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SOME PROPERTIES OF A PREPARATION OF RAT COLON PERFUSED *IN VITRO* THROUGH THE VASCULAR BED

By D. S. PARSONS and G. POWIS*

From the Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU

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SUMMARY

1. A technique is described for the perfusion of the isolated colon of the rat, involving the infusion of an appropriate fluid through both the inferior and the superior mesenteric arteries. During neither the preparation nor the subsequent perfusion is the colon without an adequate supply of oxygen. The preparation remains histologically intact and metabolically viable and is capable of actively transporting ions for up to 5 hr.

2. The addition of at least 3 g albumin/100 ml. perfusate is necessary to prevent the formation of large quantities of serosal exudate. With erythrocytes added to the vascular perfusate the preparation appears to be adequately oxygenated as judged by measurements of the rate of glycolysis. The mean rate of oxygen utilization over 4 hr is $9{\cdot}2 \pm 0{\cdot}3$ (4) μmole.hr^{-1}.g^{-1} fat free dry weight.

3. Ion transport rates approaching those found *in vivo* are found only after the administration of an antihistamine substance to the colon donor rat before operation. In the absence of an antihistamine substance there appears to be an ultrafiltration of the plasma fluid into the lumen.

4. Vasodilatory substances accumulate in the recycled perfusate. In a 'single pass' perfusion, the transport capacity of the preparation decreases at high perfusion pressures. It is suggested that this is due to some form of autoregulation whereby perfusate is shunted away from the epithelium into deeper layers as the pressure is increased.

5. With CO_2 absent from the vascular infusate there is an increase in the net lumen to blood flux of total CO_2. This increased flux is accompanied by an equivalent amount of cation, comprising Na^+ and K^+ in the ratio of 12:1.

6. The presence of ammonium in the lumen, a physiological constituent of the contents of rat distal colon *in vivo*, has a marked inhibitory effect upon the secretion of CO_2 into the contents of the lumen of the colon.

* Present address: Department of Pharmacology, The University, Glasgow, W. 2.

INTRODUCTION

Although vascular perfusion of amphibian intestine has been successfully achieved, the perfusion of the vascular bed of the mammalian intestine has proved a notoriously difficult technique and attempts prior to the middle of the last decade were only sporadic and attended by little success. (For a review, see Parsons & Prichard, 1968). From this date on, however, there has been an increasing flow of work most of which has only partially solved the problems of vasoconstriction, hypermotility and progressive destruction of the mucosal epithelium which bedevil work in this area. Hohenleitner & Senior (1967) reported the perfusion of dog intestine with Ringer solution containing sheep erythrocytes or dextran, although only by adding drugs could they overcome problems of hypermotility. Grenier, Wong, Kachelhoffer, Barth, Baruthio, Clavert & Weiss (1967) were more successful in their attempts with dog intestine; after 3 hr perfusion with homologous blood the tissue was capable of normal function when transplanted back into another dog.

Working with rat intestine, Kavin, Levin & Stanley (1967) observed vasoconstriction and fluid accumulation in the lumen of the intestine perfused *in situ* with a medium of bovine erythrocytes in dextran-Ringer. Dubois, Vaughan & Roy (1968) perfused the isolated intestine of rat with bovine erythrocytes in albumin-Ringer for 5 hr. Although the tissue exhibited both oxygen and glucose utilization, there were signs of detachment of the mucosa from tips of the villi. Short studies, up to 30 min, concerned with lymph flow were undertaken by Lee & Duncan (1968), who perfused the isolated intestine of rat with homologous blood, using pentobarbitone to inhibit motility. With a similar preparation, Windmueller & Spaeth (1969) demonstrated that in the absence of drugs to inhibit motility, there was a net secretion of fluid into the intestinal lumen. More recently, the same authors have reported the perfusion of the vascular bed of the rat intestine *in situ* with homologous blood. In these experiments, hypermotility, hyperaemia and epithelial necrosis were reduced by the addition of noradrenaline and glucocorticoids to the vascular perfusate (Windmueller, Spaeth & Ganote, 1970). The transport of solutes between the intestinal lumen and the blood stream is a process which appears to be very sensitive to the effects of surgical operation and the measurement of transport provides a means of evaluating the success of the preparation. Despite, or perhaps because of this, transport by the vascularly perfused intestine of mammals has received little critical attention.

Few attempts have been made to perfuse the vascular bed of the colon, possibly because the organ is provided with a double blood supply through

both the inferior and the superior mesenteric arteries. There have been some efforts to perfuse the colon through one artery alone, although little attention has been paid to oxygenating the perfusate and no functional or metabolic studies have been undertaken (Garry, Holmes & Wishart, 1957; Bouillin, Costa & Brodie, 1967). The colon is a tissue particularly useful for the study of transepithelial ion transport uncomplicated by effects due to the concomitant movement of organic solutes such as glucose. In the small intestine, glucose must be added to the fluid in the intestinal lumen as a substrate for fluid and hence ion transport. In the case of rat colon, in contrast the mucosal face of the epithelial cells is impermeable to monosaccharides (see, for example, Parsons, 1967; Parsons & Paterson, 1965; Heaton & Parsons, 1965).

The present paper describes a procedure for the perfusion of the entire vascular bed of the rat colon by a method which involves no interruption in the supply of oxygen. The problems of the choice of perfusate and the conditions of perfusion receive particular attention. Metabolic, histological and functional criteria indicate that the preparation remains viable for up to 5 hr. Some interrelations between the transport of different ions are investigated and discussed. It is hoped that in this study some of the problems associated with perfusion of the intestinal vascular bed have been solved and that the method may be generally applicable to the perfusion of all regions of the mammalian intestine.

METHODS

Apparatus. The apparatus (Fig. 1) consisted of two independent circuits, one for the perfusion of the vascular bed and one for the circulation of fluid through the colonic lumen. The lumen circuit comprised two water-jacketed glass reservoirs connected by means of a three-way tap to a peristaltic pump (Harvard model 600–000). A second three way tap distal to the pump allowed the lumen of the colon to be completely flushed out. The luminal fluid passed through the colon and was then either returned to the reservoir through a tube dipping below the level of the fluid or collected separately. The fluid level in the reservoir was 10 cm above the midpoint of the colon.

The oxygenator-reservoir in the vascular circuit comprised a 250 ml. polyethylene bottle rotating at 60 rev/min. The perfusate formed a film over the wall when it was exposed to 95 % (v/v) oxygen and 5 % (v/v) carbon dioxide. The perfusate was drawn from the reservoir by a non-pulsatile roller pump (Varioperpex, LKB 12000) and filtered through a compressed stack of V 75 Fipoca nylon filter elements with a filtration size of 13–43 μ (Rellumit, Ltd). The heat exchanger through which the perfusate next passed consisted of a coil of nylon tubing (Portex, Nylon 6, 1·9 × 2·76 mm), followed by a 1 ml. glass bubble trap, both immersed in the liquid paraffin of the perfusion reservoir. The perfusion pressure was measured as the height of a column of saline in the vertical limb of a T tube inserted between the heat exchanger and the bubble trap. The venous outflow was set at the level of the midpoint of the colon and the effluent collected in a 10 ml. graduated glass centrifuge

tube fitted with a two way tap fused at the bottom. The venous effluent could then be either collected or returned to the oxygenator-reservoir.

The isolated colon was held extended in a vertical position, caudal end above, by two springs clips embracing the cannulae placed in the lumen. The arterial and venous cannulae were held by two adjustable Perspex clips. Both the pairs of spring and Perspex clips were mounted on small pot magnets which could be positioned as required upon a 9 × 22 cm steel plate which fitted upright inside the glass perfusion reservoir. The reservoir was filled with liquid paraffin, maintained at 38° C by an outer water jacket. The lumen inflow and outflow tubes and the vascular outflow tube passed over the upper edge of the reservoir.

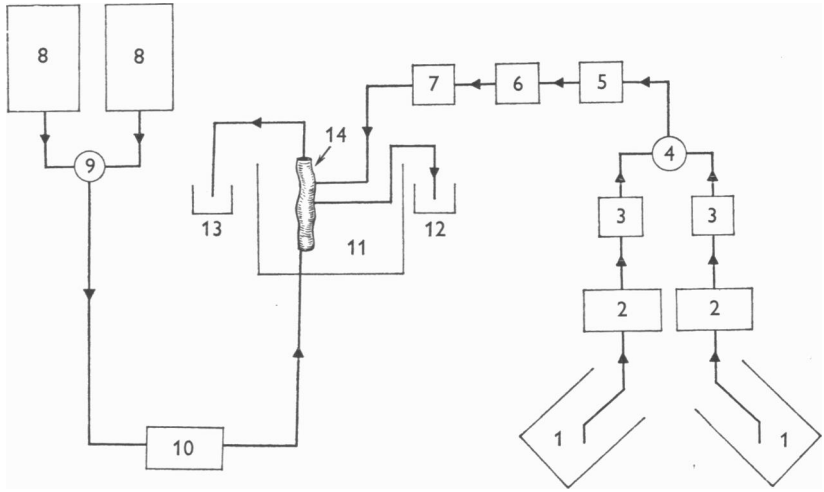

Fig. 1. Apparatus for the perfusion of the isolated colon of rat. The double vascular circuit is included to enable the vascular perfusate to be changed: (1) oxygenator-reservoir, (2) roller pump, (3) filter, (4) three-way tap, (5) heat exchange, (6) manometer, (7) bubble trap, (8) lumen reservoirs, (9) three-way tap, (10) peristaltic pump, (11) organ bath filled with liquid paraffin, (12) collection of vascular effluent, (13) collection of lumen circuit (alternatively lumen fluid may be returned to appropriate reservoir (8)), (14) colon.

Portex nylon 6 tubing (Portex, Ltd, Hythe, Kent) was used where possible for both the vascular (1·9 × 2·76 mm) and lumen circuits (2·44 × 3·24 mm). Where flexibility was desired, Tygon vinyl tubing, formulation S-50-HL (US Stoneware Co.) was used as for the immediate inflow and outflow of both circuits. The arterial cannula was fashioned from Portex polyethylene tubing (PP 190, 1·9 × 1·7 mm) drawn out over a small gas flame, whilst the venous cannula was a square ended length of the same tubing. The lumen cannulae were fashioned from 3·5 mm glass tubing with a small constriction just behind the tip, which had a 60° bevel.

The advantage of maintaining the colon in a vertical position was that it was more easily drained and, when priming with luminal fluid, all the trapped gas could be displaced.

The apparatus was easily dismantled for cleaning and was soaked in Pyroneg (Diversey, Ltd, Cockfoster, Herts.) after each use.

Operative procedure. A male albino rat, 250 g, fasted 18 hr, was anaesthetized with

intramuscular Nembutal, 6 mg/100 g body wt., and the trachea cannulated. Where required, the antihistamine promethazine hydrochloride (May & Baker), 2 mg/100 g body wt. was administered intravenously, most easily into the dorsal vein of the penis. The abdomen was opened by a mid line incision and the intestines displaced to the right. The aorta and inferior vena cava were cleared of overlying tissue and the tips of a blunt pair of forceps inserted between the two vessels, both above and below the level of the left ileolumbar artery. Double ties were made around the base of this artery on the inner ventral aspect of the aorta and the artery divided. A colour coded ligature was placed around the middle caudal artery, which was not cut because of its close proximity to the vena cava. After ligating the left renal artery, the left renal vein overlying the aorta was cut between ties. The aorta and vena cava were separated as before up to the level of the coeliac axis, dividing between ties the internal spermatic arteries, the right renal arteries and the four small lumbar arteries. Springing from the dorsal surface of the aorta, the lumbar arteries were hidden from view but could be identified by their increased resistance to blunt dissection. Considerable variation occurs in the site of origin of the inferior mesenteric artery near the bifurcation of the aorta and in the distribution of other vessels. The coeliac nerve plexus and intestinal lymphatics around the superior mesenteric artery were cleared. The superior mesenteric vein was exposed and dissected free above the level of the middle colic vein by cutting the omental attachments of the pancreas to the transverse colon and by cutting across the coeliac ganglion. The splenic artery was tied and the splenic vein divided between ties close to its junction with the superior mesenteric vein.

A tie was placed around the coelio-caecal junction and a transverse incision made in the wall of the colon below this point. The colonic contents were flushed out with 20 ml. warm bicarbonate-Ringer infused through a cannula tied into the lower end of the descending colon. After flushing, a second cannula was tied into the proximal end. Single ties were placed around the base of the mesenteric arteries supplying the ileum and jejunum and their accompanying veins. The caecum, ileum and jejunum were then removed. The omental attachment of the duodenum and upper jejunum to the transverse colon was cut and single ties placed around the blood vessels supplying the duodenum. The duodenum was then removed.

The animal was given 250 i.u. heparin intravenously and the right common iliac artery tied and cut distally. The left common iliac artery was dissected free and a polyethylene cannula tied into its distal end, pointing cephalad. A cannula was tied into the superior mesenteric vein and the blood allowed to escape. The arterial cannula was connected to an extension of the arterial inflow and perfusion started at a rate of 1 ml./min. The dorsal aorta was tied above the superior mesenteric artery and cut proximally. The colon was arranged in position on the perfusion plate which was now transferred to the perfusion apparatus and the arterial extension tubing was removed. The circulation of luminal fluid was instituted at this point. The whole operative procedure took about 45 min and at no stage was the colon without a supply of oxygenated blood.

Circulation and perfusion fluids. The basic medium of nearly all the solutions used was Krebs bicarbonate-Ringer, pH 7·4 (Krebs & Henseleit, 1932), in which Ca^{2+} was reduced to 1·25 mM. The vascular perfusate also contained glucose, 200 mg/100 ml., benzyl penicillin, 20 μ/ml., and streptomycin sulphate, 40 μ/ml. Bovine serum albumin (Miles-Serevac) dialysed against 2 × 10 volumes Krebs bicarbonate saline, pH 7·4, for 24 hr at 4° C, and bovine erythrocytes (see below) were added as specified. Preliminary work indicated that vascular flow rates above 1·5 ml./min led to a rapidly increasing vascular resistance and tissue oedema, although calculation reveals that, in the absence of erythrocytes, flow rates of at least 2·5 ml./min saline

are required to provide the colon with sufficient oxygen. Consequently in experiments where no erythrocytes were used, oxygenated bicarbonate-Ringer was recirculated, at a rate of 10 ml./min, through the lumen to provide oxygen to the mucosal cells in the manner originally described by Fisher & Parsons (1949). In all other experiments the flow rate of the Ringer through the lumen was 3 ml./min.

When investigating the effects of the inclusion of erythrocytes in the vascular perfusate, bovine erythrocytes, washed at least thrice in 5 volumes of buffered saline, were added to give a packed cell volume (pcv) of 7 %. Unless otherwise specified, in all the experiments the albumin concentration of the infusate was 3 % (w/v) and erythrocytes were added to give a pcv of 7 %. 1 ml. samples were collected from the vascular and luminal reservoirs at 30 min intervals and immediately deproteinized with 3·5 % perchloric acid.

Oxygen utilization. In one series of experiments the luminal fluid was oxygenated whilst in another series it was recirculated but not oxygenated, and closed from the atmosphere by a layer of liquid paraffin. To determine oxygen utilization, arterial and venous samples of perfusate were collected at 15 min, 1 hr and every hour up to 5 hr. The oxygen content of these samples and of the luminal fluid in the experiments with closed luminal circulation was determined by the manometric method of van Slyke & Neill (1924).

Histology and electron microscopy. Small portions of tissue taken for histological examination were fixed in Bouin's fixative and sections stained with haematoxylin and eosin or with periodic acid-Schiff reagent (PAS). Tissue for electron microscopy was fixed in 6 % glutaraldehyde in cacodylate buffer – 1 % $CaCl_2$ and post-osmicated in 1 % OsO_4, 1 % $Na_3FE(CN)_6$. Tissue fat free dry weight (ffdw) and water content were determined by the method described by Parsons & van Rossum (1961).

Haemodynamic experiments. In the study of the relationship between the transport capacity and haemodynamics, the arterial, venous and lumen outflow pressures were varied separately, each in a single experiment. The arterial pressure was altered by varying the flow rate. The value at which any two of the parameters were held constant were: arterial pressure at 50 cm H_2O, venous pressure at 0 cm H_2O and lumen outflow pressure at 10 cm H_2O, all measured from the mid-point of the colon.

Net ion transport. In studies upon net ion transport, 20 ml. of the appropriate Ringer, containing polyethylene glycol 1,2-^{14}C (New England Nuclear Corp.) 0·1 μc, 25 mg/100 ml., were recirculated through the lumen for 1 hr. At the end of this period 5 ml. of the fluid was collected anaerobically and the remainder washed into a 50 ml. volumetric flask with isotonic mannitol solution, aided by gentle air pressure. The lumen was flushed with about 20 ml. of an isotonic solution of carbon dioxide-free mannitol and then primed with the new luminal fluid at a rate of 10 ml./min. As soon as all the gas had been replaced a closed luminal circuit was formed by immersing the outflow tube beneath the liquid paraffin seal in the lumen reservoir and the flow rate reduced to 3 ml./min. Four experimental periods of 1 hr were employed which, together with an initial 15 min for equilibration, resulted in a total perfusion time of 4½ hr. The initial and final luminal solutions were assayed for the different ions, total CO_2, radioactivity and the pH determined at 37° C using a thermostated cell. From a knowledge of the concentration and total radioactivity of the luminal contents, an estimate of the final volume of the luminal contents was obtained.

In studies designed to determine the importance of CO_2 on transport processes the vascular perfusate was prepared with either Krebs-bicarbonate or phosphate-Ringer, pH 7·4, using the appropriate gas mixture. The perfusates were alternated over the four experimental periods. The order of the perfusion in two of the experiments was reversed in another two. In one set of four experiments the luminal fluid

was Krebs bicarbonate-Ringer and in another set phosphate-Ringer. The effect of ammonium ions in the lumen fluid was investigated by alternating a solution of Krebs bicarbonate-Ringer plus ammonium chloride, 15 mM, with a similar solution containing choline chloride, in each of two experiments.

The ammonium concentration in the faecal fluid of the rat was estimated by homogenizing faecal pellets in saline containing polyethylene glycol, 1,2-^{14}C as a volume marker.

Analytical methods. L(+)-lactate was determined by the lactic dehydrogenase method (Hohorst, 1963) and glucose by the glucose oxidase method of Bergmeyer & Bernt (1963). Ammonium was determined by the enzymatic method of Kirsten, Gerez & Kirsten (1963). Sodium and potassium were determined using an automated integrating flame photometer (EEL 227). Chloride was assayed using an Aminco-Cotlove Chloride Titrator and total CO_2 by the manometric method of van Slyke & Neill (1924). Samples were assayed for radioactivity using a Beckman Liquid Scintillation system.

Transepithelial potential difference. The transepithelial potential difference was measured through salt bridges (Tygon tubing filled with Ringer-4 % agar) leading from the lumen and vascular circuits to a pair of calomel electrodes. The electrodes and the salt bridges were both adequately shielded. Potential differences were measured with a Vibron electrometer (Model 33B-2, Electronic Instruments, Ltd) and recorded on a Servoscribe potentiometric recorder (Smith's Industries, Ltd). The asymmetry potential of the circuit was checked at intervals during the experiment.

Presentation of results. A net secretion of an ion, that is net accumulation in the luminal circuit, is indicated by the prefix '+' and a movement in the opposite direction, net absorption, by the prefix '−'. Net HCO_3^- fluxes are presented in terms of the total CO_2 flux. Although the fall in luminal pH over 1 hr of recirculation is significant (forty-four determinations ± S.E. of mean from 7·44 ± 0·01 to 7·39 ± 0·02, $P < 0·05$), such a change in pH represents only a 0·6 % change in the contribution of H_2CO_3 to the total CO_2. Net solute transport is defined as the sum of the net anion and cation fluxes and is used as a measure of the transport capacity of the preparation.

RESULTS

Recovery of infusate from venous effluent. The addition of albumin at concentrations of 3 g/100 ml. and above to the vascular perfusate greatly increases the recovery of the arterial infusate via the venous effluent over 5 hr of perfusion (Fig. 2). The combined recovery of the venous effluent and the serosal exudate collected from the floor of the perfusion reservoir is in all cases complete, thus the mean of the volume of combined venous effluent and exudate in 5 experiments of 5 hr duration was ± S.E. of mean, 100 ± 0·7 % of volume infused.

Tissue metabolism. The rate of production of lactate by the aerobic perfused colon is reduced by 41 % when erythrocytes are added to the vascular perfusate, even though dissolved oxygen is continually being supplied to the mucosal surface from the lumen fluid (Table 1). The fraction of the glucose taken up by the tissue and converted to lactate is similarly decreased by 41 %.

The rate of oxygen utilization by the colon with a closed luminal circuit

(mean of 4 ± S.E. of mean) is 9·2 ± 0·3 μmole/g ffdw.hr over the first 4 hr of perfusion, rising to 13·1 ± 1·2 hr μm/g ffdw/hr in the fifth hr. With an oxygenated fluid flowing through the lumen, the rate of utilization of oxygen from the vascular fluid during the first 4 hr is only reduced (mean of 4 ± S.E. of mean) to 6·7 ± 0·4 μmole/g ffdw.hr.

Morphological changes. With the vascular bed perfused with medium containing both albumin and erythrocytes the preparation remains

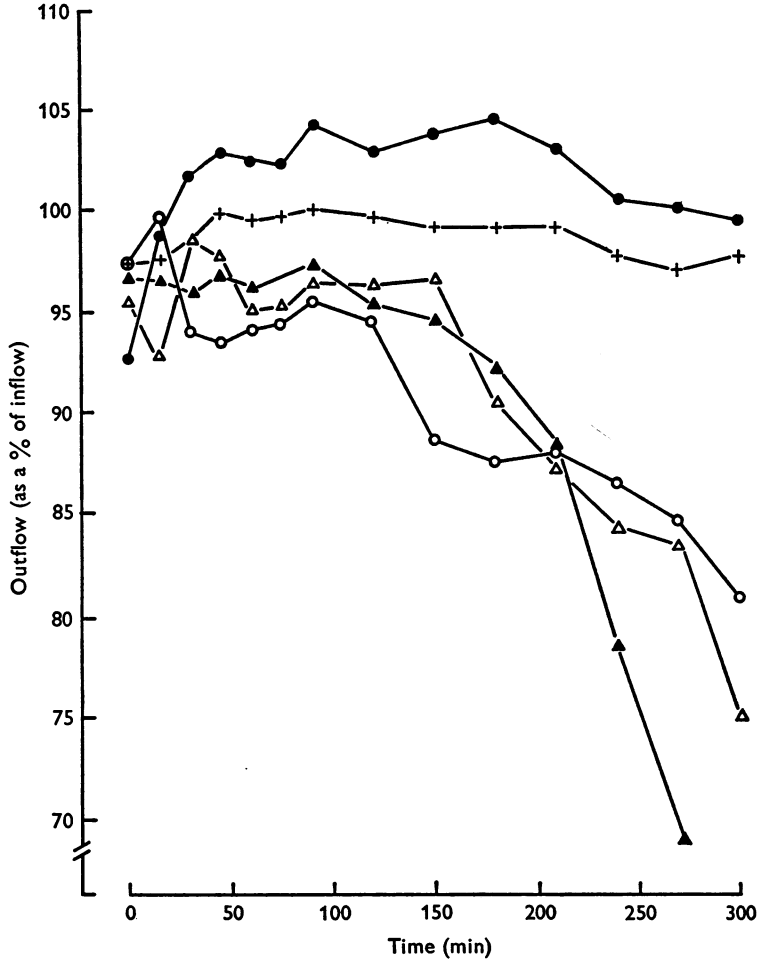

Fig. 2. Influence of composition of infusate on venous outflow: ○, no albumin (99·1); ▲, 1 % albumin (102·0); △, 2 % albumin (98·8); +, 3 % albumin (99·8); ●, 4 % albumin (102·1).

Figures in parentheses represent the mean total recovery, including sweat, over the 5 hr of perfusion, expressed as a percentage of the inflowing volume.

macroscopically normal for up to 5 hr with no leaks and exhibiting a uniform degree of perfusion. Injection of indian ink or latex at the end of this period likewise reveals a perfusion which appears uniform both to macroscopic and microscopic inspection. Motility is low, the preparation exhibiting a gentle swaying motion for the first hour only.

Histological appearances of the colon after 5 hr perfusion are those of an essentially undamaged tissue with but slight oedema in the submucosal region and exhibiting a decrease in the mucus content of the goblet cells

TABLE 1. Aerobic glycolysis by the rat colon perfused *in vitro*. Vascular infusate Krebs-Ringer bicarbonate as described in Methods. Albumin concentration 3 g 100 ml.$^{-1}$ 4 hr perfusion. Values are means ± S.E. of means

Addition to vascular infusate	Vascular flow rate ml./min	Number of observations	Lactate output	Glucose utilization	Contribution of aerobic glycolysis to glucose utilization (%)
			μmole/g/ffdw.hr		
Albumin	1·0	4	647 ± 24	424 ± 17	76 ± 2
Albumin	2·8	3	458 ± 33	445*	47*
Albumin-erythrocytes (7 % pcv)	1·0	4	382 ± 26	542 ± 33	36 ± 4

* A single 1 hr experiment.

TABLE 2. Hydration of rat colon wall. 4–5 hr. Values are means ± S.E. of means. For the experiments *in vitro* the arterial infusate was Krebs-Ringer bicarbonate. *In vivo*, luminal fluid recirculated as described by Parsons (1956). Luminal fluid *in vivo* and *in vitro*, NaCl 125 m-mole/l., $NaHCO_3$ 25 m-mole/l., pH 7·4. Vascular perfusate 100 ml., recycled

Addition to arterial infusate	Number of observations	g H_2O g ffdw^{-1}
	A. In vitro	
None	7	11·38 ± 0·63
Albumin		
1 g 100 ml.$^{-1}$	1	10·18
2 g 100 ml.$^{-1}$	1	9·62
3 g 100 ml.$^{-1}$	6	8·37 ± 0·56
4 g 100 ml.$^{-1}$	1	8·01
Albumin (3 g 100 ml.$^{-1}$)-erythrocytes:		
pcv 7 %	10	6·56 ± 0·15
pcv 30 %	6	4·83 ± 0·12
	B. In vivo	
Treatment		
Fasted 18 hr, control	11	3·95 ± 0·07
Fasted 18 hr. Lumen contents recirculated for 5 hr	6	4·82 ± 0·17

as compared with the *in vivo* preparation (PAS-stained sections). The absence of erythrocytes and a reduced albumin concentration in the vascular infusate yields the appearances of an increasing oedema and the eventual detachment of the mucosal epithelium. These histological changes are reflected in the extent of the tissue hydration under the differing conditions (Table 2).

Electron microscopy reveals no significant differences between the ultrastructure of the 5 hr perfused colon and that *in vivo*. In particular, the nuclei, endoplasmic reticulum, mitochondria and microvilli of the epithelial cells remain unchanged in appearance.

Ion transport by the perfused colon

Vascular perfusate recycled. Net solute transport by the perfused colon is significantly depressed compared with the rates found using an *in vivo* preparation described by Parsons (1956), but K^+ transport remains largely unaltered ($P < 0{\cdot}05$) (Table 3). Administration of the antihistamine promethazine to the donor rat before establishing the preparation results in a threefold increase in the net solute transport, which still remains, however, lower than the comparable level *in vivo* ($P < 0{\cdot}05$). Analysis of the combined results reveals a significant correlation between the net fluxes of all four ionic species (Table 4). When promethazine is not given there occurs a net secretion into the lumen of a fluid containing ions in the molar proportions of Na^+ 145, Cl^- 122, K^+ 5 and total CO_2 36. These quantities are very close to those existing in the arterial infusate. Attempts to demonstrate the simultaneous passage of infused albumin into the colonic lumen in these experiments were unsuccessful although the finding of an appearance of glucose in the lumen is further support for the idea that an ultrafiltration of the vascular perfusate into the lumen was occurring. Variations in the vascular flow rate from 0·5 to 1·4 ml./min have no effect upon the net solute transport.

The undesirability of recycling the vascular perfusate was also evident from the changes found in concentrations of electrolytes in the perfusate over the period of $4\frac{1}{2}$ hr. Both Na^+ and Cl^- concentrations in the infusate are increased by about 5 mM ($P < 0{\cdot}02$) whilst the total concentration of CO_2 falls to 21 mM ($P < 0{\cdot}001$). K^+ levels are not changed. The decrease in total CO_2 and the consequent change in pH is unsatisfactory, for preliminary experiments showed that small changes of this magnitude could produce large alterations in the fluxes of other ions (Fig. 3).

Single pass of vascular perfusate. To minimize these effects, experiments were performed in which the perfusate passed through the intestinal vascular bed only once. The lumen fluid was also changed from simple bicarbonate-saline to Krebs bicarbonate saline. It soon became apparent, how-

TABLE 3. Ion transport by the rat colon perfused *in vitro*. Transport measured as described in the text. Values are means ± S.E. of means. Lumen fluid NaCl 125 mM, $NaHCO_3$ 25 mM, pH 7·4. Vascular perfusate 100 ml., recycled. Transport rates *in vivo* measured as described by Parsons (1956)

	Number of observations	μ mole/g ffdw. hr			Total CO_2	Total solute
		Na^+	Cl^-	K^+		
In vivo control	8	−1365 ± 141	−1440 ± 150	+97 ± 16	+152 ± 19	−2555 ± 293
Perfused control	20	−304 ± 49	−336 ± 44	+81 ± 5	+34 ± 22	−526 ± 96
Promethazine*	16	−789 ± 25	−724 ± 28	+60 ± 6	−128 ± 20	−1582 ± 65
Promethazine* and papaverine†	8	−891 ± 44	−916 ± 44	+53 ± 4	−153 ± 28	−1907 ± 85

* 20 mg/kg body wt. to rat before operation.
† 10 mg/100 ml. vascular perfusate.

TABLE 4. Relationship between ions transported by rat colon *in vitro*. Values are of means ± S.E. of means. Parameters of regression lines relating individual results in Table 3 (forty-four pairs of observations). $Y = (\text{slope} \times X) + \text{intercept}$

X	Y	Slope	Intercept	Coefficient of linear correlation	P*
Na^+	Cl^-	0·846 ± 0·0082	−86 ± 35	0·920	< 0·001
Na^+	K^+	0·037 ± 0·0017	+90 ± 7	0·540	< 0·001
Na^+	CO_2	0·246 ± 0·0074	+83 ± 30	0·634	< 0·001
Cl^-	CO_2	0·311 ± 0·0066	−123 ± 28	0·738	< 0·001
CO_2	K^+	0·153 ± 0·0029	+77 ± 2	0·796	< 0·001

* Calculated by the method given in Snedecor & Cochran (1967).

ever, that transport by this new preparation was now highly dependent upon haemodynamic events.

Effects of hydrostatic pressure. Changes in the hydrostatic pressure in the lumen have little effect upon net solute transport (Fig. 4) and the pressure head of 10 cm, necessary to sustain circulation through the lumen, was retained.

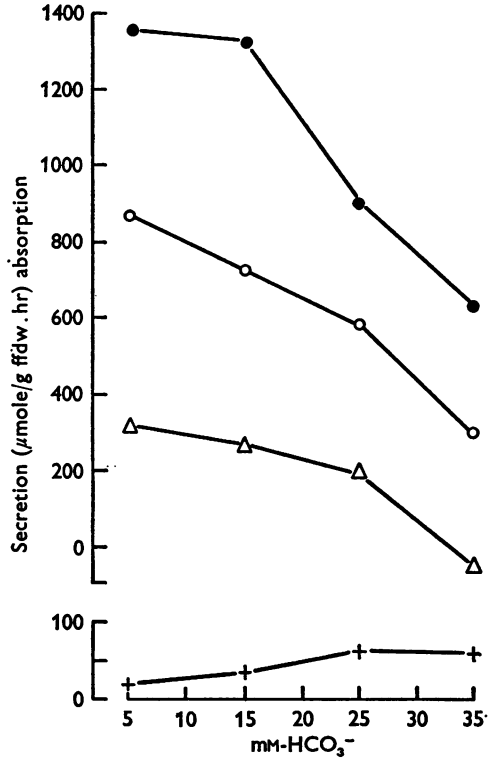

Fig. 3. Effect of varying vascular HCO_3^- upon ion transport by the perfused rat colon, gassed with O_2, 5 % CO_2. ●, Na^+; △, total CO_2; ○, Cl^-; +, K^+. (Each value mean of two experiments.)

Small variations in the venous pressure have only a small effect upon the transport capacity but changes in arterial pressure are of a greater consequence. The results of a further investigation involving the use of perfusates with different haematocrit values are shown in Fig. 5. The findings show that the maximum absorptive capacity occurs at the lowest vascular infusion pressure. It is possible that the phenomenon is attributable to a form of vascular autoregulation for both the absence of Ca^{2+} and the presence of papaverine in the infusate increase absorption at high haematocrit values and largely reduce the dependence upon vascular flow. A

haematocrit value of 7 % and an arterial infusion rate of 0·7 ml./min were chosen as optimum.

Transepithelial potential difference and active transport. The transport of different ions and the values of the transepithelial potential difference over the 4 hr of perfusion are shown in Table 5. Over the 4 hr none of the changes are significant ($P > 0{\cdot}10$). It is clear that Na^+, being transported against both a voltage and a chemical potential gradient, is subjected to

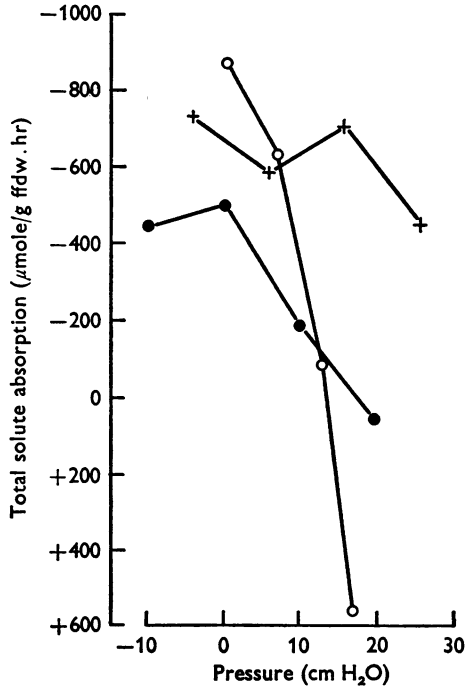

Fig. 4. Effect of varying lumen distension and venous and arterial pressure upon total solute absorption by the perfused rat colon. Except where specified, venous outflow pressure is 0 cm H_2O, luminal outflow pressure 10 cm H_2O, arterial inflow pressure 50 cm H_2O. Abscissa represents the changes in pressure: +, luminal, ● venous, ○ arterial.

'active transport' as defined by Rosenberg (1948). HCO_3^- is similarly 'actively' accumulated in the lumen against a chemical and voltage gradient. With 5 ml. Krebs bicarbonate saline instilled into the lumen of the perfused colon and left to equilibrate for 4 hr, the K^+ concentration of the contents rises to 9·3 mM. With a known plasma K^+ concentration of 4·5 m-mole/kg H_2O and a mean transepithelial potential difference of 10·3 mV, the equilibrium K^+ concentration is 6·7 mM, assuming a passive distribution. That the actual concentration gradient observed is greater is not, however, a conclusive demonstration of active K^+ secretion into the

lumen, for if the colon were impermeable to K^+, fluid absorption or otherwise the release of K^+ from cellular debris could produce similar results. An investigation of Cl^- transport will be reported elsewhere.

Interactions between carbon dioxide and ion transport. If, in the vascular infusate, the Krebs–Ringer HCO_3^- (gassed with O_2 95% (v/v)/CO_2 5% (v/v) is replaced by Krebs–Ringer phosphate (gassed with O_2, 100% (v/v) a significant increase in the absorption of Na^+, K^+ and total CO_2

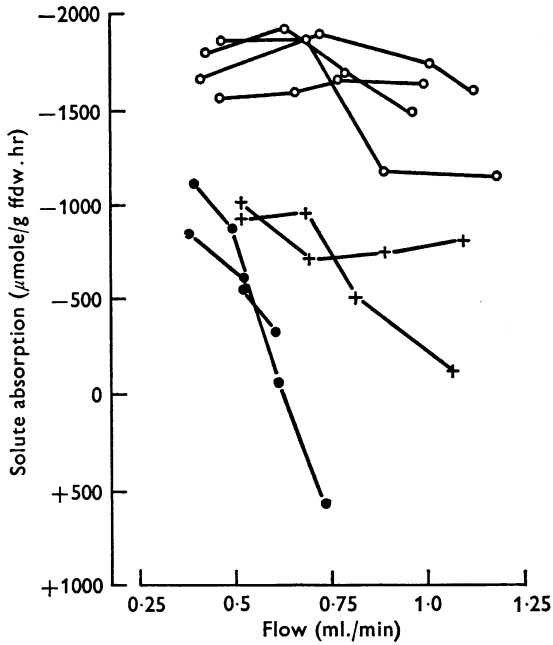

Fig. 5. Effects of changes in haematocrit and vascular flow rate upon total solute absorption by perfused colon. ○ 7% haematocrit, + 15% haematocrit, ●, 30% haematocrit.

(Table 6) from the lumen is found. The over-all effect is an increased net HCO_3^- flux from the lumen to the blood accompanied by an equivalent cation flux in which the ratio of Na^+: K^+ is 12:1, a ratio lower than in the luminal solution. Analysis of variance shows a large net absorption of CO_2 during the first hour, with little subsequent change.

In other experiments, in which Krebs–Ringer phosphate, pH 7·4, was present in the lumen, a significant decrease in the blood to lumen flux of total CO_2 was found when CO_2 was absent from the vascular infusate. No significant changes in the net fluxes of any other ion or in the luminal pH were observed. It was found that in all the experiments in which CO_2 was absent from the vascular infusate there was a noticeable increase in the

vascular resistance and that the effect was irreversible. This finding was particularly striking in the experiments in which Krebs–Ringer phosphate was present in the luminal fluid. The increase in vascular resistance was associated with a precipitous drop in the absorption rates during the third and fourth periods.

The results from the experiments in which CO_2 is present in the vascular perfusate may be combined to determine the effect of exogenous CO_2 from the luminal fluids, with CO_2 present in the vascular infusate. Analysis of

TABLE 5. Variation of transport and transepithelial potential difference with time. Values are of means of three experiments ± S.E. of means. Animals pre-treated with promethazine, lumen fluid Krebs-Ringer bicarbonate

	Time hr			
	1	2	3	4
Solute	μ-mole/g ffdw. hr			
Na^+	−867 ± 18	−916 ± 13	−869 ± 19	−851 ± 19
Cl^-	−781 ± 41	−901 ± 97	−862 ± 123	−881 ± 109
K^+	+36·7 ± 9·4	+24·5 ± 6·2	+14·7 ± 5·8	+19·2 ± 9·8
Total CO_2	+32 ± 22	+105 ± 32	+94 ± 11	+105 ± 27
	mV (blood positive)			
Potential difference	+12·4 ± 1·4	+11·9 ± 1·3	+9·8 ± 1·1	+10·0 ± 1·0

variance of the results reveals a significant increase in the net blood to lumen flux of total CO_2 in the absence of luminal CO_2. There also appears to be a decreased Na^+ absorption, but this effect only appears in the last 2 hr of perfusion and may be associated with a general deterioration in the condition of the preparation.

Results from similar experiments carried out in the absence of Ca^{2+} or in the presence of papaverine in the vascular infusate exclude the possibility that the increased concentration of phosphate in the luminal fluid could be chelating Ca^{2+} in these experiments and indirectly affecting transport through the vascular shunting mechanism noted previously.

Luminal ammonium and ion transport. It appears that ammonium is normally present in high concentrations in the faeces of the rat (Table 7). Experiments were therefore undertaken to examine the effects of this cation on net ion transport across the rat colon. In two perfusions choline or ammonium chloride (15 mM) was added alternately to the luminal solution. The order of addition was reversed in the two experiments. The results are shown in Table 8. Compared with the control values taken from the data shown in Table 5, the addition of choline chloride to the lumen fluid has the effect of increasing Cl^- absorption ($P < 0{\cdot}05$). Ammonium

TABLE 6. Effect of transmural orientation of CO_2 bicarbonate upon ion transport by rat colon perfused *in vitro*. Experimental design as described in Methods. Units of net flux, μ mol/g ffdw.hr. Each value represents mean ± S.E. of mean of eight observations. Initial pH of all luminal fluids, 7·40. Additions to basic medium of vascular fluid were as described in 'Methods'

Basic medium		Net flux			Total	Change in
Fluid in lumen	Vascular fluid	Na^+	Cl^-	K^+	CO_2	luminal pH
Krebs-Ringer bicarbonate	Krebs-Ringer bicarbonate	−731 ± 42	−716 ± 43	+13·8 ± 7·7	+34 ± 25	−0·18 ± 0·07
	Krebs-Ringer phosphate	−1035 ± 67	−814 ± 59	−10·9* ± 3·6	−358* ± 19	+0·01 ± 0·04
Krebs-Ringer phosphate	Krebs-Ringer bicarbonate	−538 ± 86	−652 ± 90	+5·5 ± 3·9	+366 ± 30	0·35 ± 0·02
	Krebs-Ringer phosphate	−625 ± 88	−711 ± 74	−2·9 ± 3·8	+156* ± 16	−0·25 ± 0·02

* Analysis of variance reveals a significant effect due to the replacement of bicarbonate by phosphate ($P < 0·05$).

chloride, however, causes an inhibition of HCO_3^- secretion with a consequent reduction of Cl^- absorption. K^+ secretion is also significantly depressed. It is clear from these findings that under physiological conditions, the ammonium of the colonic contents could have significant effects upon bicarbonate transport.

TABLE 7. Ammonium concentration in the faecal fluid of the rat. Each value is the mean ± S.E. of mean of four determinations

Ascending colon	Transverse colon (mM)	Descending colon
12·4 ± 1·6	13·9 ± 2·0	38·7 ± 5·0

TABLE 8. Effect of luminal ammonium upon ion transport by the rat colon perfused *in vitro*. Lumen fluid Krebs bicarbonate Ringer, pH 7·4, plus either choline or ammonium chloride, 15 mM. Each value represents the mean ± S.E. of mean. Control values are means of data given in Table 5. Units of net fluxes are μ mole/g ffdw.hr

Addition	Number of periods	Na^+	Cl^-	K^+	CO_2	NH_4^+	Change in lumen pH
Control	12	−875 ± 11	−856 ± 44	+12·5 ± 7·4	+84 ± 14	—	—
Choline	4	−862 ± 50	−1076† ± 89	+11·6 ± 1·8	+75 ± 23	—	+0·01 ± 0·03
Ammonium	4	−805 ± 39	−813* ± 51	+4·1* ± 1·0	−124* ± 37	−177 ± 100	+0·05 ± 0·05

* Significantly different from control, $P < 0·05$.

† Significantly different from in the presence of choline chloride, $P < 0·05$.

DISCUSSION

Viability of the preparation. The apparatus and technique described enable the isolated colon of the rat to be maintained *in vitro* for up to 5 hr. Preliminary investigations revealed the necessity of adding at least 3 g albumin/100 ml. perfusate to prevent the formation of large quantities of serosal exudate. Bovine erythrocytes were favoured as possessing a low cellular K^+ and a low metabolic rate, as well as proving remarkably resistant to haemolysis (Bernstein, 1954; Bishop & Surgenor, 1964; Whittam, 1964).

After perfusion under optimal conditions for up to 5 hr, the appearances of the tissue with light and electron microscopy are normal and the increase in tissue hydration slight. The utilization of oxygen is constant over the first 4 hr of perfusion and corresponds to levels reported by other workers (Lohmann, Graetz & Langen, 1966; Rosenthal & Lasnitzki, 1928). The increased oxygen utilization found in the final hour of perfusion may possibly result from a stimulation of respiration produced by increase of sodium within the mucosal cells, and evidently reflects a deterioration in

the condition of the preparation. It is known that an increased rate of respiration is commonly associated with a high proportion of damaged cells in the preparation (Bronk & Parsons, 1965) and there is now considerable evidence for the view that Na pumping processes, acting through the production of ADP, are important factors in determining the rate of mitochondrial O_2 utilization (see, for example, Whittam & Wheeler, 1970).

Dickens & Weil-Malherbe (1941) first demonstrated a distally increasing Pasteur effect in the rat intestine, with a fourfold increase in the rate of lactate production by the colonic mucosa under anaerobic conditions. The contribution of intestinal smooth muscle to lactate production by the whole colon is said not to exceed 20% (Sherratt, 1968). The rate of lactate production thus offers a useful index of the extent of oxygenation of the colonic mucosa. Only in the presence of erythrocytes is the rate of glycolysis nearly as low as the rate observed *in vivo*, despite the fact that oxygen is continually present in the fluid bathing the mucosal surface. Allowing for the fact that the smooth muscle contributes about 45% to the dry weight of the colon, an estimate of the rate of lactate production by the colonic mucosa alone is 400 μ-mole/g dry wt. hr, a value slightly above the aerobic values reported elsewhere (Rosenthal & Lasnitzki, 1928; Dickens & Weil-Malherbe, 1941; Parsons & Paterson, 1965). The direction of blood:lumen concentration ratio of endogenously produced lactate of 16:1 is the same as the distribution of lactate reported by Wilson (1956) for the small intestine and by Parsons & Paterson (1965) for rat colonic mucosa. Supplying oxygen to the vascular rather than the mucosal face of the absorbing cells, whilst apparently ensuring a greater efficiency of oxygenation, has the additional advantages of maintaining oxygen and carbon dioxide gradients across the cell similar to those occurring *in vivo*. The use of a closed lumen circuit also facilitated the study of net CO_2 transport.

Functionally, the preparation has proved capable of sustaining an 'active' Na^+ transport for up to 5 hr, although pretreatment with promethazine is necessary to produce rates of transport of the magnitude of those reported by other workers both *in vivo* (Curran & Schwartz, 1960; Edmonds, 1967*a*, *b*) and *in vitro* (Curran & Lindemann, 1967). It is possible that promethazine prevents histamine released during the trauma of operation from combining with receptors in the intestine. Promethazine is believed to prevent increases in permeability of cell layers by inhibiting Ca^{2+} mobilization from the cell surface (Judah, 1962; Judah, Ahmed & McLean, 1964) and it might therefore prevent the ultrafiltration of plasma fluid into the lumen via intercellular channels. The antihistaminic must be administered to the rat before operation; indeed, the presence of promethazine in the vascular perfusate may be undesirable for an effect of the

substance is said to be an inhibition of Na^+ and K^+ transport (Judah *et al.* 1964).

How is it possible to explain the finding that absorption from the colon in which the vascular perfusate is recycled is independent of the effects of perfusion pressure, whereas if the perfusate passes in a single passage such effects are very marked? It is possible that in the latter case the colon exhibits a form of autoregulation whereby at high infusion pressures blood is shunted from the submucosal vascular bed into that of the deeper muscle layers. A partial ischaemia and an inefficient washout of the accumulated ions could then account for the decreased rates of absorption. This hypothesis is supported by the finding that if the tone of the smooth muscle of the vascular bed is inhibited by the addition of papaverine or by the omission of Ca^{2+} from the infusate, then the effect is abolished. The decrease in net absorption which is found at increased vascular infusion pressures may mean that an ultrafiltration into the lumen from the vascular bed may be present.

It appears that material which inhibits autoregulation may accumulate in the vascular perfusate when this is recycled. Phemister & Handy (1927) have demonstrated that exposure to a trauma such as pumping, imparts strong vasodilator properties to whole blood, and that this is associated with the disruption of erythrocytes. Venous blood from a normally perfused resting organ *in vivo* is also known to possess vasodilatory properties. (For a review, see Haddy & Scott, 1968). The effect is usually very labile, lasting less than 2 min upon storage (Jelliffe, Wolf, Berne & Eckstein, 1957), but there have been reports of such an activity which is stable for at least 30 min (Anrep & von Saalfeld, 1935). A decreased vascular pH is also known to be vasodilatory in the dog intestine (Mohamed & Bean, 1951; Fleisch, Sibul & Ponomarev, 1932) and could contribute to the effects observed here.

Ion transport. An intriguing aspect of HCO_3^- transport is its relationship to the transport of other ions. Sladen & Dawson (1968) have found that the presence of HCO_3^- in the intestinal lumen stimulates the absorption of Na^+ and water by the human jejunum. This effect has been observed with rat jejunum, but not ileum, *in vitro* by Sladen, Parsons & Dupré (1968) and Parsons (1971). It is possible that Na^+ transport is reduced in conditions which lower the intracellular pH, HCO_3^- could then buffer such changes and thus stimulate the transport of Na^+. This explanation has been suggested for the frog skin (Funder, Ussing & Wieth, 1967) and the turtle bladder (Gonzalez, Shamoo & Brodsky, 1969). In the present study the replacement of HCO_3^- in the lumen fluid by phosphate appears to have no effect upon Na^+ transport by the colon. The removal of CO_2 from the vascular infusate increases the absorption of Na^+ and K^+, a finding which

is in direct contrast to the results obtained in amphibian tissues (Cooperstein & Hogben, 1959; Funder *et al.* 1967). It is not possible to advance the theory that the effects of HCO_3^- in the present experiments are mediated through its buffering properties unless one assumes that in the colon Na^+ transport is differently affected by intracellular pH than in other tissues. In view of the ubiquity of the Na^+ transport mechanism, this may be unlikely. The fact that it is the increased net lumen to blood HCO_3^- flux *per se* which is important is suggested by the finding that when this flux is abolished by removing the HCO_3^- from the luminal circuit then removing CO_2 from the vascular infusate has no effect. It would seem that the extra HCO_3^- flux into the blood can 'drag' an equivalent amount of cation across with it.

The physiological implications of an inhibition of HCO_3^- secretion by NH_4^+ are of interest. That NH_4^+ reaches high concentrations in the faecal fluid of the distal colon of the rat *in vivo* and that such concentrations are sufficient to abolish net CO_2 secretion *in vitro* is clear. The technique of faecal dialysis employed in humans by Wrong, Metcalfe-Gibson, Morrison, Ng & Howard (1965) reveals total CO_2 levels also of the order of 40 mM. Although it is difficult to extrapolate from these data, it is possible that some stimulation of HCO_3^- absorption is occurring, for in the dog colon in the absence of NH_4^- equilibrium concentrations for CO_2 of 70 mM are reached (d'Agostino, Leadbetter & Schwartz, 1953; Swallow & Code, 1967). Although no comparable data are available for the human colon, it is not unreasonable that similar values should be found. The data reported for other regions of the intestine are essentially comparable in the dog (d'Agostino *et al.* 1953; Swallow & Code, 1967; DeBeer, Johnston & Wilson, 1935; Kinney & Code, 1964) and human (McGee & Hastings, 1942; Phillips & Summerskill, 1966). The normal level of NH_4^+ found in human faecal fluid is 14 mM (Wrong *et al.* 1965). It therefore appears, as also suggested by Carter & Parsons (1971), that NH_4^+ must be regarded as a physiological constituent of the fluid in the colonic lumen. From this it follows that net reabsorption, rather than secretion, of CO_2 is the usual physiological event across the mucosal epithelium of this part of the intestine. The apparent stimulation of the absorption of HCO_3 in the presence of NH_4^+ in the colonic lumen could be produced in one of two ways: either by the effects of an increased secretion of H^+ ions into the lumen during NH_4^+ absorption or by a reduction in the flux of HCO_3 in the direction blood into lumen. It is difficult to distinguish between the two effects (D. S. Parsons & G. Powis, in preparation).

Ammonium itself appears to be distributed across the wall of the perfused colon passively according to the prevailing potential and pH gradients. This is in contrast to the findings in the hamster ileum where NH_4^+ is

actively transported from the mucosal to the serosal fluids (Mossberg & Ross, 1967). However, we believe our experiments do not exclude the existence of some pumping mechanism for NH_4^+ in colonic mucosal cells. If NH_4^+ is trapped by erythrocytes, formal demonstration of an 'active' transepithelial transport may be difficult.

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