Small intestinal permeability

1. Effects of ischaemia and exposure to acetyl salicylate

J. G. C. KINGHAM, P. J. WHORWELL, AND C. A. LOEHRY

From the Department of Medicine, Royal Victoria Hospital, Bournemouth

SUMMARY Permeability of the small intestinal mucosa was estimated using a perfusion technique after either a period of ischaemia or exposure to acetyl salicylate. It was shown that these procedures increased the passive permeability of the mucosa to macromolecules while maintaining normal mucosal selectivity. Histologically, there was derangement of the epithelial cell layer of the villous tips without damage to the epithelial basement membrane or subepithelial structures. It is concluded that the epithelial cell layer is purely rate limiting with no selective function and that the role of selectivity must be ascribed to either or both of the other mucosal barriers, the capillary and the epithelial basement membrane.

In normal conditions the gastrointestinal tract is an important site of loss of plasma constituents. Fluid, small solutes, and macromolecules are all lost into the intestinal lumen either by active secretion or by passive exsorption. The loss of significant amounts of important plasma constituents such as albumin (Wetterfors, 1965), immunoglobulins (Barth *et al.*, 1964), insulin (Danforth and Moore, 1959), and vitamin B12 (Loehry and Creamer, 1969) into the intestinal lumen is well recorded.

The leakage of water soluble substances from the intestinal mucosa is largely a passive process. It has been shown by Loehry *et al.* (1970) that the degree of exsorption of any particular substance depends directly on its plasma concentration and inversely on its molecular size.

The mucosal barrier is obviously a very effective one, the *milieu intèrieure* being maintained except in severe intestinal disease. The barrier is not only very impermeable—that is, rate limiting—but also highly selective. An analogy may be drawn with the renal glomerulus, which is also a highly selective barrier, though much more permeable, the selectivity in this instance being imparted by the glomerular basement membrane. There are three distinct anatomical barriers between the circulating plasma and the intestinal lumen, any or all of which may be responsible for rate limiting and selective functions of the intestinal mucosa: these are the capillary endothelium, the epithelial basement membrane, and the epithelial cell layer.

Two of the proposed barriers, the epithelial and

endothelial cell membranes, are lipid membranes, perforated by aqueous pores and therefore permeability will be determined by the absolute number of pores while selectivity depends on the distribution of pore size. The remaining barrier, the epithelial basement membrane, is a tightly woven mesh of fibres; similarly, its permeability and selectivity will be governed by the frequency and dimensions of the gaps between the fibres.

In view of the difficulty of separating these three anatomical barriers to determine their individual permeability characteristics, it was felt the permeability of the mucosa as a whole should be measured after producing selective damage to any one of the three barriers. In the present series of experiments we sought to damage only the epithelial cell layer of the small intestinal mucosa. The permeability of the mucosa to various tracer substances was observed after a period of ischaemia and after acetyl salicylate exposure. These two experimental models were studied because they not only produce mucosal epithelial damage, but are also common clinical situations.

Methods

Permeability was measured using a perfusion technique in female albino rabbits (body weight 2.5-3.5 kg). The animals were anaesthetised with pentobarbitone (Nembutal) (30 mg/kg) and hydration maintained with normal saline *via* an intravenous cannula. A midline longitudinal incision was made from xiphisternum to pubis and the renal pedicles

Fig. 1 Changes in permeability of the small intestine after the period of ischaemia by comparison of the clearances of the tracer substances into control and ischaemic loops. ● = control. × = ischaemic.

Tracer substance	MW	Mean clearance $\pm$ SD (ml/min/cm)		Significance of difference $P =$	%
		Control	Ischaemia		
PVP	33000	0.000026 $\pm$ 0.000005	0.000157 $\pm$ 0.000029	<0.001	500
Inulin	5000	0.000043 $\pm$ 0.000008	0.000247 $\pm$ 0.000066	<0.001	470
Vitamin B ₁₂	1300	0.00015 $\pm$ 0.00003	0.00092 $\pm$ 0.00010	<0.001	510
Creatinine	120	0.0012 $\pm$ 0.0002	0.0034 $\pm$ 0.0008	<0.001	180
Urea	60	0.0063 $\pm$ 0.0013	0.0060 $\pm$ 0.0010	>0.4	-5

Table 1 Average clearances, with standard deviations from mean, of each of the tracer substances into control and ischaemic loops. (Results also expressed as percentage increase in clearance after ischaemia compared with control.)

were ligated on both sides to prevent renal excretion during the experiment. Four 15 cm loops of small intestinal at varying levels between duodenum and ileocaecal valve were individually cannulated with inflow and outflow cannulae of 4 mm PVC tubing. Care was taken to avoid mucosal trauma and impairment of the blood supply to the cannulated loops. The loops were replaced in the abdominal cavity and gently washed out with normal saline.

When the perfusates were clear, two loops were

designated control and the other two experimental. For the ischaemic procedure the two experimental loops were rendered ischaemic for two hours by clamping off their local mesenteric blood supply. At the end of this period the clamps were removed. In the case of the acetyl salicylate (ASA) procedure, the mucosa of the two experimental loops was exposed to a unionised solution of ASA at pH 2.6 for 45 minutes.

During the ischaemia or the ASA exposure, the

rabbits were given a slow intravenous infusion of the substances whose exsorption into the intestine was under study. The infusion contained 6 g urea, 3 g creatinine, 3 uCi ^{57}Co vitamin B12, 60 uCi ^3H inulin, and 50 uCi 125I polyvinylpyrrolidone (PVP). At the time of the anaesthetic the rabbits had been given 100 ug unlabelled vitamin B12 in order to flood the body stores.

At the end of the two hours' ischaemia or 45 minutes' ASA exposure, the loops were washed out with normal saline. The loops were then perfused at a rate of 2 ml/min from a reservoir containing normal saline with PEG 0.8 g/l as a non-absorbable marker. Samples were collected over four periods of 15 minutes for estimation of the exsorbed tracer substances.

A 10 ml blood sample was taken from the jugular vein of the side opposite to the intravenous infusion at the start and finish of the perfusion. The starting and finishing plasma levels of the tracer substances were estimated and averaged.

After the perfusion a full thickness biopsy of each intestinal segment was taken for histological examination. The rabbits were then killed and the small intestine removed in order to accurately measure and localise the segments.

Biopsies were fixed in 10% formol saline for 24 hours, processed, sectioned, and stained with Ehrlich's haematoxylin and eosin.

Permeability was assessed by calculating the clearance of each of the tracers into the perfused segments where

$$\text{clearance} = \frac{\text{perfusate concn} \times \text{perfusion rate}}{\text{plasma concn} \times \text{length of a segment}}$$

Selectivity was measured by gel fractionation of the PVP in perfusate and plasma and comparison of the two fractions (Loehry *et al.*, 1970).

Ischaemia

Results

Figure 1 demonstrates the changes in permeability of the small intestine after a period of ischaemia by comparison of the clearances of the tracer substances into control and ischaemic loops. Table 1 shows the average clearances, with the standard deviations from the mean, of each of the tracer substances into the control and ischaemic loops. The results are also expressed as a percentage increase in clearance after ischaemia compared with control.

It is evident from Fig. 1 and Table 1 that ischaemia produces a large increase in intestinal permeability to PVP, insulin, and vitamin B12, a moderate increase in permeability to creatinine, but is without effect on

permeability to urea.

Table 1 shows that, for substances greater in size than creatinine, there was a constant increase in clearance after ischaemia. Thus, for larger molecules, permeability was increased but selectivity unaltered.

Confirmation of this maintenance of selectivity is given by the PVP fractionation curves seen in Fig. 2. This figure shows the clearances into the control and ischaemic loops of the individual fractions of PVP as they came off the column. The clearances are plotted against tube number where tube number corresponds to a particular molecular weight which is known from the calibration of the column.

Fig. 2 Clearances into the control and ischaemic loop of the individual fractions of PVP as they came off the column plotted against the tube number. The tube number corresponds to a particular molecular weight, which is known from calibration of the column. Slope of control curves, 0.033 ± 0.002 ; slope of ischaemic curves, 0.031 ± 0.003 — $P > 0.1$.

The two sets of clearances give curves of almost identical slope, indicating that, in both intestinal loops, selection is taking place in favour of the smaller molecules. Thus, although the permeability of the ischaemic loop is greater than that of the control, the selectivity is not significantly different.

It was felt that the different behaviour of both creatinine and urea was a reflection of their small molecular size. It appeared that for tracer substances above a critical size the effect of ischaemia was to cause a constant relative increment in permeability, whereas for substances below this size the effect of ischaemia diminished progressively as the size of the molecule decreased, and that, for a substance as small as urea, the effect was negligible.

In order to put this hypothesis to the test, the clearance of a substance of a molecular size between

that of creatinine and urea was measured. ^{14}C labelled thiourea was used, as this is water soluble, neither highly ionised nor actively transported, and is known to permeate thin artificial lipid membranes at a rate similar to that of urea (Holz and Finkelstein, 1970). The results of these experiments show clearly that, for a substance intermediate in size between urea and creatinine, both the clearance and the percentage increase in clearance fall between those respective figures for urea and creatinine (Table 2).

HISTOLOGY

There were marked changes in the villous tips of the ischaemic intestine. The epithelial cells were degenerate and in the process of sloughing off the underlying basement membrane into the intestinal lumen. Over the crests of the villi the microvillous brush border had disappeared and the intercellular membranes were indistinct. The lamina propria appeared normal. The epithelial changes decreased down the sides of the villi; the lower half of the villi and the crypts appeared to be undamaged.

Discussion

Total ischaemia of the small intestine causes both functional and morphological derangement of the mucosa and ultimately necrosis of the whole viscus. Chiu *et al.* (1970) studied the effects of occlusion of the superior mesenteric artery of dogs. Mucosal morphological changes were noted after as little as five minutes and worsened as ischaemia time increased. The earliest change was the development of the subepithelial Gruenhagen's space, usually at the apex of the villus; this space then extended, lifting the epithelium off the lamina propria, so separating the epithelial cells from their basement membrane, and eventually there was denuding of the villi, the crests being affected first. The villi were more susceptible to ischaemia than the crypts. Once the epithelial cell layer had lifted off from the lamina propria the mucosal permeability barrier broke down and intraluminally administered D-tubocurarine passed across the mucosa, normal mucosa being impermeable to this substance. The subepithelial Gruenhagen's space contained fluid that had escaped from both capillaries and intestinal lumen. These workers

felt that the development of this space was an important factor leading to cell death by physically separating the epithelial cells from their nutrient supply. There is usually full recovery of mucosal function if ischaemia time is less than two hours, because, although the villus epithelial cells are killed, the crypt cells survive and, after two to three days, migrate upwards to repopulate the villi (Robinson *et al.*, 1966; Röttger and Oran, 1969). Active transport of sugars and amino acids by the epithelial cells is abolished almost immediately by ischaemia (Robinson and Mirkovitch, 1972). Electron microscopical studies of dog acute ischaemic ileum shows changes within five minutes; the earliest changes are intracellular, but by 30 minutes the intercellular spaces have increased, the intercellular membranes have fractured, and the epithelial cells have lifted from the basement membrane (Brown *et al.*, 1970). The intercellular spaces are dilated with lakes of protein-containing fluid.

In the current study there were obvious gross changes produced by two hours' ischaemia. The light microscopic changes noted are in accord with those noted by other observers (Chiu *et al.*, 1970; Robinson *et al.*, 1966; Röttger and Oran, 1969)—namely, separation of epithelial cells from one another and from the basement membrane with maximal damage at the villous crests, and progressively less damage down the sides of the villi towards the crypts.

Several studies have investigated changes in mucosal permeability after ischaemia. Davenport and Barr (1973) found no increase in H^+ and Na^+ permeability after vasopression induced ischaemia in dog stomach pouches. However, it is doubtful whether their methods actually produced mucosal ischaemia. Dorricott *et al.* (1973) used a similar experimental model and again found no increased mucosal permeability to Na^+ and H^+ ; they estimated the degree of ischaemia produced by comparing the gastric aminopyrine clearance of ischaemic pouches (16%) with normal pouches (100%). Complete ischaemia therefore was not achieved. Little work has been done regarding macromolecular permeability after ischaemia, but Zuidema *et al.* (1962) have shown increased faecal loss of labelled PVP (MW 40 000) in dogs after two hours' occlusion of the superior mesenteric artery. Increased faecal PVP loss

Tracer substance	MW	Mean clearance $\pm$ SD (ml/min/cm)		Percentage increase in clearance
		Control loop	Ischaemic loop	
Creatinine	120	0.0012 $\pm$ 0.0002	0.0034 $\pm$ 0.0008	180
Thiourea	76	0.003 $\pm$ 0.0006	0.0057 $\pm$ 0.0005	60
Urea	60	0.0063 $\pm$ 0.0013	0.0060 $\pm$ 0.0010	-5

Table 2 Mean clearances, with standard deviations, of creatinine, ^{14}C thiourea, and urea into the ischaemic and control loops. (Results also expressed as percentage increase in clearance after ischaemia.)

after occlusion of the superior mesenteric artery has also been recorded in man (Zuidema *et al.*, 1962).

The permeability changes induced by ischaemia in the present study are marked. From Table 1 it can be seen that the passive blood to lumen flux of substances with molecular weights between 1300 and 80 000 increases by 500%. Although mucosal permeability is increased, the natural selectivity of the mucosa is retained; this finding is borne out by the results of PVP clearance curves, for ischaemic and normal bowel are the same.

It seems reasonable to assume that the increase in mucosal permeability is related to the morphological damage seen under the microscope. Thus, destruction of the epithelial cell layer at the villous crests and less severe epithelial cell changes in the upper half of the villous sides lead to a substantial increase in macromolecular permeability, but have no effect on the selectivity of the mucosal barrier.

This phenomenon may be explained if it is postula-

ted that ischaemia basically affects the epithelial cell membrane and tight junctions which are rate limiting, but has little or no effect on the selective barriers of the intestinal mucosa.

Acetyl salicylic acid exposure

Results

Figure 3 demonstrates the changes in permeability of the small intestine after exposure to unionised ASA by comparison of the clearances of the tracer substances into control and ASA-treated segments. Table 3 shows the average clearances, with the standard deviations from the mean, of each of the tracer substances into the control and unionised ASA-treated loops. The results are also expressed as a percentage increase in clearance after ASA exposure.

It is evident that exposure to unionised ASA

Fig. 3 Changes in permeability of the small intestine after exposure to unionised ASA by comparison of the clearances of the tracer substances into control and ASA treated segments. ● control. × = ASA pH 2.6.

Tracer substance	MW	Mean clearance $\pm$ SD (ml/min/cm)		Percentage increase in clearance	P values
		Control	ASA pH 2.6		
PVP	33000	0.000010 $\pm$ 0.000002	0.000086 $\pm$ 0.000029	740	<0.001
Inulin	5000	0.000025 $\pm$ 0.000007	0.000206 $\pm$ 0.000075	720	<0.001
Vitamin B ₁₂	1300	0.00011 $\pm$ 0.00002	0.00104 $\pm$ 0.00022	810	<0.001
Creatinine	120	0.0015 $\pm$ 0.0006	0.0040 $\pm$ 0.0014	170	<0.001
Urea	60	0.0045 $\pm$ 0.0015	0.0042 $\pm$ 0.0011	8	>0.3

Table 3 Average clearances, with standard deviations from mean, of each of tracer substances into the control and unionised ASA treated loops. (Results are also expressed as percentage increase in clearance after ASA exposure)

greatly increases mucosal permeability to PVP, inulin, and vitamin B12, moderately increases permeability to creatinine, but is without effect on urea permeation.

From Table 3 it can be seen that for substances greater in size than creatinine there was a constant relative increase in clearance after exposure to unionised ASA. This indicates that selectivity for large molecules is unchanged. There was a moderate increase in creatinine clearance but no increase in urea clearance. Confirmation of this maintenance of selectivity is given by the results of PVP fractionation. Figure 4 shows the clearances into the control and ASA exposed segments of the individual fractions of PVP as they came off the Sephadex column plotted against the tube number, where the tube number corresponds to a particular molecular weight known from calibration of the column. The two clearance curves have very similar slopes indicating that in both intestinal loops selection is taking place in favour of the smaller molecules. Thus ASA exposure produces no change in selectivity despite the increase in permeability.

HISTOLOGY

Exposure of the mucosa to unionised ASA produced marked morphological changes. There was gross derangement of the mucosa with destruction of the villous epithelial cells, mainly at the crests, separation and lifting of the cells from the basement membrane, and in places partial denuding of the villous tips. The epithelium of the base of the villi and the crypts appeared undamaged.

Neither ionised ASA nor acidified saline had any visible effects on mucosal morphology.

Discussion

Most of the work done to study the role that acetyl salicylic acid (ASA) plays in increasing mucosal permeability has been carried out on the gastric mucosa. Davenport (1965, 1966) treated denervated gastric pouches in dogs with 20 mM ASA at pH 2.0 and 20 mM ASA buffered to pH 5.0. He showed that unionised (acid) ASA, but not ionised ASA (neutral) caused an increase in mucosal perm-

Fig. 4 Clearances into the control and unionised ASA exposed segments of the individual fractions of PVP as they came off the Sephadex column plotted against the tube number. The tube number corresponds to a particular molecular weight which is known from calibration of the column. Slope of control curves, 0.033 $\pm$ 0.002; slope of ASA curves, 0.032 $\pm$ 0.005—P > 0.6.

eability to hydrogen, sodium, potassium, and chloride ions.

Frenning (1971), studying the increased permeability induced in cat gastric mucosa by ASA, suggested that the effect was produced by a widening of the mucosal intercellular spaces and loosening of the tight junctions.

The studies of Flemstrom *et al.* (1973) showed an increase in frog gastric mucosal permeability to electrolytes, raffinose, and dextran (MW 30 000) caused by ASA. They produced similar results with both 5 and 10 mM ASA at pH 3.0, but found no increase in permeability after 1 mM ASA at pH 3.0, 10 mM ASA at pH 7.0, or control solution at pH 3.0. They calculated the resulting equivalent pore size after unionised ASA exposure to be 40 Å and suggested that the increased permeability was intercellular rather than transcellular.

In a study of volunteer subjects by Beeken (1967) ingestion of pharmacological doses of acetyl salicylate has been shown to increase protein loss into the gut as measured by the ⁵¹Cr-Albumin faecal clearance technique. The study did not suggest whether the albumin loss was gastric or small intestinal.

The most comprehensive study is that carried out by Davenport (1965) using *in vivo* dog gastric pouches to investigate fluid production caused by acetic and salicylic acids. The theory put forward by Davenport (1965) is that unionised acetyl salicylic acid (ASA) increases mucosal permeability to hydrogen ions which back-diffuse from the gastric lumen into the gastric mucosa. The hydrogen ions then cause cell damage with secondary histamine release. The histamine causes increased mucosal capillary permeability with a resultant increase in the passage of plasma from the circulation into the gastric lumen. In summary, Davenport's argument states that mucosally applied noxious agents such as unionised ASA cause increased mucosal permeability to plasma macromolecules by releasing histamine which will increase capillary permeability. Although the idea is attractive, his experimental evidence is not conclusive, as his results do not show whether or not capillary permeability was increased by ASA.

The permeability changes induced by unionised ASA in the present study are considerable with increases around 750% in passive plasma to lumen flux of molecules ranging in molecular weight from 1300 to 80 000. These percentage increases are in the same order, indicating that selectivity has been retained. The results of PVP fractionation confirm that natural mucosal selectivity has not been lost despite the increase in permeability. As is the case in the ischaemia experiments, the creatinine permeability is only moderately increased—170%—and

urea permeability is no greater than in the control loops.

Permeability was to all intents and purposes unaffected by exposure to ionised ASA at pH 7.0 and acidified saline at pH 2.6 (unpublished observations). These findings are in accord with those of Davenport (1964), Davenport (1966), and Flemstrom and Marsden (1973).

The morphological changes in the mucosa induced by unionised ASA are essentially damage to the epithelial cell layer, and the changes in permeability are in degree but not in quality; there is a large increase in macromolecular permeability, but no alteration of selectivity. It would seem, then, that the epithelial cell barrier is rate-limiting and non-selective, and that ASA is without effect on the selective barriers of the intestinal mucosa.

Conclusions

Permeability and selectivity are best understood by the pore theory—the greater the number of pores the more permeable is a membrane, and the greater the size the less selective. It is probable that there is a normal distribution of pores in the intestinal mucosa with the great majority having a radius of 4–8 Å (Fordtran *et al.*, 1965; Dillard *et al.*, 1965), but also a smaller proportion of large pores to allow passage of macromolecules. This is an oversimplification of the state of affairs, however, as there are three separate anatomical barriers which summate to give this overall picture. The purpose of the present series of experiments was to evaluate the barrier function of the epithelial cell layer of the mucosa.

The results have shown that ischaemia and ASA exposure increase the permeability of the small intestinal mucosa for substances greater in size than creatinine, the percentage increase in permeation is constant, thus indicating that selectivity is unchanged. Fractionation of the PVP to calculate the individual clearances of the fractions confirms this unaltered selectivity. For substances smaller in size than, and including, creatinine, the percentage increase in permeability diminishes with decreasing molecular size, and, for a substance as small as urea, there is no appreciable increase in permeability. Histologically, ischaemia and ASA exposure produce derangement of the epithelial cell layer of the villous tips with separation and lifting of the cells from the basement membrane and partial destruction of the apical and lateral cell membranes.

Thus, it must be concluded that the epithelial cell layer is purely rate limiting and non-selective, as damage to this barrier affects only rate limitation and not selectivity. This may best be visualised by an

increase in the number of pores but no alteration in their dimensions. Therefore, the role of selectivity in the intestinal mucosa must be ascribed to the capillary or the epithelial basement membrane or perhaps both.

We wish to thank the Department of Biochemistry and Histopathology for their technical assistance. J.G.C.K. thanks the Wessex Regional Health Authority for a grant to support this work.

References

- Barth, W. F., Wochner, R. D., Waldmann, T. A., and Fahey, J. L. (1964). Metabolism of human gamma macroglobulins. *Journal of Clinical Investigation*, **43**, 1036-1048.
- Beeken, W. L. (1967). Effect of salicylates on gastrointestinal protein loss in normal subjects. *Gastroenterology*, **53**, 894-99.
- Brown, R. A., Chiu, C. J., Scott, H. J., and Gurd, F. N. (1970). Ultrastructural changes in the canine ileal mucosal cell after mesenteric arterial occlusion: a sequential study. *Archives of Surgery (Chic.)*, **101**, 290-7.
- Chiu, C. J., McArdle, A. H., Brown, R., Scott, H. J., and Gurd, F. N. (1970). Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic and metabolic reappraisal. *Archives of Surgery (Chic.)*, **101**, 478-83.
- Danforth, E., and Moore, R. O. (1959). Intestinal absorption of insulin in the rat. *Endocrinology*, **65**, 118-23.
- Davenport, H. W. (1964). Gastric mucosal injury by fatty and acetylsalicylic acids. *Gastroenterology*, **46**, 245-53.
- Davenport, H. W. (1965). Damage to the gastric mucosa; effects of salicylates and stimulation. *Gastroenterology*, **49**, 189-96.
- Davenport, H. W. (1966). Fluid produced by the gastric mucosa during damage by acetic and salicylic acids. *Gastroenterology*, **50**, 487-99.
- Davenport, H. W., and Barr, L. L. (1973). Failure of ischemia to break the dog's gastric mucosal barrier. *Gastroenterology*, **65**, 619-624.
- Dorricott, N. J., Eisenberg, H., and Silen, W. (1973). Effect of intra-arterial vasopressin on canine gastric mucosal permeability. *Gastroenterology*, **65**, 625-29.
- Flemström, G., and Marsden, N. V. B. (1973). Dextran permeability, electrical properties, and H⁺ secretion in isolated frog gastric mucosa after acetylsalicylic acid. *Gastroenterology*, **64**, 278-84.
- Dillard, R. L., Eastman, H., and Fordtran, J. S. (1965). Volume flow relationship during the transport of fluid through the human small intestines. *Gastroenterology*, **49**, 58-66.
- Fordtran, J. S., Rector, F. C., and Ewton, M. F. (1965). Permeability characteristics of the human small intestine. *Journal of Clinical Investigation*, **44**, 1935-1944.
- Frenning, B. (1971). The disappearance of acetylsalicylic acid (ASA) in aqueous solution from the cat's stomach and its influence on the transmucosal ion transport in the innervated non-secreting stomach. *Acta Physiologica Scandinavica*, **83**, 235-246.
- Holz, R., and Finkelstein, A. (1970). The water and non-electrolyte permeability induced in thin lipid membranes by the polyene antibiotics Nystatin and Amphotericin B. *Journal of General Physiology*, **56**, 125-145.
- Loehry, C. A., Axon, A. T. R., Hilton, P. J., Hider, R. C., and Creamer, B. (1970). Permeability of the small intestine to substances of different molecular weight. *Gut*, **11**, 466-70.
- Loehry, C. A., and Creamer, B. (1969). Vitamin B₁₂ excretion by the rat small intestine. *Gut*, **10**, 662-664.
- Robinson, J. W. L., Antonioli, J. A., and Mirkovitch, V. (1966). The intestinal response to ischaemia. *Naunyn-Schmiedelberg's Archiv für experimentelle Pathologie und Pharmakologie* **255**, 178-191.
- Robinson, J. W. L., and Mirkovitch, V. (1972). The recovery of function and microcirculation in small intestinal loops following ischaemia. *Gut*, **13**, 784-89.
- Röttger, P., and Oran, M. (1969). Histochemische und biochemische Untersuchungen zur Frage des Enzym-Gehaltes der Dunndarmschleimhaut nach temporärer Ischaemie. *Verhandlungen der Deutschen Gesellschaft für Pathologie*, **53**, 189-195.
- Wetterfors, J. (1965). Albumin: investigations into the metabolism, distribution and transfer of albumin under normal and certain pathological conditions with special reference to the gastro-intestinal tract. *Acta Medica Scandinavica*, **177**, suppl. 430.
- Zuidema, G. D., Turcotte, J. G., Wolfman, E. F., and Child, C. G. (1962). Metabolic studies in acute small-bowel ischemia. *Archives of Surgery (Chic.)*, **85**, 130-135.