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THE UTILIZATION OF GLUCOSE AND PRODUCTION OF LACTATE BY *IN VITRO* PREPARATIONS OF RAT SMALL INTESTINE: EFFECTS OF VASCULAR PERFUSION

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SUMMARY

1. The rate of metabolism of glucose to lactate has been measured in a number of non-vascularly perfused preparations of rat jejunum *in vitro*. The glucose and lactate metabolism was measured simultaneously and under conditions such that the uptake of glucose and the appearance of lactate were linearly related to time.

2. It is found that there is no difference between the rates at which rings of rat jejunum utilize glucose during the first 45 min of anaerobic or aerobic incubation. During the first 15 min of incubation between 60–70 % of the metabolized glucose was converted to lactate under aerobic conditions; this value increased to 80–90 % during the subsequent 30 min. During the period 0–15 min of incubation, lactate production was found to be higher under anaerobic than under aerobic conditions but after this initial period the rate of lactate production was the same under aerobic and anaerobic conditions.

3. For segments of rat jejunum, maintained *in vitro* by the recirculation of nutrient fluid through the intestinal lumen, neither the rate of production of lactate, nor the utilization of glucose, was stimulated if the preparation was maintained under anaerobic rather than aerobic conditions. The direct delivery of gas into the intestinal lumen in the form of a stream of bubbles (segmented circulation) reduced both the utilization of glucose and the production of lactate under aerobic conditions. However, no effect on glucose metabolism was observed under anaerobic conditions. The finding of a Pasteur effect with the segmented-circulated preparation, but not with the simple recirculated preparation, is associated with the lower rate of aerobic lactate production in the former preparation. Reasons are given for supposing that under conditions of segmented circulation, the luminal compartment is better stirred, thereby increasing access of O_2 to the tissue.

4. A preparation of rat small intestine perfused through the vascular bed is described. With this preparation the rate of glucose utilization is significantly lower than that for recirculated preparations and the rate of lactate production is substantially less than that of the other preparations studied.

5. With the preparation perfused through the vascular bed, and with glucose, 10 mM, present only in the vascular medium the addition of erythrocytes to the vascular infusate causes a significant reduction in both glucose utilization and in the rate of lactate production. The addition of erythrocytes to produce an haematocrit of 40% (v/v) causes a greater reduction in glucose utilization and lactate production than is found for an haematrocrit of 15%. About 10% of the lactate produced appears in the luminal contents. With an haematocrit of 15%, the O_2 consumption of the whole wall of the jejunum was found to be 6·4 μmole O_2 g dry wt.$^{-1}$ min^{-1}, equivalent to a value for the Q_{O_2} of 8·6 μl. O_2 mg dry wt.$^{-1}$ hr^{-1}. The uptake of O_2 was almost entirely from the vascular infusate.

6. With the vascularly perfused preparation, an increased glucose utilization and an increased lactate production is found when glucose is present in the lumen and also when the concentration of gluosce in the vascular bed is raised to 28 mM in the absence of luminal glucose. It is suggested that in the rat jejunum some of the lactate is formed in a compartment that is readily accessible to glucose in the lumen but is accessible to glucose in the vascular bed only when the glucose is present at a high concentration.

7. In an Appendix the influence of an unstirred layer on the delivery of O_2 to a tissue is considered in relation to the limiting thickness of the tissue. It is shown that for a tissue with a respiration rate similar to that of the jejunal intestinal mucosa, the presence of an unstirred layer of thickness greater than about 5×10^{-2} cm will lead to the tissue being anaerobic at depths greater than about 100 μm below the tissue surface.

INTRODUCTION

Recently doubt has been thrown on the 'classical' view that a characteristic of the *in vitro* metabolism of rat jejunum is a high rate of lactate production under aerobic conditions of incubation and the absence of a Pasteur effect (Dickens & Weil-Malherbe, 1941; Wilson & Wiseman, 1954; Wilson, 1956; Lohmann, Graetz & Langen, 1966; Sherratt, 1968). Lamers & Hülsmann (1972) found that a vascularly perfused preparation of the whole small intestine of the rat produced more lactate and utilized more glucose if incubated under anaerobic than under aerobic conditions. Moreover, Windmueller & Spaeth (1972) found that there was no net

production of lactate by a vascularly perfused preparation that included the whole small intestine, caecum and proximal colon. The metabolism of glucose to lactate, under aerobic and anaerobic conditions, has therefore been re-examined in a number of non-vascularly perfused *in vitro* preparations of rat jejunum. In contrast to most previous investigations the utilization of glucose and the production of lactate were measured simultaneously and care has been taken to ensure that under the conditions of incubation the uptake of glucose by the tissue and the appearance of lactate in the incubation medium were linearly related to time.

We also present the first measurements of utilization of glucose and production of lactate that have been obtained with a vascularly perfused preparation of rat intestine that consists solely of jejunum.

Vascularly perfused preparations of intestine differ in several ways from other *in vitro* preparations: oxygen and glucose can be supplied from the vascular bed; tissue glucose metabolism can be studied in the absence of transepithelial glucose transport; and it is unlikely that there will be a high concentration of glucose at the base of the epithelial cells (Fisher & Parsons, 1953). We have tried to discover which, if any, of the above factors are responsible for the differences found when the results obtained for the metabolism of the vascularly perfused jejunum are compared with other *in vitro* preparations of rat jejunum.

METHODS

Animals. Male Wistar rats were supplied by OLAC Ltd, Bicester, Oxon. The rats were fed on Oxoid breeding diet. Experiments, upon unfasted rats usually of 200–250 g body wt., were performed between 11 a.m. and 3 p.m.

Incubation and perfusion fluids. The composition of the Krebs–Ringer bicarbonate medium was as follows (mM): NaCl, 120; KCl, 4·5; $MgSO_4$, 1·0; Na_2HPO_4, 1·8; NaH_2PO_4, 0·2; $NaHCO_3$, 25; $CaCl_2$, 1·25. When gassed with 95% O_2, 5% CO_2 (v/v) (aerobic experiments), or with 95% N_2, 5% CO_2 (v/v) (anaerobic experiments), the medium reached a pH of 7·51.

Bovine erythrocytes were washed twice with 3 vol. isotonic saline at 4° C, and then once with 3 vol. Krebs–Ringer bicarbonate medium which had been gassed with 95% O_2, 5% CO_2 (v/v) and contained glucose at a concentration of 5 mM. Packed cells were stored for not more than 5 days in dialysis bags placed in vessels containing 6 vol. of the above Krebs–Ringer bicarbonate medium. On the day of use the cells were washed twice with 4 vol. Krebs–Ringer medium without glucose.

Bovine serum albumin (Pentex), concentration 7·5 g/100 ml., was dialysed against 2 × 10 vol. Krebs–Ringer bicarbonate medium at 4° C, for at least 24 hr before use. In all cases the concentration of albumin in the vascular perfusate was 3 g/100 ml.

Preparation and incubation of rings of jejunum. Rings of 2 mm width were cut from a 30 cm segment of proximal jejunum. The rings were randomized in a dish of ice-cold saline (9 g NaCl/l.). Batches of rings, two for each period and 15 rings per batch, were incubated at 37° C for 15, 30 and 45 min. Incubation was terminated by rapid homogenization of the tissue and incubation medium, and deproteinization

by the method of Somoygi (1945). A batch of rings was taken to determine the initial amounts of glucose and lactate in the tissue.

Procedure for recirculation. The apparatus of Fisher & Parsons (1949) was modified such that a single incubation medium bathed both the mucosal and serosal surfaces of the length of intestine. The operative procedure was similar to that described by Parsons & Volman-Mitchell (1974). In experiments using the segmented-circulated preparation (Fisher & Gardner, 1974) the pump (Peripump, Schuco Scientific Ltd) that controlled the rate of flow of liquid through the lumen of the intestine was switched on to the 'input' side and bubbles of the appropriate

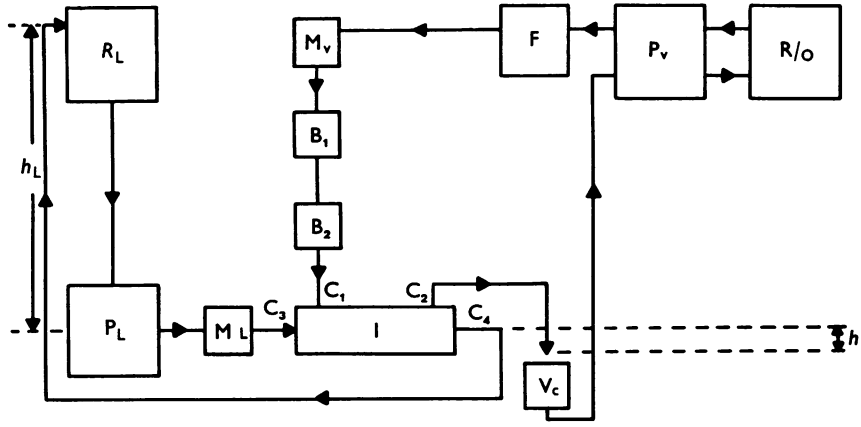

Fig. 1. Apparatus for vascular perfusion of rat jejunum *in situ*. B_1 and B_2, bubble traps; C_1 and C_2, aortic and venous cannulae; C_3 and C_4, luminal cannulae; F, filter; h_L, height of luminal reservoir above intestine, about 15 cm; h_v, distance tip of venous cannular below portal vein, about 5 cm; I, intestinal segment; M_L, luminal manometer; M_v, vascular manometer; P_L, luminal peristaltic pump; P_v, vascular peristaltic pump; R_L, luminal reservoir; $R/_O$, reservoir oxygenator; V_c, collection vessel for venous effluent.

gas mixture injected just distal to the pump. In experiments using ileum, the 30 cm segments terminated about 20 cm above the ileo-caecal valve.

Samples of incubation medium, taken at 10 min intervals over the period 20–50 min (jejunum) or 30–60 min (ileum), were deproteinized by the method of Somoygi (1945).

Vascular perfusion – apparatus. The apparatus and perfused intestine (Fig. 1) were maintained at 37° C in a cabinet somewhat similar to that described by Hems, Ross, Berry & Krebs (1966). The cannulae were positioned with the aid of pot magnets and the reservoir/oxygenator and filter were as described by Parsons & Powis (1971) except that a siliconized glass bottle was used as a reservoir.

One channel of the peristaltic pump (Gilson minipuls HP_4) which gave a virtually non-pulsatile flow was used for pumping the arterial inflow whilst a second channel was used to return the venous effluent to the reservoir. Arterial pressure was measured with an anaeroid dial gauge, and was corrected for the resistance of the tubing between the site of the gauge and the tip of the arterial cannula. Venous pressure was maintained at 5–10 mmHg (6·8–13·6 cmH_2O).

Where possible nylon 6 tubing (Portex Ltd) was used to connect the various components of the perfusion apparatus. Tygon vinyl tubing, formulation S-50-HL (U.S. Stoneware Co.), was used where more flexible tubing was required. Both the aorta and superior mesenteric vein were cannulated with nylon tubing of dimensions 0·75 mm i.d. and 0·94 mm o.d.

The medium that passed through the lumen of the intestine (luminal fluid) was recirculated by means of a small peristaltic pump (Peripump, Schuco Scientific Ltd) which gave a pulsatile flow. The transmural distension pressure (15–20 cm saline), which was applied to the intestinal segment to prevent hypermotility, was measured with a saline manometer and adjusted by altering the height of the reservoir which held the luminal fluid (h_L, Fig. 1).

Vascular perfusion – operative procedure. The procedure most nearly resembles that of Dubois, Vaughan & Roy (1968), but differs chiefly in that the intestine is left *in situ* and the aorta is cannulated by the method of Gerber & Remy-Defraigne (1966). In preliminary experiments the superior mesenteric artery was cannulated directly and the blood supply to the intestine consequently interrupted for up to 5 min although O_2 was available from the lumen during this period. In such preparations there was considerable destruction of the mucosal epithelium, and the venous effluent did not appear to be appreciably deoxygenated.

The rat, anaesthetized with intramuscular sodium pentobarbitone (Nembutal 60 mg/kg body wt.), was positioned on the operating table inside the cabinet. The abdomen was opened and the bulk of the intestinal tract was exteriorized and covered with a plastic-backed gauze pad wetted with warm isotonic saline.

The duodenum was gently pulled away from the colon and the right colic artery and vein were ligated. A glass cannula was tied into the lumen of the intestine about 3 cm from the duodenal–jejunal flexure. The portion of jejunum proximal to the site of insertion of the cannula was gently pulled under the colon. Vessels supplying blood to the duodenum and the above section of jejunum were tied off and a ligature was tied around the duodenum just distal to the point of entry of the bile duct. Most of the duodenum was then excised.

The middle colic artery and vein were ligated. The lumen of the intestine was flushed out with 30 ml of Krebs–Ringer bicarbonate medium at 37° C and a second cannula tied into the lumen about 30 cm distal to the first. A loose tie was placed around the vessels leading to the remainder of the small intestine, the caecum and the proximal colon. 250 i.u. heparin were injected into the right femoral vein.

Loose ligatures were placed around the aorta, just above and below the origin of the superior mesenteric artery. The right and left renal arteries were tied. The aorta was occluded with a fine bulldog clamp, just below the point of departure of the superior mesenteric artery and the arterial cannula pushed up to the clamp and tied in. The clamp was removed and perfusate flow started at 1·5 ml. min^{-1}, or about 5 ml. min^{-1} g dry $wt.^{-1}$ (a similar value for the jejunal blood flow *in vivo* was obtained by Windmueller & Spaeth, 1975). The intestine was isolated by tying the loose ligatures around the aorta and the vessels leading to the ileum, caecum and proximal colon. The flow of luminal fluid was started and a venous cannula pushed up into the superior mesenteric vein and tied in.

The venous effluent for the first 15 min of perfusion was discarded after which time it was returned to the reservoir and recirculated.

Vascular perfusion – conduct of experiments. In order to minimize the utilization of glucose by erythrocytes the vascular perfusate was pre-incubated for 2 hr before the start of perfusion. It was found that the utilization of glucose by the erythrocytes in the subsequent hour was 6 times lower than if pre-incubation was for 30–45 min.

For glucose and lactate determinations 0·5 ml. samples of vascular perfusate and luminal medium were taken, alternately at intervals of 5 min usually over the period 20–55 min, and were deproteinized with perchloric acid (35·5 g $HClO_4$/l.).

Analytical methods. Glucose was determined by the glucose oxidase method of Bergmeyer & Bernt (1963). L-(+)-lactate was measured by the method of Hohorst (1963). The O_2 content of samples was determined by the method of Van Slyke & Neill (1924).

Histology. Small pieces of intestine were fixed in Bouin's fixative and the longitudinal sections stained with haematoxylin and eosin.

Expression and calculation of results. With the exception of the rings, glucose metabolism is expressed with respect to the final dry weight of the tissue. The blotted tissue was dried in an air oven at 105° C overnight. The results obtained with rings are expressed with respect to the initial dry weight of the tissue, which was derived from the initial wet weight by the use of accompanying measurements of the ratio of the wet weight to the dry weight.

When the uptake of glucose increases linearly with time it is assumed that the tissue is in a steady state of metabolism and the slope of the line relating uptake of glucose to time is taken as a measure of the rate of metabolism of glucose by the tissue ('glucose utilization'). The rate of production of lactate was similarly measured in the steady state from the linear relationship existing between the lactate appearing and time. In the vascular perfusion experiments the steady-state rates of glucose uptake and lactate appearance calculated for the vascular perfusate and luminal medium were summed to give the over-all rates of glucose utilization and lactate production. Where appropriate the measured concentrations of glucose and lactate in the vascular perfusate were corrected for the effects of the metabolism of the erythrocytes. The rate of conversion of glucose to metabolites other than lactate is given by:

$$\mu\text{mole glucose utilized g dry wt.}^{-1}\ \text{hr}^{-1} - \tfrac{1}{2}\ (\mu\text{mole lactate produced g dry wt.}^{-1}\ \text{hr}^{-1}).$$

The results obtained with the rings over the initial period of 0–15 min were calculated from the changes in the amounts of glucose and lactate in the tissue and incubation medium during that time. In this case the tissue may not have been in a steady state.

RESULTS

Non-vascularly perfused preparations

Rings of jejunum. The time the rings spent in the ice-cold isotonic saline had little effect upon their subsequent metabolism. Glucose metabolism as judged by lactate production was directly proportional to the initial dry weight of the tissue.

There was no significant difference between the rates at which the rings utilized glucose under aerobic and anaerobic conditions of incubation (Table 1). This was the case whether the initial concentration of glucose in the incubation medium was 5 or 10 mM or whether the measurements were made over the period 0–15 or 15–45 min.

Lactate production was found to be higher under anaerobic rather than aerobic conditions only during the first 15 min of incubation. The rate of production of lactate by the rings was the same whether the concentration of glucose in the incubation medium was 10 or 28 mM.

TABLE 1. Utilization of glucose and production of lactate by rings of rat jejunum incubated under aerobic and anaerobic conditions. Values are of means ± S.E. of mean, or where the results are from only two experiments, the two results and their mean are shown

Period of measurement (min)	Initial glucose concentration (mM)	No. of experiments		Glucose utilization (μmole g dry wt.$^{-1}$ hr^{-1})		Lactate production (μmole g dry wt.$^{-1}$ hr^{-1})		Proportion of glucose converted to lactate (%)	
		Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
0–15	5	5	4	424 ± 24	429 ± 21	555 ± 65	760 ± 23*	66 ± 7	89 ± 3*
	10	4	5	552 ± 58	556 ± 45	728 ± 67	1024 ± 29***	68 ± 9	94 ± 6*
	28	4	2	—	—	809 ± 35	982, 1144, 821	—	—
15–45	5	5	4	307 ± 16	335 ± 38	498 ± 11	617 ± 66	82 ± 3	92 ± 2*
	10	4	5	402 ± 25	395 ± 16	718 ± 34	760 ± 22	90 ± 5	96 ± 3
	28	4	2	—	—	733 ± 26	682, 673, 691	—	—

For the comparison of the corresponding aerobic and anaerobic measurements; P, where significant, denoted as follows: *, $P < 0{\cdot}05 > 0{\cdot}02$; ***, $P < 0{\cdot}01 > 0{\cdot}001$.

TABLE 2. Utilization of glucose and production of lactate by rat jejunum recirculated through the lumen under aerobic and anaerobic conditions. Results were calculated from measurements made between 20 and 50 min under aerobic incubation conditions, and 20 and 40 min under anaerobic incubation conditions. Flow rate through the lumen: 35 ml./min. Values are of means ± S.E. of mean

Incubation conditions	No. of experiments	Initial glucose concentration (mM)	Glucose utilization (μmole g dry wt.$^{-1}$ hr^{-1})	Lactate production (μmole g dry wt.$^{-1}$ hr^{-1}	Proportion of glucose converted to lactate (%)
Aerobic	6	5	460 ± 32	748 ± 72	82 ± 7
Anaerobic	4	5	434 ± 32	864 ± 45	100 ± 3
Aerobic	4	10	736 ± 50	1288 ± 115	87 ± 3*
Anaerobic	4	10	600 ± 37	1157 ± 82	96 ± 2
Aerobic	4	20	—	1186 ± 67	—
Anaerobic	3	20	—	1056 ± 193	—

For the comparison of the corresponding aerobic and anaerobic measurements; P, where significant, denoted as follows: *, $P < 0{\cdot}05 > 0{\cdot}02$.

TABLE 3. Utilization of glucose and production of lactate by rat small intestine recirculated with segmented flow through the lumen under aerobic and anaerobic conditions. Flow rate of bubbles of gas, 95% O_2, 5% CO_2 (v/v), through the lumen 10 ml./min, flow of liquid through the lumen 13–14 ml./min. In all cases the initial concentration of glucose in the incubation medium was 10 mM. Values are of means ± S.E. of mean

Region of intestine	Incubation conditions	No. of experiments	Period of measurement (min)	Glucose utilization (μmole g dry wt.$^{-1}$ hr^{-1})	Lactate production (μmole g dry wt.$^{-1}$ hr^{-1})	Proportion of glucose converted to lactate (%)
Jejunum	Aerobic	5	20–50	518 ± 29	834 ± 45	81 ± 2
Jejunum	Anaerobic	4	20–40	569 ± 45	1112 ± 51***	98 ± 4***
Ileum	Aerobic	5	30–60	327 ± 5	404 ± 37	62 ± 5
Ileum	Anaerobic	4	30–45	343 ± 27	688 ± 24****	102 ± 11***

For the comparison of the corresponding aerobic and anaerobic measurements; P, where significant, denoted as follows: ***, $P < 0{\cdot}01 > 0{\cdot}001$; ****, $P < 0{\cdot}001$.

Jejunum recirculated through lumen. The effects of anaerobiosis upon the metabolism of this more intact preparation of intestine which was neither cooled nor deprived of O_2 before incubation were similar to those obtained with the rings, except that the production of lactate was never increased when the tissue was incubated under anaerobic conditions (Table 2).

Small intestine recirculated with segmented flow through lumen. In both jejunum and ileum, the rate of production of lactate but not the utilization

of glucose was stimulated if the preparation was maintained under anaerobic rather than aerobic conditions (Table 3).

The introduction of bubbles of gas into the lumen of the segment of intestine did not affect glucose metabolism under anaerobic conditions but the utilization of glucose and the production of lactate under aerobic conditions were both reduced ($0{\cdot}01 < P > 0{\cdot}001$ in both cases for the comparison of the appropriate data in Tables 2 and 3). Consequently the finding of a Pasteur effect with the segmented-circulated preparation, but not with the recirculated preparation, seems to be a consequence of the lower aerobic lactate production by the former preparation.

TABLE 4. Tissue hydration and recovery of arterial inflow in venous effluent. Flow rate of vascular perfusate in all cases 1·5 ml./min. Values are of means ± S.E. of mean with the number of experiments in parentheses, or as the mean of two results, with the results in parentheses

Preparation	Haematocrit (%)	Glucose concentrations (mM) Vascular medium	Glucose concentrations (mM) Luminal medium	Flow rate of luminal fluid (ml./min)	Venous recovery (% of arterial inflow)	Tissue hydration (g H_2O/g dry wt.)
Fresh tissue	—	—	—	—	—	3·55 ± 0·10 (6)
A	0	10	0	12	97 ± 1 (3)	3·78 ± 0·06 (3)
B	0	10	0	12†	95 + 1 (3)	3·79 ± 0·18 (3)
C	15	10	0	2	93 + 0·3 (4)	3·54 ± 0·08 (4)
D	40	10	0	2	96 ± 2 (4)	3·11 ± 0·06 (4)**
E	40	10	0	2	96 ± 1 (3)	3·45 ± 0·16 (3)
F	40	10	10	2	96 ± 2 (3)	3·46 ± 0·20 (3)
G	40	10	28	2	99 ± 1 (3)	3·63 ± 0·07 (3)
H	40	28	0	2	96 ± 3 (3)	3·55 ± 0·03 (3)
I	40	28	10	2	96 (99, 94)	3·69 (3·69, 3·69)

**, $P < 0{\cdot}02 > 0{\cdot}01$ for the comparison with fresh tissue.

† Bubbles of gas, 95 % O_2, 5 % CO_2 (v/v) introduced into the lumen at a rate of 3 ml./min.

With both recirculated and segmented-circulated jejunum, there was a reduction in the uptake of glucose by the tissue and in the appearance of lactate in the medium after 40 min of incubation under anaerobic conditions. This was not seen if the tissue was incubated under aerobic conditions.

With the exception of the proportion of glucose converted to lactate under anaerobic conditions, all the results shown for ileum in Table 3 were significantly lower ($P < 0{\cdot}01$ in all cases) than the corresponding results for jejunum. The stimulation of lactate production by anaerobiosis was greater in the ileum (70 %) than in the jejunum (33 %).

Vascularly perfused rat jejunum

Some characteristics of the preparation. Values for the recovery of the arterial inflow in the venous effluent measured at the end of the experimental period and for the hydration of the tissue after perfusion are shown in Table 4. Gross leakage of fluid from the vascular perfusate into the lumen of the perfused jejunum was not responsible for the incomplete recovery of the arterial inflow in the venous effluent for no such secretion was detectable when [^{14}C]polyethylene glycol (mol. wt. 4000) was used as a non-absorbable marker for the volume of the luminal fluid. The conditions adopted for perfusion did not give rise to oedema of the tissue wall.

The perfusion pressure rose slightly during the experiments (Fig. 2), and was increased relatively little when the haematocrit of the vascular perfusate was 40 % rather than 15 %.

After perfusion for 1 hr the tissue histology was essentially normal although a small number of cells appeared to have become detached from the epithelium.

Changes in the amounts of glucose in the vascular perfusate were usually, and of lactate, always, linearly related to time over the period 20–50 min (Figs. 3*A*, *B*). When glucose was added only to the vascular perfusate, the highest value for the mean concentration of glucose in the medium that passed through the lumen after 45 min of perfusion was $0{\cdot}12 \pm 0{\cdot}04$ m-mole/l. (4) (preparation C, Table 5). The appearance of lactate in the luminal fluid was linearly related to time after perfusion for 35 min. The rate of appearance of lactate in the luminal fluid was 10–15 % of the total rate of lactate appearance.

Oxygen consumption. In two experiments with 15 % haematocrit in the vascular medium, O_2 consumption was 5·48 and 7·28 μmole g dry wt.$^{-1}$ min^{-1} (mean Q_{O_2} 8·58 μl. mg dry wt.$^{-1}$ hr^{-1}). The uptake of O_2 was almost exclusively from the vascular perfusate rather than from the fluid in the closed luminal circuit, and was the same whether measured over the period 20–25 or 45–50 min (cf. Parsons & Powis, 1971). On average 22 % of the O_2 delivered to the tissue in the vascular perfusate was utilized.

Oxygen supply. Comparison of preparations A and B (Table 5) shows that a large increase in the delivery of O_2 to the lumen of the perfused segment of jejunum (preparation B) had no significant effect upon the utilization of glucose, the production of lactate or the proportion of glucose converted to lactate. However, when erythrocytes were present in the vascular perfusate (preparation C) the rate of lactate production was reduced below the levels found with preparation A ($P < 0{\cdot}001$), and preparation B ($P < 0{\cdot}05 > 0{\cdot}02$). The utilization of glucose was significantly reduced below that found with preparation A ($P < 0{\cdot}02 > 0{\cdot}01$).

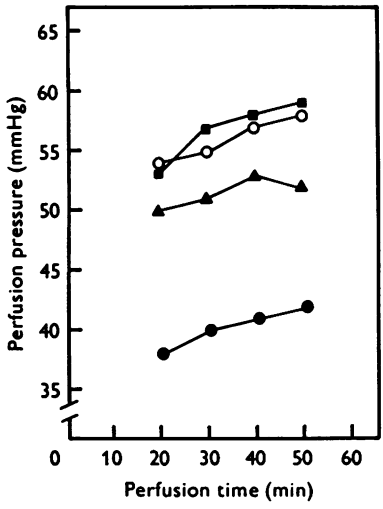

Fig. 2

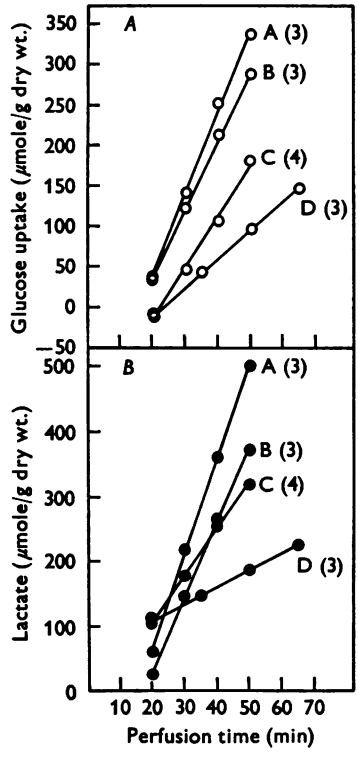

Fig. 3

Fig. 2. Vascular perfusion of rat jejunum. Effect of perfusion time on perfusion pressure. In all cases flow rate of vascular perfusate 1·5 ml./min, and luminal distension pressure 15–20 cm saline. ●, haematocrit 15 %, glucose in vascular medium 10 mM, no glucose in lumen, mean of two experiments. ○, haematocrit 40 %, glucose in vascular medium 10 mM, no glucose in lumen, mean of three experiments. ▲, haematocrit 40 %, glucose in vascular medium 28 mM, no glucose in lumen, mean of three experiments. ■, haematocrit 40 %, glucose in vascular medium 10 mM, glucose in lumen, 10 mM, mean of three experiments.

Fig. 3. Vascular perfusion of rat jejunum. In all cases vascular perfusate contained glucose 10 mM, and bovine serum albumin 3 g/100 ml. Flow rate of vascular perfusate 1·5 ml./min. Flow rate of Krebs–Ringer bicarbonate (no glucose) through the lumen: preparations A and B, 12 ml./min; preparations C and D, 2 ml./min. In preparation B bubbles of gas, 95 % O_2, 5 % CO_2 (v/v), were introduced into the lumen at 3 ml./min. Preparations A and B, haematocrit of vascular perfusate 0, preparation C 15 %, preparation D 40 %. Number of experiments in parentheses. (*a*) Relationship between the uptake of glucose from the vascular perfusate/g dry wt. of perfused jejunum, and time. (*b*) Relationship between the amount of lactate in the vascular perfusate/g dry wt. of perfused jejunum, and time.

TABLE 5. Effect of O_2 supply upon utilization of glucose and production of lactate by rat jejunum perfused through the vascular bed *in situ*. Flow rate of vascular perfusate in all cases 1·5 ml./min. Flow rate of liquid through the intestinal lumen: preparations A and B, 12 ml./min; preparations C and D, 2 ml./min. In preparation B bubbles of gas, 95 % O_2, 5 % CO_2 (v/v), were introduced into the lumen at a rate of 3 ml./min. Initial concentration of glucose in the vascular perfusate 10 mM. No glucose added to the luminal fluid. Values are of means ± S.E. of mean. Where appropriate, the results have been corrected for the metabolism of the erythrocytes

		Rate of O_2 delivery (μl./min)				μmole g dry wt.$^{-1}$ hr^{-1}			
Preparation	Haemato-crit (%)	Vascular side	Luminal side	Total	No. of experi-ments	Glucose utilization	Lactate production	Conversion of glucose to metabolites other than lactate	Proportion of glucose converted to lactate (%)
A	0	27	216	243	3	594 ± 33	897 ± 12	146 ± 29	76 ± 4
B	0	27	3066	3093	3	509 ± 43	702 ± 95	158 ± 6	68 ± 3
C	15	120	36	156	4	392 ± 44	438 ± 27	173 ± 55	59 ± 8
D	40	275	36	311	4	222 ± 20	144 ± 28	149 ± 28	34 ± 9

If the haematocrit of the vascular medium was increased to 40% (preparation D), then the rates of utilization of glucose and production of lactate were significantly lower than the values found in the presence of 15% haematocrit (preparation C) ($P < 0{\cdot}02 > 0{\cdot}01$ and $P < 0{\cdot}001$, respectively).

The rate at which glucose was converted to metabolites other than lactate was the same in all preparations.

Effects of orientation and concentration of glucose. The addition of glucose to the luminal fluid of the perfused intestine resulted in an increased utilization of glucose and production of lactate by the tissue (compare preparations E and F, Table 6).

When the concentration of glucose in the luminal fluid was increased from 10 to 28 mM there was no further stimulation of glucose utilization or of lactate production (compare preparations F and G, Table 6).

When the concentration of glucose in the vascular medium was raised to 28 mM (preparation H) lactate production was higher than when the concentration of glucose in this medium was 10 mM (preparation E), occurring at a rate similar to that found in preparations F, G and I, where glucose was also present in the luminal fluid.

DISCUSSION

Non-vascularly perfused preparations of rat small intestine. A feature of our experiments is that unlike previous work glucose and lactate metabolism was investigated under conditions in which the tissue was apparently in a steady state with respect to the uptake of glucose and the output of lactate. Rings of rat jejunum exhibit a Pasteur effect only during the first 15 min of incubation. This suggests that changes within the tissue during incubation may have been one of the factors responsible for the inability of previous workers (Dickens & Weil-Malherbe, 1941; Wilson & Wiseman, 1954; Lohmann *et al.* 1966; Sherratt, 1968) to find a Pasteur effect in this tissue.

A Pasteur effect was also found with the segmented-circulated preparation of intestine, but not with a similar preparation in which only liquid passed through the lumen of the intestine. In the latter preparation the oxygen delivered to the lumen was adequate to support a Q_{O_2} of 126 μl. mg dry wt.$^{-1}$ hr^{-1}, which is one order of magnitude above the likely Q_{O_2} of the tissue (Bronk & Parsons, 1965). An effect of presence of the gas bubbles may therefore be an increased stirring of the luminal compartment (Fisher & Gardner, 1974), with a consequent reduction in the thickness of the unstirred layer adjacent to the epithelium. A reduction of the thickness of this stationary layer will increase the access of O_2 to the tissue (see Appendix).

TABLE 6. Effects of the orientation and concentration of glucose upon utilization of glucose and production of lactate by rat jejunum perfused through the vascular bed *in situ*. In all cases the flow rate of the vascular perfusate was 1·5 ml./min, and that of the luminal fluid 2 ml./min. Vascular perfusate contained penicillin G, 10,000 u./100 ml., streptomycin, 5 mg/100 ml., bovine serum albumin, 3 g/100 ml. and erythrocytes to give a haematocrit of 40 %. Values are of means ± S.E. of mean, or where only two experiments were performed the two results and their mean are shown. The results are corrected for the metabolism of the erythrocytes. Satisfactory measurements of glucose utilization were not obtainable with preparation I because the uptake of glucose from the vascular perfusate and luminal fluid was never linearly related to time

	Initial glucose concentrations (mM)			(μmole g dry wt.$^{-1}$ hr^{-1})			
Preparation	Vascular medium	Luminal medium	No. of experiments	Glucose utilization	Lactate production	Conversion of glucose to metabolites other than lactate	Proportion of glucose converted to lactate (%)
E	10	0	3	274 ± 30	221 ± 36	163 ± 13	40 ± 3
F	10	10	3	401 ± 31*	421 ± 59*	190 ± 27	52 ± 5
G	10	28	3	379 ± 77	499 ± 53**	130 ± 60	69 ± 9*
H	28	0	3	420†	439 ± 49*	239†	43†
I	28	10	2	—	484 (472, 496)	—	—

For the comparison with values obtained with preparation E; P, where significant, denoted as follows: *, $P < 0{\cdot}05 > 0{\cdot}02$; **, $P < 0{\cdot}02 > 0{\cdot}01$.

† Single experiment.

In no case was lactate production significantly higher under aerobic than anaerobic conditions of incubation. It is possible that the finding of an 'inverse Pasteur effect' by Lohmann *et al.* (1966) is related to our finding that the metabolism of glucose tends to fall off during the incubation of the tissue under anaerobic, but not under aerobic conditions.

Under anaerobic conditions and when the concentration of glucose in the incubation medium was 10 mM it is unlikely that the availability of glucose was limiting its utilization by the tissue (see Leese & Bronk, 1975), for raising the concentration of glucose in the incubation medium to 28 mM did not increase lactate production. The rate of glucose utilization obtained under anaerobic conditions with 10 mM glucose in the incubation medium may thus be taken as a measure of the maximal rate of glucose metabolism by the tissue. Since there was no difference between the utilization of glucose under anaerobic and aerobic conditions of incubation it would appear that glucose utilization under aerobic conditions was similarly maximal. This also seems to be true in the ileum despite the presence of quite a large Pasteur effect in this tissue.

Vascularly perfused intestine

Viability. The viability of the preparation would appear to have been maintained over a period of 1 hr as judged by the constancy of the O_2 consumption, the uptake of glucose by the tissue, and the appearance of lactate in the incubation medium; the small variation in perfusion pressure; the absence of oedema, and the essentially normal histology found at the end of the experiment.

Regulation of metabolism. When the haematocrit of the vascular perfusate was 40% and glucose was absent from the fluid in the lumen of the intestine, the utilization of glucose and the production of lactate by the vascularly perfused preparation was clearly not taking place at a maximal rate, for much higher rates of these processes could be obtained if erythrocytes were absent from the vascular perfusate.

Effect of haematocrit. An explanation of the finding that an increase in the haematocrit of the vascular perfusate from 15 to 40% reduced glucose utilization and lactate production, despite an apparent surplus of oxygen at the lower haematocrit, is that the distribution of blood flow within the jejunal wall was such that the supply of O_2 to a region of the tissue was inadequate when the haematocrit was 15%. Such an explanation does not necessarily require the presence of shunts of the type described by Spanner (1932). Another possible contributory factor could be a reduction in the villous haematocrit as a result of plasma skimming (Jodal & Lundgren, 1970).

Possible explanations for the behaviour of the non-vascularly perfused preparations

Two factors can increase the utilization of glucose and the production of lactate by the vascularly perfused preparation above the basal level found with glucose in the vascular medium (10 mM). These are (*a*) the addition of glucose to the luminal medium, as has been found *in vivo* (Csaky & Ho, 1966; Förster, Meyer & Ziege, 1972), and (*b*) increasing the concentration of glucose in the vascular perfusate to 28 mM (Table 6). During absorption under non-perfused conditions *in vitro* monosaccharides such as glucose galactose, etc., accumulate within the intestinal wall. Thus Fisher & Parsons (1953) found that during absorption by rat jejunum from 28 mM glucose in the lumen the average tissue concentration was in excess of 50 mM. The presence of glucose at the luminal pole of the intestinal epithelial cell and the consequent accumulation of glucose within the tissue may therefore contribute to the high rates of glucose metabolism found with the non-vascularly perfused preparations of rat jejunum.

In the vascularly perfused preparation, both an increased glucose utilization and an increased lactate production is found when either glucose (10 mM or more) is present in the lumen or when there is a high (28 mM) concentration of glucose in the vascular inflow. It is possible that the increased glucose metabolism that is found during absorption under our conditions of vascular perfusion occurs in a compartment easily accessible to glucose absorbed from the lumen. If so, this compartment is not accessible to glucose at 10 mM in the vascular bed although it may be accessible to glucose at 28 mM in the vascular bed (see also Sherratt, 1968).

One interpretation of the data shown in Table 5 is that the oxygen requirement of rat jejunum cannot be satisfied by the delivery of large amounts of oxygen exclusively via the lumen of the intestine. It is possible that the access of O_2 to deeper regions of the intestinal wall, such as the crypts, might be limited by the presence of an unstirred layer of fluid in the lumen (see Appendix). Although the effect of erythrocytes in the vascular perfusate in reducing glucose utilization and lactate production may be mediated through an increase in the oxygenation of the tissue, there is no effect upon the proportion of glucose that is converted to metabolites other than lactate, a fraction which should reflect oxygen consumption. It is found that values for this fraction are lower in a number of non-vascularly perfused preparations (Table 7).

A limitation in the supply of oxygen would presumably result in a fall in the ATP/ADP ratio, which if sufficiently large might result in deinhibition of phosphofructokinase, and a rate of glucose utilization similar to

that found under anaerobic conditions. B. J. Parsons (1959) found that incubation of everted sacs for 1 hr resulted in a reduction in the total adenine nucleotide content of the tissue together with a decreased ATP/ADP ratio. However, tissue adenine nucleotide levels that might favour deinhibition of phosphofructokinase have also been found upon incubation of isolated cells (Iemhoff, Van Den Berg, De Pijper & Hülsmann, 1970) and slices of intestinal mucosa (Bronk & Leese, 1973) and it is difficult to see how an inadequate oxygenation could be involved in these cases.

TABLE 7. Utilization of glucose and production of lactate by preparations of rat jejunum *in vitro*. Aerobic conditions of incubation. Initial concentration of glucose in the single incubation medium, or in the vascular perfusate and luminal fluid, 10 mM. Haematocrit of vascular perfusate, 40%. Figures in parentheses are the period of time (min) over which measurements were made. Values are of means ± S.E. of mean and are taken from Tables 1, 2, 3 and 6

		(μmole g dry wt.$^{-1}$ hr^{-1})			
Preparation	No. of experiments	Glucose utilization	Lactate production	Conversion of glucose to metabolites other than lactate	Proportion of glucose converted to lactate (%)
Vascularly perfused (20–55)	3	401 ± 31	421 ± 59	190 ± 27	52 ± 5
Rings (0–15)	4	552 ± 58	728 ± 67*	188 ± 69	68 ± 9
Rings (15–45)	4	402 ± 25	718 ± 34***	43 ± 22***	90 ± 5***
Recirculated (20–50)	4	736 ± 50***	1288 ± 115***	92 ± 18*	87 ± 3***
Segmented-recirculated (20–50)	5	518 ± 29*	834 ± 45***	101 ± 13**	81 ± 2***

For the comparison with the vascularly perfused preparation, P, where significant, denoted as follows: *, $P < 0{\cdot}05 > 0{\cdot}02$; **, $P < 0{\cdot}02 > 0{\cdot}01$; ***, $P < 0{\cdot}01 > 0{\cdot}001$.

In conclusion, it is evident that whatever the mechanism involved, the only preparation of rat jejunum that exhibited a low rate of glucose utilization and converted a relatively small proportion of the glucose utilized to lactate was one in which the vascular bed was perfused with a medium containing a high content of erythrocytes. With this preparation the presence of glucose in the lumen or high concentrations (28 mM) of glucose in the vascular bed stimulates glucose utilization and lactate production and raises questions with respect to compartmentation within the intestinal mucosa of substrate that can be metabolized as well as absorbed from the lumen.

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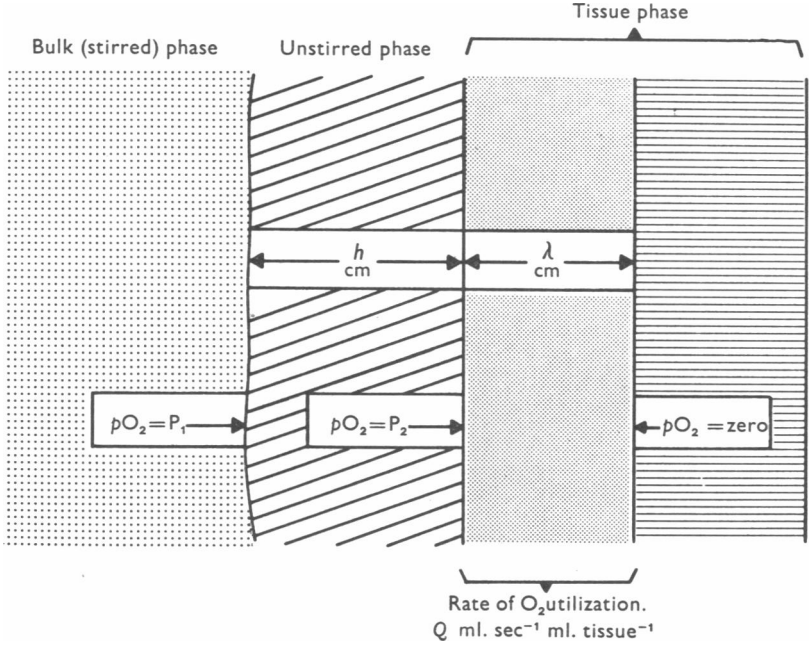
APPENDIX

A NOTE ON THE INFLUENCE OF AN UNSTIRRED LAYER ON THE DELIVERY OF OXYGEN TO A TISSUE AND HENCE ON THE EFFECTIVE 'LIMITING TISSUE THICKNESS'

We attempt to answer the question: Is the diffusion of O_2 through unstirred layers of incubating medium in practice likely to be rate limiting to the respiration of a tissue? We show that the limiting thickness of the tissue may in practice be reduced to an extent that is related to the thickness of the unstirred layer.

Assume a tissue in the form of a plane sheet with uniform respiration rate of Q vol. vol^{-1} sec^{-1} independent of O_2 pressure and exposed on one side to a fluid medium (Appendix Fig. 1). Suppose the medium is divided into two compartments. One, the bulk phase, well stirred and containing O_2 at a uniform pressure of P_1 units (see below), the other, a stationary



Appendix Fig. 1. Model tissue in the form of a plane sheet, the left-hand side of which is exposed to a fluid medium composed of two phases. The well-stirred phase is separated from the tissue by a stationary, unstirred, phase of the fluid medium, h cm thick. O_2 diffuses into the tissue and is consumed at a constant rate. Beyond the 'limiting thickness', λ cm below the surface of the tissue, all the O_2 that diffuses in has been consumed and at all greater depths the partial pressure of O_2 is zero.

unstirred phase of thickness h cm, adjacent to the sheet and through which there is a flux of O_2 (J) in the direction normal to the plane of the tissue and at a rate determined by Fick's law with diffusion coefficient D_1, $cm^2\ sec^{-1}$. If the diffusion coefficient of O_2 within the tissue is D_2 then in the plane distant λ cm from and parallel to the tissue surface, the partial pressure of O_2 will be zero when

$$\lambda = \{2P_2.D_2/Q\}^{0.5} \quad \text{(see Warburg, 1923, and Höber, 1945),} \qquad (1)$$

where P_2 is the partial pressure of O_2 at the surface of the tissue, i.e. at the fluid-tissue interface (Appendix Fig. 1). Then the steady-state influx of O_2 into unit area of tissue is given by

$$J = \lambda Q \text{ ml. sec}^{-1}.$$

λ is the limiting tissue thickness as defined by the Warburg equation (1). Also, the flux of oxygen through the unstirred layer in the steady state is

$$J = D_1 (P_1 - P_2)/h \text{ ml. sec}^{-1},$$

hence

$$P_2 = P_1 - h\lambda Q/D_1 \text{ ml. ml.}^{-1}.$$

Inserting this value for P_2 in the Warburg eqn. (1) and solving for λ gives.

$$\lambda = \{(hD_2/D_1)^2 + 2P_1D_2/Q\}^{0.5} - hD_2/D_1 \text{ cm.}$$

Consider a specific example. For a flat sheet of epithelium with a respiration rate of 10 μl. mg dry wt.$^{-1}$ hr^{-1} (Bronk & Parsons, 1965), and suppose 4 g tissue = 1 g dry wt. and 1 g tissue = 1 ml. tissue = 0·25 g dry weight giving $Q = 2{\cdot}5$ ml. ml. tissue^{-1} hr^{-1} = $7{\cdot}10^{-4}$ ml. O_2 ml. tissue^{-1} sec^{-1}. For P_1, the partial pressure of O_2 in the bulk phase it is convenient to use units of volume of O_2 per unit volume of solution which take into account both the pressure in atmospheres and the solubility of the oxygen. Assume P_1 the O_2 pressure in the bulk phase, 0·025 ml. ml.$^{-1}$ (i.e. p_{O_2} around 700 mm Hg) and $D_1 = 2 \times 10^{-5}\ cm^2\ sec^{-1}$ and $D_2 = 10^{-5}\ cm^2\ sec^{-1}$ equivalent to the value of Krogh for O_2 diffusion in muscle (Warburg, 1923) of $1{\cdot}4 \times 10^{-5}$ ml. $O_2\ cm^2\ min^{-1}$ with 1 atm pressure difference. It then appears that the effective limiting thickness of the tissue is related to the thickness of the unstirred layer as follows:

Thickness of unstirred layer, h (μm)	Limiting thickness of tissue, λ (μm)
0	261
1	261
10	257
100	221
250	165
500	112
1000	64
5 mm	14
1 cm	7

The thickness of the whole wall of adult rat jejunum (excluding the villi) is about 150 μm and the villi stand 250–300 μm high (D. S. Parsons, unpublished observations). The unstirred layer thickness may vary from 100 μm to more than 500 μm; *in vivo* the thickness may be even greater because of mucous secretion (see Winne, 1973, and Dietschy & Westergaard, 1975).

REFERENCES

BERGMEYER, H. U. & BERNT, E. (1963). D-glucose: determination with glucose oxidase and peroxidase. In *Methods of Enzymatic Analysis*, ed. BERGMEYER, H. U., pp. 123–130. Weinheim: Verlag Chemie.

BRONK, J. R. & LEESE, H. J. (1973). Changes in the adenine nucleotide content of preparations of the rat small intestine *in vitro*. *J. Physiol.* **235**, 183–196.

BRONK, J. R. & PARSONS, D. S. (1965). The polarographic determination of the respiration of the small intestine of the rat. *Biochim. biophys. Acta* **107**, 397–404.

CSAKY, T. Z. & HO, P. M. (1966). The effect of potassium on the intestinal transport of glucose. *J. gen. Physiol.* **50**, 113–128.

DICKENS, F. & WEIL-MALHERBE, H. (1941). Metabolism of normal and tumour tissue. 19. The metabolism of intestinal mucous membrane. *Biochem. J.* **35**, 7–15.

DIETSCHY, J. M. & WESTERGAARD, H. (1975). The effect of unstirred water layers on various transport processes in the intestine. In *Intestinal Absorption and Malabsorption*, ed. CSÁKY, T. Z. New York: Raven.

DUBOIS, R. S., VAUGHAN, G. D. & ROY, C. C. (1968). Isolated rat small intestine with intact circulation. In *Organ Perfusion and Preservation*, ed. NORMAN, J. C. pp. 863–875. New York: Appleton-Century-Crofts.

FISHER, R. B. & GARDNER, M. L. G. (1974). A kinetic approach to the absorption of solutes by isolated perfused small intestine. *J. Physiol.* **241**, 211–234.

FISHER, R. B. & PARSONS, D. S. (1949). A preparation of surviving rat small intestine for the study of absorption. *J. Physiol.* **110**, 36–46.

FISHER, R. B. & PARSONS, D. S. (1953). Glucose movements across the wall of the rat small intestine. *J. Physiol.* **119**, 210–223.

FÖRSTER, H., MEYER, E. & ZIEGE, M. (1972). The intestinal absorption of glucose with simultaneous determination of the arterioportal glucose concentration difference. *Revue Europ. Étud. clin. biol.* **17**, 958–964.

GERBER, G. B. & REMY-DEFRAIGNE, J. (1966). DNA metabolism in perfused organs. II. Incorporation into DNA and catabolism of thymidine at different levels of substrate by normal and X-irradiated liver and intestine. *Archs int. Physiol. Biochim.* **74**, 785–806.

HEMS, R., ROSS, B. D., BERRY, M. N. & KREBS, H. A. (1966). Gluconeogenesis in the perfused rat liver. *Biochem. J.* **101**, 284–292.

HÖBER, R. (1945). *Physical Chemistry of Cells and Tissues*, chap. 24. London: Churchill.

HOHORST, H. J. (1963). L-(+)-lactate: determination with lactic dehydrogenase and DPN. In *Methods of Enzymatic Analysis*, ed. BERGMEYER, H. U., pp. 266–270. Weinheim: Verlag Chemie.

IEMHOFF, W. G. J, VAN DEN BERG, J. W. O., DE PIJPER., A. M. & HÜLSMANN, W. C. (1970). Metabolic aspects of isolated cells from rat small intestinal epithelium. *Biochim. biophys. Acta* **215**, 229–241.

JODAL, M. & LUNDGREN, O. (1970). Plasma skimming in the intestinal tract. *Acta physiol. scand.* **80**, 50–60.

LAMERS, J. M. J. & HÜLSMANN, W. C. (1972). Pasteur effect in the *in vitro* vascularly perfused rat small intestine. *Biochim. biophys. Acta* **275**, 491–495.

LEESE, H. J. & BRONK, J. R. (1975). Lactate formation by rat small intestine in vitro. *Biochim. biophys. Acta* **404**, 40–48.

LOHMANN, K., GRAETZ, H. & LANGEN, P. (1966). The metabolism of the small intestine. In *Current Aspects of Biochemical Energetics*, ed. KAPLAN, N. O. & KENNEDY, E. P., p. 111. New York: Academic Press.

PARSONS, B. J. (1959). Studies of the effect of triethyltin sulphate on transport and metabolism in the small intestine of the rat. *J. Physiol.* **148**, 117–126.

PARSONS, D. S. & POWIS, G. (1971). Some properties of a preparation of rat colon perfused *in vitro* through the vascular bed. *J. Physiol.* **217**, 641–663.

PARSONS, D. S. & VOLMAN-MITCHELL, H. (1974). The transamination of glutamate and aspartate during absorption *in vitro* by small intestine of chicken, guinea-pig and rat. *J. Physiol.* **239**, 677–694.

SHERRATT, H. S. A. (1968). The metabolism of the small intestine. Oxygen uptake and L-lactate production along the length of the small intestine of the rat and guinea-pig. *Comp. Biochem. Physiol.* **24**, 745–761.

SOMOYGI, M. (1945). Determination of blood sugar. *J. biol. Chem.* **160**, 69–73.

SPANNER, R. (1932). Neue Befunde über die Blutwege der Darmwand und ihre funktionelle Bedeutung. *Morph. Jb.* **69**, 394–454.

VAN SLYKE, D. D. & NEILL, J. M. (1924). The determination of gases in blood and other solutions by vacuum extraction and manometric measurement: I. *J. biol. Chem.* **61**, 523–573.

WARBURG, O. (1923). Versuche an überlebendem Carcinomgewebe (Methoden). *Biochem. Z.* **142**, 317–333.

WILSON, T. H. (1956). The role of lactic acid production in glucose absorption from the intestine. *J. biol. Chem.* **222**, 751–763.

WILSON, T. H. & WISEMAN, G. (1954). Metabolic activity of the small intestine of the rat and golden hamster (*Mesocricetus auratus*). *J. Physiol.* **123**, 126–130.

WINDMUELLER, H. G. & SPAETH, A. E. (1972). Fat transport and lymph and plasma lipoprotein biosynthesis by isolated intestine. *J. Lipid Res.* **13**, 92–105.

WINDMUELLER, H. G. & SPAETH, A. E. (1975). Metabolism of glutamine and glutamic acid during their intestinal transport *in vivo*. *Fedn Proc.* **34**, 880.

WINNE, D. (1973). Unstirred layer, source of biased Michaelis constant in membrane transport. *Biochim. biophys. Acta* **298**, 27–31.