Ce-spheres were $12 \pm 0{\cdot}15$ μ diameter (mean $\pm$ S.E.) and the ^{51}Cr-spheres were $17 \pm 0{\cdot}16$ μ diameter. This size difference was highly significant ($P < 0{\cdot}0001$, unpaired t test).

Thus the distribution of microspheres between the mucosa and submucosa

TABLE 2. *The relative distribution of ^{141}Ce and ^{51}Cr microspheres in* 11 *cats*

	^{141}Ce as % total injected	^{51}Cr as % total injected	Paired S.E.
Mucosa	47·4	26·3*	(1·72)
Submucosa	27·5	50·0*	(2·08)
Mucosa + submucosa	74·7	76·3	(1·39)
Muscle layer	7·8	7·7	(0·52)
Total intestine	82·6	84·0	(1·22)
Mesentery	1·37	1·05	(0·06)
Lymph nodes	3·33	2·92	(0·19)
Head of pancreas	1·02	0·79	(0·22)
Colon	11·2	10·9	(1·22)
Mesocolon	0·56	0·52	(0·42)

(* $P < 0{\cdot}0001$, paired t test)

depended on the size of the microspheres, while the distribution in all other areas and in the combined mucosa plus submucosa did not depend on the microsphere size. This suggests that the mucosa and submucosa are not parallel-coupled sections but are in series, with the mucosal section being of smaller diameter than the submucosal section. Thus larger spheres are trapped in the submucosa while smaller ones pass further into the mucosa. On this hypothesis, we predicted that if the microspheres were given when the vessels were constricted, subsequent dilatation of the vessels should cause some microspheres to move from the submucosa into the mucosa. This prediction was tested.

Effects of vasopressin followed by isoprenaline infusions

In 7 cats, superior mesenteric arterial flow was recorded and vasopressin was infused intravenously. During infusions of (10 mU/min)/kg, the flow decreased from (31 ml/min)/kg to (10 ml/min)/kg (paired S.E. $\pm 2{\cdot}6$; $P<0{\cdot}001$, paired t test). When the flow was steady at this low level, ^{141}Ce-spheres were given into the superior mesenteric artery followed 2–3 min later by ^{51}Cr-spheres. A 40–50 cm length of intestine was then removed while the infusion of vasopressin was continued. The position of the excised piece was not critical since the proportional weights and microsphere distributions were shown to be similar in all parts. The vasopressin was then stopped and isoprenaline was infused into the superior mesenteric artery. The dose was increased until maximal vasodilatation was obtained. Flow increased to ($49 \pm 5{\cdot}5$ ml/min)/kg but this cannot be compared to the control flow since half of the small intestine had been removed. After the flow had been steady for 5–10 min, the animal was killed and the remaining portions of the intestine were removed. The distribution of the two types of microsphere in the mucosa and submucosa during vasopressin infusion and after subsequent vasodilatation by isoprenaline are shown in Table 3. It can be seen that isoprenaline caused a highly significant movement of both microspheres from the submucosa into the mucosa.

Microspheres in the liver and lungs

In all the experiments, the liver and lungs were counted to determine the proportion of microspheres which passed through the mesenteric organs and were trapped in the liver or lungs. The proportion of the injected dose which was found in the liver was $0{\cdot}20 \pm 0{\cdot}005\%$ for ^{141}Ce and $0{\cdot}03 \pm 0{\cdot}001\%$ for ^{51}Cr-spheres. No detectable radioactivity was present in the lungs. It is concluded that less than 1% of the

TABLE 3. *Relative distributions of ^{141}Ce and ^{51}Cr-microspheres injected during vasopressin infusion into the superior mesenteric artery*

	During vasopressin	After subsequent isoprenaline	Paired S.E.
^{141}Ce in mucosa	51·0	63·1*	(1·13)
^{141}Ce in submucosa	49·0	36·9*	(1·13)
^{51}Cr in mucosa	20·9	44·4*	(1·30)
^{51}Cr in submucosa	79·1	55·6*	(1·30)

(* $P<0{\cdot}0001$, paired t test)

Part of the intestine was removed during vasopressin infusion and the remainder after subsequent vasodilatation by isoprenaline. The mean radioactivity in the mucosa and submucosa for 7 cats is expressed as a percentage of the sum in the two layers.

blood flow passed through vessels larger than the ^{141}Ce microspheres (12 μ) in control experiments or after maximal vasodilatation of the mesenteric organs.

Histological studies

The most notable feature of the sections of intestine and sheets of submucosa after India ink injection was the almost complete absence of capillary-sized vessels in the submucosa except close to the mucosal border. These small vessels close to the mucosal border appeared to pass into the mucosa when serial sections were examined. An example of the type of picture seen is shown in Figure 2. No vessels appeared to pass from the submucosa to the muscle layer and the branches to the muscle arose from the arteries on the peritoneal surface of the muscle. The sheets of submucosa showed a dense plexus of vessels. These observations confirm those of other workers (Barlow, 1952; Boulter & Parks, 1960). The submucosal vessels were larger than 30 μ diameter except for the small branches to the mucosa. No arterio-venous anastomoses were seen (confirming Jacobson & Noer, 1952) and this is confirmed by the small proportion of radioactivity in the liver. If any of these vessels (larger than 30 μ) were arterio-venous anastomoses, microspheres would be expected to pass through them.

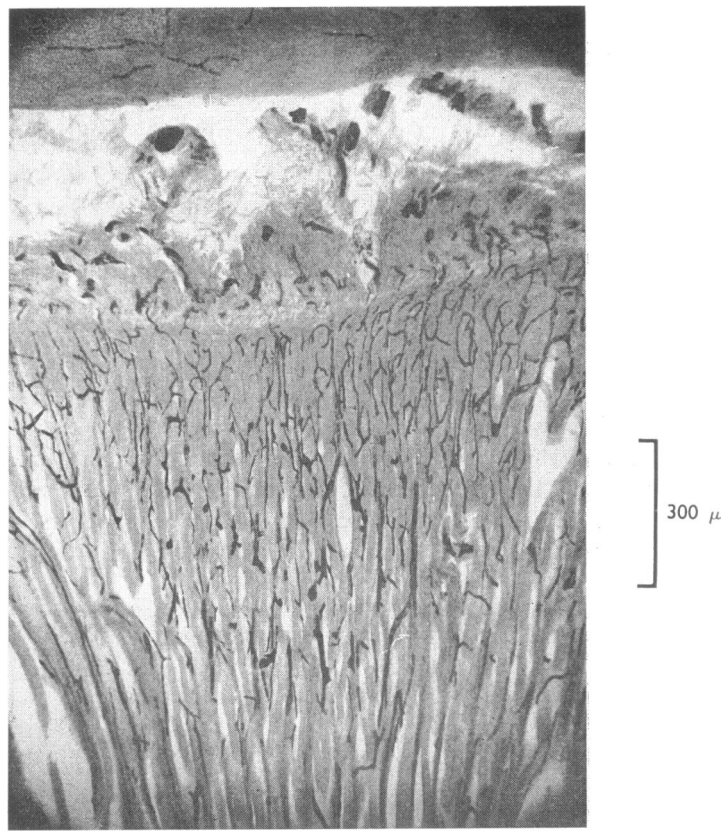

FIG. 2. Histological section of submucosa and mucosa after injection of India ink. Note the relative absence of capillary-sized vessels in the submucosa except close to the mucosal border and the absence of vessels from submucosa to muscle.

Discussion

The use of radioactive microspheres to study fractional distribution of cardiac output or blood flow to some region is based on the assumptions that the spheres are uniformly mixed with the blood, that they have the same rheological properties as the red cells, that they become impacted in some section of the precapillary bed depending on their size and thus that they are distributed proportionally to the blood flow in each of the parallel-coupled sections of the vascular bed (Wagner *et al.*, 1969). If a series of determinations of flow distribution are to be made by repeated injections of several types of microspheres, it must be shown that any one measurement is not modified by the previously administered spheres. In some situations, these assumptions are clearly not valid ; for example, portal flow to the liver cannot be measured by intra-arterial injection of microspheres since the liver is in series and not in parallel with the other mesenteric organs. Also if the spheres are small enough to pass through any part of the bed, the distribution will not accurately reflect flow. In most studies, some indication of the validity of the assumptions can be gained by administration of microspheres labelled with two different isotopes and of two different sizes. The spheres should be given simultaneously in some control experiments and one after the other in other control experiments. If the distribution of these two types is closely similar in the areas being studied and if no significant radioactivity passes through the bed to be trapped in the next series-coupled capillary bed (liver or lungs), the assumptions are mainly justified.

In the mesenteric vascular bed, these criteria are fulfilled in the intestine as a whole, the mesentery, lymph nodes, pancreas, colon and mesocolon and our data confirm and extend earlier work with microspheres varying in size from 15 to 80 μ diameter (Delaney, 1969 ; Kaihara, Van Heerden, Migita & Wagner, 1968). Therefore it appears reasonable to suggest that the distribution of spheres in these regions is a valid measure of blood flow (Table 1). These values are in substantial agreement with data obtained by other methods (Delaney, 1969 ; Ericsson, 1971 ; Folkow & Neil, 1971 ; Goodhead, 1969 ; Lundgren & Wallentin, 1964 ; Ross, 1971c). The values for pancreatic blood flow reflect only the proportion supplied by the superior mesenteric artery and the contribution from the coeliac artery was not measured. The criteria were also fulfilled for the muscle layer of the intestine and for the combined mucosa plus submucosa. The muscle received 7·8% of the total radioactivity giving a mean flow of (2·0 ml/min)/kg body weight, (8·0 ml/min)/100 g intestine or (16 ml/min)/100 g muscle. The combined mucosa and submucosa received 74% of the total radioactivity giving a mean flow of (19 ml/min)/kg body weight, (77 ml/min)/100 g intestine or (154 ml/min)/100 g mucosa plus submucosa. These values are in good agreement with those obtained from the ^{85}Kr wash-out studies in intestine (Kampp & Lundgren, 1968) and colon (Hultén, 1969) but much lower than the value for muscle flow reported by Grim & Lindseth (1958). No significant radioactivity was found in the liver or lungs and this suggests that less than 1% of the superior mesenteric arterial flow passes through arterio-venous shunts larger than 12 μ. This proportion is even smaller than that reported by Delaney (1969) and is substantially smaller than that reported by Grim & Lindseth (1958). In the latter experiments, the authors suggest some leaching of the isotope from the spheres occurred.

The distribution of microspheres between the mucosa and submucosa did not

meet the criteria set out above in that the distribution was dependent on the size of the spheres. Grim & Lindseth (1958) also observed this but no explanation was suggested. If the microspheres in the submucosa were impacted in some part of a parallel-coupled vascular bed in the submucosa, it is extremely unlikely that they would move into the mucosa when the vascular bed was subsequently dilated. On the other hand, if the submucosal vessels were in series with the mucosal vessels, the site of impaction of the spheres would depend on the relative sizes of the spheres and vessels as they passed from submucosa to mucosa. This would explain the greater proportion of ^{141}Ce-spheres (12 μ) compared to ^{51}Cr-spheres (17 μ) in the mucosa and the movement of both types of spheres when the vascular bed was dilated.

We conclude from these arguments that a portion of the microspheres in the submucosa were trapped in vessels which subsequently passed into the mucosa. However, some microspheres were trapped in the submucosa even during maximal vasodilatation of the intestine (Table 3). This could be due either to a true parallel-coupled submucosal section or to the fact that 37% of the vessels passing from the submucosa to mucosa are smaller than 12 μ diameter even when maximally dilated. The second possibility seems more likely for several reasons. Ross (1971c) showed in his ^{86}Rb uptake studies that only 7–12% of the superior mesenteric arterial flow passed through the submucosa. If no contamination occurred during separation of the intestinal layers, this would represent true nutritional flow to the submucosa. When Grim & Lindseth (1958) used 12 μ spheres, only 7% were trapped in the submucosa ; this value is lower than ours but their experiments were on dogs. The histological observations show very few capillary-sized vessels within the submucosa, except close to the mucosal border where a large number of small vessels arise and run into the mucosa.

Thus these observations, together with those by other workers discussed above, suggest that the vascular architecture in the intestine involves only two parallel-coupled sections, one to the muscle and the other through the submucosa to the mucosa. The vessels in the submucosa are in series with those in the mucosa and submucosal shunts do not exist. This is illustrated diagrammatically in Figure 3. If this hypothesis is correct, redistribution from mucosa to submucosa during autoregulatory escape or infusions of vasoactive drugs cannot occur. This does not

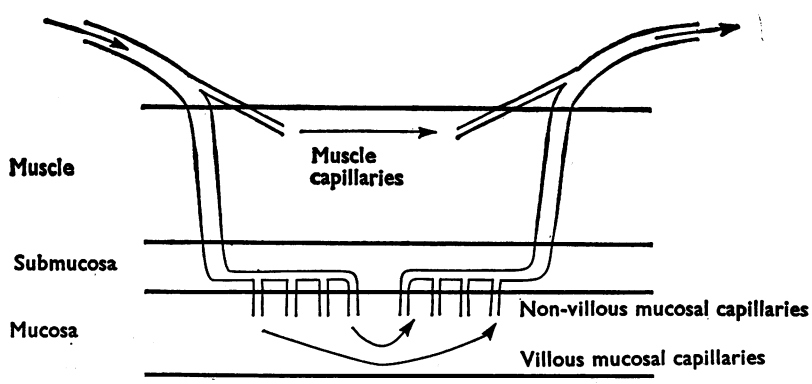

FIG. 3. Diagrammatic representation of the two parallel-coupled vascular circuits in the intestinal wall and the series-coupled sections between the submucosa and mucosa.

exclude the possibility of a redistribution within the mucosa, for example from villous to non-villous mucosa, but at present there is little evidence for this except the prolonged decrease in capillary filtration coefficient during sympathetic nerve stimulation (Folkow *et al.*, 1964, see **Introduction**). Adrenaline cannot be dilating the intestine by opening submucosal shunts as previously suggested (Greenway & Lawson, 1966) and it seems likely that α- and β-adrenoceptors have opposite effects on the same vascular smooth muscle as was shown to be the case in the spleen (Greenway & Stark, 1970).

On this hypothesis that there are no submucosal shunts, the sizes of the vessels between the submucosa and mucosa can be calculated from the microsphere data (Table 3). In the constricted vascular bed, 21% of these vessels are >17 μ, 30% are between 12 and 17 μ and 49% are <12 μ diameter while in the dilated bed, 44% are >17 μ, 19% are between 12 and 17 μ and 37% are <12 μ diameter.

When microspheres are used in pharmacological investigations on distribution of blood flow in organs, controls to validate the method for the particular areas being studied are essential. If the blood vessels in the areas studied are in series rather than in parallel, the method is invalid. We have shown that this is true in the intestinal wall and another situation where the problem should be studied is in the distribution between cortex and medulla of the kidney. Microspheres are being used to study this distribution but no controls of the type described in this paper have been carried out.

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