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A PREPARATION OF SURVIVING RAT SMALL INTESTINE FOR THE STUDY OF ABSORPTION

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Despite the physiological importance of the activities of the intestinal mucosa, there have been few successful attempts to study it in isolation, and the impression has arisen that it is difficult or impossible to set up a satisfactory surviving intestine preparation apart from the body and still with a viable mucosa. The objects of this paper are to show that this is not so, to describe a preparation which can be set up with uniform success, and to describe its major properties.

In outline, the preparation to be described is made by cannulating at both ends a segment of small intestine in an ether-anaesthetized rat, and setting up a closed circulation through its lumen of an oxygen-saturated, CO_2-bicarbonate-buffered fluid *before* the circulation through the intestinal wall is interrupted. The segment is suspended in a bath of oxygenated Ringer, and absorptive processes are followed by sampling the fluid circulating through the lumen (inner fluid) and the fluid bathing the exterior of the intestine (outer fluid).

The success of the technique is believed to depend on the feature that there is no time at which the mucosal cells are deprived of an adequate supply of oxygen.

METHODS

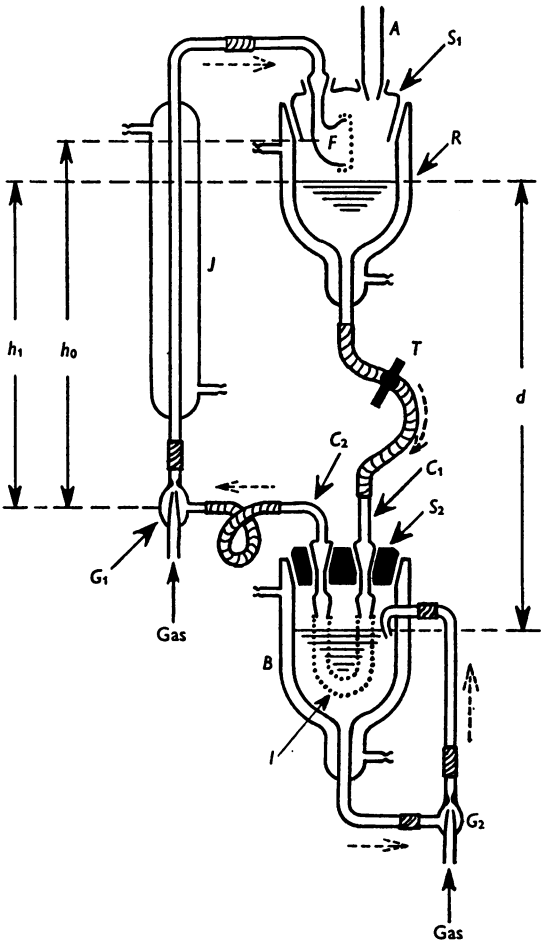
Animals. Male albino rats of Wistar stock weighing 200–300 g. are used. The animals are taken off the stock diet 24 hr. before use, and are provided with plain water and 5% glucose in water. They usually take about 100 ml. of the glucose-water, so that their calorie intake is reasonably maintained, but at the same time the small intestine is rendered largely free of solid contents.

Anaesthesia. Induction is effected by placing the animal in a small box containing a cotton-wool pad on which ether has been poured. Light anaesthesia is maintained with an Oxford Vaporizer adapted for use with small animals.

Preparation of segments. Two segments from each animal are usually set up, the whole of the small intestine except the duodenum being used. The duodenum has been excluded in the work so far carried out because of difficulties in mobilizing it, and for fear of harmful traction on the blood vessels or the intestine.

The abdomen is opened in the midline, the duodenal-jejunal flexure identified, and the intestine divided at this point. A cannula directed caudad is tied into the jejunum, the length of upper

segment required is decided, the intestine is again divided at the appropriate place, and the upper cannula of the lower segment is tied in. The upper segment is now washed free of debris with a gentle stream of warm 0·9% NaCl directed caudad, and a second cannula is tied into its lower end. (If the lower cannula is tied in earlier, it tends to block during washing.)



Text-fig 1. Circulation unit; for explanation see text.

The operating table, which is mounted on a trolley, is now brought up to one of the 'circulation units' described below, in which the inner and outer fluids are maintained at 38° C. and are kept oxygenated. The upper segment cannulae are connected into the inner fluid circuit and circulation is started in the direction from upper to lower end of the segment. The mesentery of this segment is now clamped and divided peripherally to the clamp, the freed segment is dipped into 300–400 ml. of warm saline to remove traces of blood, and is then set up in the outer fluid bath. The procedure for the lower segment is the same. The time taken, from completion of induction of anaesthesia to setting up of the second segment, averages 10 min.

Circulation units. Text-fig. 1 shows diagrammatically the layout of a circulation unit. The inner fluid passes from the double-walled glass reservoir R through a rubber tube furnished with a screw-clamp T to the glass tube C_1. Half-way along C_1 is a standard glass cone, and at the lower end

there is a smaller standard cone. The upper cone seats in a tapered hole in the ebonite stopper S_2. The inner fluid passes from C_1 through the loop I to a second similar glass tube C_2. At the beginning of an experiment I is a glass U-tube, connected by short lengths of rubber tubing. Later it is replaced by a segment of intestine. The fluid passes from C_2 to G_1, which is a small gas-injector which introduces a stream of gas bubbles into the liquid flowing through it. The inner fluid then passes via the jacketed vertical tube J and the antifrothing device F back to the reservoir R. F is a small hemispherical glass bowl with a short side tube emerging tangentially at the bottom of the bowl. The opening of the bowl is covered by cheese-cloth impregnated with paraffin-wax. This device is held by a short length of rubber tubing on to an angled glass tube fitting into a standard tubulure in the glass stopper S_1 of the reservoir R. S_1 carries a second standard tubulure into which fits the air condenser A.

When gas is injected through G_1 the mean density of the fluid in the portion G_1JF of the inner circuit falls to a value much below that of the fluid in the portion RTC_1IC_2, so that fluid is forced to circulate in the direction shown by the dotted arrows so long as gas is supplied to G_1 and T is open. The pressure differential determining the rate of flow is $(h_1 - ah_0)$, where h_0 and h_1 are the heights indicated on Text-fig. 1, and a is the fraction of the volume of the upflow tube which is occupied by liquid. With $h_1 = 35$ cm., $h_0 = 40$ cm. and tubes of *c.* 4 mm. internal diameter, inner fluid circulation rates of 35–45 ml./min. are obtained.

The distance d in Text-fig. 1 is equal to the difference, in terms of head of water, between the pressures inside and outside the intestine, i.e. is the distension pressure. Some circulation units have been made up in which the distance d can be varied without affecting the pressure differential determining the rate of flow.

The outer fluid passes from the double-walled bath B through a vertical tube at the bottom of the bath to a gas-injector G_2 and thence, through a vertical return tube, back into the bath by way of a horizontal tube sealed through its walls. The gas introduced into the bath by the injectors is carried away by an air-condenser (not shown in the figure) fitting into a third tapered hole in the stopper S_2.

Water is circulated through the jackets of B, J and R at high speed from a thermostatically controlled reservoir maintained at 39° C. With a circulation rate of 4 l./min./unit, there is less than 0·05° C. difference between the inner fluid temperatures of six units heated in parallel, and there is no detectable variation of temperature with time. The temperature of the inner and outer fluids is maintained at 38° C. by this arrangement. The set of six circulation units normally used is mounted on a rack designed for rapid dismounting of components for cleaning, and supported on a stand so that the baths B are at the height of the mobile operating table.

Setting up of intestinal segments on circulation units. With the inner circuits completed by glass U-tubes at I, 50 ml. of the appropriate inner fluid are introduced into each unit through S_1, and 50 ml. of the appropriate outer fluid are introduced into B through S_2. T is opened and 5% CO_2 in O_2 is introduced through G_1 and G_2, the flow being adjusted to give a regular pattern of gas bubbles and liquid films in the upflow tubes. The inner and outer fluids attain steady temperatures in 5–10 min. from the beginning of circulation.

To set up an intestinal segment, T is closed, S_2 is lifted out of B, and the rubber connexion of the glass U-tube to C_1 is taken off, S_2 being held well above the level of G_1 to prevent back flow of inner fluid. Inner fluid in the glass U-tube and beyond is returned to R by flowing through the U-tube, which is then disconnected from C_2. The intestinal cannulae, which have internal tapers corresponding to the lower cones on C_1 and C_2, are then connected in place of the glass U-tube, being held on by short rubber tubes. The clamp T is opened to start fluid circulating through the intestine, and, after the intestine has been freed and washed, as already described, it is introduced into B and the stopper S_2 replaced. Samples of inner and outer fluids can be withdrawn at any time by means of pipettes introduced through S_1 and S_2.

Composition of circulation fluids. On all experiments, so far, the circulation fluid for both circuits has been basically a Krebs bicarbonate medium (containing 0·0012 M-phosphate), made up according to the directions given by Umbreit, Burris & Stauffer (1945, p. 194), which when equili-

brated with 5% CO_2 in O_2 gives a pH of approximately 7·4. As a general rule 500 mg. glucose/100 ml. has been added to this medium. The inner circuit fluid also regularly contains 1 ml. of 0·3% phenol red/50 ml. Krebs medium. Phenol red does not pass across the intestinal wall in detectable amount in our conditions, so that it provides a convenient way of detecting the minute pinholes which occur on occasion in the neighbourhood of the ligatures securing the intestine to the cannulae.

RESULTS

Histological appearances

Sections of segments of intestine, removed from the animal immediately after cannulating and washing with saline, have been compared with sections prepared from intestine which has survived on a circulation unit for an hour. Pl. 1 illustrates comparable sections of fresh and surviving upper jejunum fixed in Bouin solution and stained with haematoxylin and eosin. It will be noted that in the higher power illustrations there is evidence in all sections of disengagement of cells from the mucosa of the tips of the villi. No distinction in this respect has been found between fresh and surviving intestine. Leblond & Stevens (1948) have recently reported the same phenomenon in normal intestine, and give reasons for supposing that continual replacement of the mucosa is a normal process.

An attempt to assess the magnitude of this mucosal shedding by determining the nitrogen passing into the inner fluid in an hour period of survival gave the answer that there was no detectable addition of nitrogen in five experiments, whilst in a sixth 16 μg. N/cm. length of intestine appeared. This corresponds to 3% of the mucosal nitrogen (mean of eight determinations is 0·5 mg. mucosal N/cm. length intestine, measured on material removed from longitudinally split segments by scraping with a blunt spatula).

Apart from this finding of cellular disengagement, the mucosa appears histologically to be in good order, and there is no indication of the gross stripping usually reported in surviving preparations (Verzár & McDougall, 1936, p. 19).

When the intestinal segment is fixed *in situ* on the circulation unit, which may be done by replacing the greater part of the inner and outer fluids, in that order, by Bouin's solution, and continuing circulation for 30 min., the conformation of the mucosa is very different from that seen in a normal fixed preparation made by dropping the excised segment into fixative. Pl. 1*a* shows the 'normal' appearance, and Pl. 1*c*, *e* show the appearances of segments fixed at different distension pressures. The appearances of Pl. 1*c*, *e* are very like those illustrated by Johnson (1912–13) and they are much more like Johnson's illustration of the conformation of the guinea-pig intestinal mucosa fixed after tying off when the intestine was normally distended with food than is Pl. 1*c*.

One other histological feature which is marked in Pl. 1*c*, *e* is the distension of the submucous space in the surviving segments. Measurements of the water content of comparable segments of small intestine before and after an hour survival period indicate that, with 500 mg. glucose/100 ml. in inner and outer fluids, the water accumulation amounts to 0·5 g./g. initial weight. Although

this amount appears large, it corresponds to only 0·02–0·04 ml./cm. intestinal length.

Transfer of water across the intestinal wall

If water as well as solutes is transferred across the intestinal wall, then the extent of this transfer, as well as the change in concentration of the solute, must be known before estimates of rates of transfer of solute can be made. No substance has so far been found which can conveniently be used for estimating volume changes in the circuits. Phenol red, for example, which is transferred trivially if at all across the mucosa, nevertheless appreciably stains it. The current procedure is to drain both circuits very carefully at the end of the period of observation into 100 ml. graduated cylinders. Estimates of initial volumes are not essential, since, in the absence of a volume change indicator, all that can be observed is the total movement of solute from beginning to end of the period of observation, and the total amount of solute originally introduced into each circuit is known. Initial volumes can be obtained, where necessary, by taking initial samples from each circuit and dividing the concentration of solute in these samples into the amount of solute originally introduced. These volumes are regularly larger than the volumes originally placed in the circuits, because some of the washing saline is inevitably trapped in the lumen and entrained in mesenteric remnants on the serosal surface of the intestine.

Measurements of initial and final volumes made in these ways, with practically identical composition of inner and outer fluids, show that a significant transfer of water takes place (Table 1) from inner to outer circuit, the magnitude

TABLE 1. Movement of water from inner fluid of normal segments of intestine, surviving for 1 hr., with originally identical inner and outer fluids containing 500 mg. glucose/100 ml.

Segment location	Water leaving inner fluid (ml.)	Length of segment (cm.)	Shift per cm. intestine (ml.)
J	10·8	37	0·29
J	10·1	45	0·22
J	12·6	63	0·20
J	8·9	49	0·18
I	8·1	42	0·19
I	7·1	42	0·17
I	6·8	49	0·14
I	4·9	48	0·10

I = ileal, J = jejunal segment.

of the shift being 0·1–0·3 ml./cm. intestine/hr. Since the initial water content of 1 cm. of intestine is about 0·05 ml. the rate of transfer corresponds to the movement of 2–6 times the initial water content of the intestine per hour. It is not surprising, in these conditions, that there should be some water retention in the tissue. The magnitude of the retention (0·02–0·04 ml./cm.) is not such as to suggest that there is any serious barrier to diffusion through the submucosal tissues. On the contrary, it is clear that, in the presence of such a swift water

current across the submucosal region, rates of appearance of solutes in the outer fluid, when corrected for the known extent of water retention in the intestinal wall, may safely be taken as good measures of the rate of disengagement of such solutes from the peripheral border of the mucosa.

Transfer of glucose across the intestinal wall

The transfer of glucose may be used to illustrate this point. Table 2 presents the results of experiments in which changes in glucose content in inner and outer fluids have been measured, the initial concentrations in the two fluids being initially the same, in the region of 500 mg./100 ml. and the survival period being 1 hr.

TABLE 2. The discrepancy between rate of disappearance of glucose from inner fluid and rate of appearance of glucose in outer fluid

Segment location	Inner fluid: disappearance rate (mg./cm./hr.)	Outer fluid: appearance rate (mg./cm./hr.)	Discrepancy
J	2·89	1·05	1·84
J	2·73	1·21	1·52
J	2·20	0·50	1·70
J	2·00	0·63	1·37
I	2·20	1·60	0·60
I	2·06	1·14	0·92
I	1·71	1·11	0·60
I	1·68	1·33	0·35
		Mean	1·11

The discrepancies in the last column of Table 2 are all in the sense to indicate an appreciable loss of glucose in passage across the intestinal wall. Further experiments were made, therefore, in which intestinal segments were maintained in the same conditions on circulation units for the same time, and the intestine killed at the end of this period by rapid disengagement from the circulation unit and plunging into water at 100° C. The total glucose in the intestine plus circulating fluids was then determined. The upper part of Table 3 gives the differences, expressed as mg. glucose, between the amounts of glucose originally introduced and the amounts recovered. Unfortunately, these figures cannot be expressed exactly in mg./cm. intestine, since the intestine contracts on immersion in boiling water. But in view of the variability of the results in Table 2, no more than an order of magnitude is needed for comparison. A column has therefore been added to Table 3 giving estimated glucose disappearance per cm., on the basis that, in our conditions, the intestine contracts on average to 80% of its initial length.

The possibility that the losses of glucose observed in these last experiments are due to events occurring in the course of killing the intestine has been controlled by similar experiments in which the intestine is removed and killed within 5 min. of setting up on the circulation unit. The results, given in the

lower part of Table 3, indicate that there is no artefact due to glucose destruction during killing of the intestine.

The mean water content per cm. of perfused intestine is 0·08 ml./cm. The mean difference between the discrepancies of Table 2 and the 60 min. utilization given under Table 3 is 0·22 mg. glucose/cm. Thus, if the glucose retained in the intestinal wall is distributed throughout its total water, it must be present in a concentration of 22/0·08 mg./100 ml., i.e. approximately 280 mg./100 ml. Since the final concentrations in the two fluids in contact with the intestine are

TABLE 3. Disappearance of glucose from the whole system, intestine plus inner and outer fluids, after different periods of survival on circulation units

Segment location	Period of survival (min.)	Disappearance of glucose (mg.)	Estimated disappearance per cm. intestine
J	60	55·2	1·21
I	60	37·9	0·83
J	60	47·8	0·94
I	60	6·0	0·22
J	60	52·0	0·88
I	60	16·6	0·62
		Mean	+0·78
J	5	−1·8	−0·03
I	5	−7·1	−0·28
J	5	5·4	0·13
I	5	1·1	0·03
		Mean	−0·04

Algebraic difference of the two means (=0·82 mg./cm.) gives estimated utilization for 55 min. The corresponding figure for 1 hr. is 0·90 mg./cm.

usually both in excess of this figure, the observed degree of retention is consistent with the view that the retained glucose is present in some fraction of the intestinal water, for example, the extra-muscular water, in a concentration approximating to that of the outer fluid. There is certainly no suggestion of any serious barrier to diffusion of glucose across the submucosal tissues.

Effects of degree of distension

In view of the necessity to use some degree of distension to ensure flow through the intestine, and since some authors (e.g. Verzár & McDougall, 1936; Sols & Ponz, 1947) have described effects of distension on absorption from intestine *in situ*, some experiments have been made to examine the effects of different degrees of distension on active glucose absorption from surviving segments.

There is a gradient of activity along the intestine in rate of active glucose absorption from inner fluid, the evidence for which is presented in a subsequent paper (Fisher & Parsons, 1949), the gradient taking the simple form that the uptake of glucose per cm. intestine per hr. is linearly related to the mean distance of the segment (i.e. of the centre of the segment) from the ileo-caecal

valve. Utilizing this gradient, one can compute a 'standardized absorption rate', i.e. the absorption rate that would have been expected had the segment in question come from a standardized location in the small intestine. Table 4 presents the results of experiments in which two segments of intestine from each animal were set up, one at 35 cm. H_2O distension pressure and the other at 10 cm. H_2O, all observed rates being corrected to a standardized mean distance of 40 cm. from the ileo-caecal valve. These results indicate that change in degree of distension is without effect over this range. As, however, segments surviving at 10 cm. distension pressure regularly show peristaltic activity, which is vigorous in jejunal segments, whereas segments at 35 cm. distension pressure are always quiescent (cf. Magee & Southgate, 1929–30), and as the peristalsis may be vigorous enough on occasion to interrupt flow, with a consequent risk of anoxia, 35 cm. H_2O distension pressure is used as a routine.

TABLE 4. Effect of degree of distension on the rate of active absorption of glucose from surviving rat small intestine

Standardized absorption rate at 40 cm. from ileo-caecal valve (mg. glucose/cm. intestine/hr.)

Animal	10 cm. H_2O distension pressure	35 cm. H_2O distension pressure
159	1·67 (I)	1·30 (J)
161	1·50 (J)	1·91 (I)
166	1·56 (I)	1·61 (J)
167	1·79 (J)	1·74 (I)
Means	1·63	1·64

It is unlikely that the peristaltic activity is in any way related to the nutrition of the intestine since it is abolished immediately by raising the distension pressure and reappears when it is lowered.

Effects of anoxia

In early experiments with surviving intestine the mesentery was clamped and cut, and the freed segment washed briefly in saline before it was connected into the inner fluid circuit of the circulation unit. Although these steps were taken as rapidly as possible, there were 20–30 sec. during which the mucosa had little or no oxygen supply. Comparing these experiments with the later ones in which there was no interruption of oxygen supply, there are two obvious differences:

(1) With interrupted oxygen supply the intestine frequently showed vigorous motor activity at 35 cm. H_2O distension pressure, which persisted for all or most of the standard hour period of observation. With continuous oxygen supply motor activity is never seen in these circumstances.

(2) With interrupted oxygen supply the phenol red of the inner fluid regularly indicated a pH shift to the acid side, more marked in jejunal than ileal segments, the pH appearing to drop continuously throughout the period of observa-

tion. With 30–40 cm. jejunal segments (the small intestine of the rat being 110–130 cm. long, of which 15–20 cm. is duodenum) the pH shift might exceed 0·5 unit, the phenol red becoming quite yellow. Such a shift is in conformity with the aerobic glycolysis quotient for rat jejunum quoted by Dickens & Weil-Malherbe (1941), provided that the lactate remains in the inner fluid. No pH shift is observed in the outer fluid.

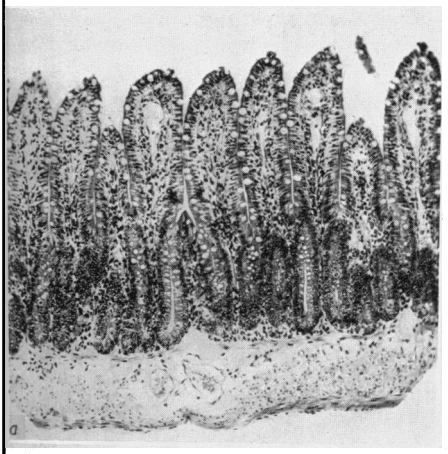
With continuous oxygen supply it is sometimes possible to detect a slight acid shift in the inner fluid, but no such shift as those regularly observed with interrupted oxygen supply has been seen in well over 100 segments of surviving intestine.

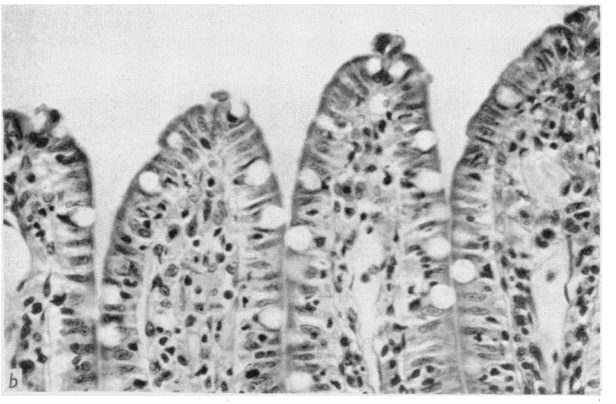
Both these observations show that brief anoxia can produce disturbances which far outlast the period of anoxia, and emphasize the importance of our precautions to avoid the briefest anoxia.

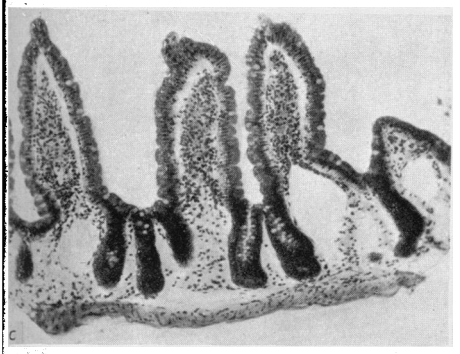
DISCUSSION

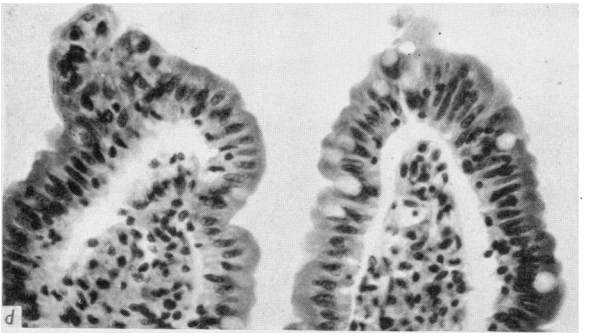
The one indication of mucosal disintegration observed in the preparation described in this paper parallels a process described in normal intestine by Leblond & Stevens (1948), who describe disengagement of fragments of mucosa at the tips of villi, the remaining mucosa sealing up behind these fragments. They link this process with the constant presence of mitoses in the crypts of Lieberkühn, a phenomenon which has been seen in all our preparations, and they have recently shown, by a radioautograph technique (Leblond, Stevens & Bogoroch, 1948) that there is good reason to believe that the view that mucosa is constantly being replaced at a high rate is correct.

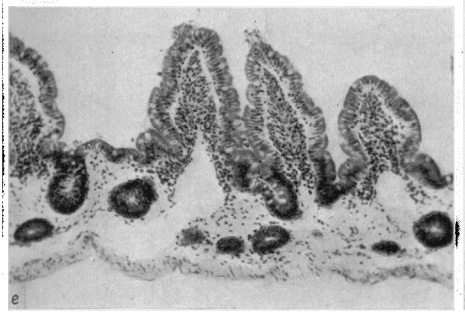
The ability of the intestine to remove glucose from the lumen when the initial concentrations of glucose on the two sides of the intestinal wall are equal, and the active translocation of water, are evidence that the cells of the mucosa of the preparation are capable of active work, but a consideration of the scale of activity is best deferred to a later paper (Fisher & Parsons, 1949). One other piece of evidence for the metabolic activity of the preparation may be cited here. The figures for glucose utilization in Table 3 correspond to Q_{O_2} values ranging from 53 to 10, if it is assumed that the glucose is completely oxidized, an assumption which appears probable since no appreciable accumulation of acid products occurs. These figures may be compared with those of Dickens & Weil-Malherbe (1941) for isolated intestinal mucosa. The activity of their preparations steadily diminished with time, the highest Q_{O_2}'s observed being 31 for jejunum and 11 for ileum. The present preparation thus shows a distinctly higher metabolic rate than the maximal rates in Dickens & Weil-Malherbe's experiments. It is also of interest that these authors observed marked aerobic glycolysis in rat small intestine, which may be linked with the present observation of a marked acid change in surviving intestine exposed to a brief anoxia. It seems unlikely that the metabolic pattern disclosed by tissue respiration

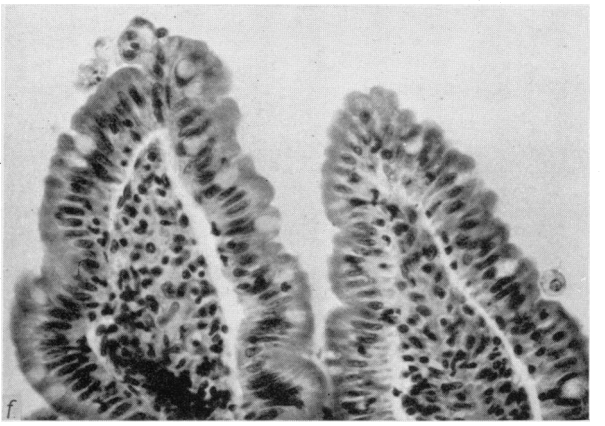












a. Normal jejunum, fixed in Bouin's solution. Haematoxylin and eosin. × 80.
b. High-power view of *a*. × 272.
c. Surviving jejunum, fixed in Bouin's solution at 10 cm. H_2O distension pressure. Haematoxylin and eosin. × 80
d. High-power view of *c*. × 272.
e. Surviving jejunum, fixed in Bouin's solution at 35 cm. H_2O distension pressure. Haematoxylin and eosin. × 80.
f. High-power view of *e*. × 272.

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experiments in which the tissue has been exposed to anoxia to the extent which is usual in such work can safely be interpreted as normal, at least in so far as the small intestine is concerned.

Despite the artificiality inherent in its divorce from the remaining tissues of the body, the surviving intestine has a number of advantages over other preparations used to study intestinal absorption. The Cori (1925) technique has the major disadvantage that the concentration in the intestine and the portion of the intestine effecting the absorption are unknown, and are open to modification by secondary factors such as gastric and intestinal motility (e.g. see Fenton, 1945). The Verzár (1936, p. 18) technique of injecting a solution into a tied-off segment *in situ* has the two disadvantages: (*a*) that maintained anaesthesia is necessary, and (*b*) that, with a readily absorbed substance, the change in concentration in the small amount of fluid that can be introduced is so large that specification of a concentration at which absorption takes place is artificial. The most recent technique, that of Sols & Ponz (1947), in which a solution of the solute to be studied is allowed to flow through a cannulated segment, suffers from the disadvantage that anaesthesia is required. All these techniques have, in addition, the disadvantage that they permit the study of one aspect only of the process of absorption, disappearance of solute from the intestinal lumen. It has already been indicated that a considerable amount of glucose taken up from the lumen may be utilized in the intestinal wall, and it is worth making a rough calculation on the basis of the data of Table 3. Taking the average rate in this table of 0·90 mg. glucose/cm./hr. as applicable to the whole 100 cm. of intestine of a 200 g. rat, the utilization amounts to 0·45 g. glucose/kg. body wt./hr. when the whole small intestine is exposed to 0·5% glucose. This is an appreciable fraction of the usually quoted figure for absorption rate, determined by the Cori technique, of 1 g./kg./hr., and raises important questions concerning the general significance of reported absorption rates.

The work reported in this paper indicates that the new technique described should be capable of analysing movement of solute from the lumen into its components of utilization and translocation. It possesses another advantage over the older methods in that it makes it possible to study the form in which the absorbed material passes from the mucosa into the underlying tissue fluids.

SUMMARY

1. Apparatus and procedure are described for instituting circulation of oxygenated fluids through and around isolated segments of rat small intestine without subjecting the tissue to anoxia during the procedure.

2. The histology of the mucosal cells of the preparation is shown to be essentially normal after survival for an hour.

3. Evidence is provided that the preparation contains actively metabolizing cells, capable of active translocation of solutes across the intestinal wall, and

that there is no appreciable barrier to diffusion across the submucosal tissues of the intestinal wall.

4. The preparation extends the possible range of studies of intestinal absorption, since it renders accessible to study the form and amount in which solutes leave the peripheral border of the intestinal mucosa.

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