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THE INTESTINAL ABSORPTION OF GLUCOSE

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It is generally agreed that glucose is absorbed from the mammalian intestine by an active process, but the details of this are still not known with any certainty. It has not even been definitely established in what form the actively transferred glucose enters the mesenteric blood. A number of authors have shown that there is a rise in the glucose concentration of the venous mesenteric blood during glucose absorption, but data are not available to show how much of the absorbed glucose could be accounted for in the mesenteric blood. Furthermore, in order to show a glucose concentration in portal blood increased above that in arterial blood, very high concentrations of glucose must be present in the intestinal lumen. Thus Magee & Reid (1931) obtained a concentration of glucose in the blood in the superior mesenteric vein 10–20 mg/100 ml. higher than in the arterial blood when a solution of 13·5% glucose was present in the intestine. With a concentration in the intestine 100 times that in the blood an enormous gradient for diffusion existed, and from such an experiment no conclusions could be drawn as to whether the glucose appearing in the mesenteric blood was actively or passively transported. Recently, Hestrin-Lerner & Shapiro (1953, 1954) claimed that glucose was absorbed in a form which was neither glucose nor lactic acid, but this claim could not be substantiated in experiments *in vitro* by Newey, Smyth & Whaler (1955). The present work deals with a quantitative investigation of the fate of glucose absorbed from the intestine *in vivo*, and with an attempt to determine in what form the absorbed glucose appears in the mesenteric blood.

In preliminary experiments a solution to the problem was sought by chemical estimation of the glucose and lactic acid in the intestinal lumen and in the blood. The glucose disappearing from the intestine was measured, and at the same time the change in the glucose and lactic acid contents of the blood passing through the intestine was determined. These attempts proved unsuccessful because of the fluctuations not only of glucose but also of lactic acid in the arterial blood during the experiment. Other difficulties were the relatively large amounts of glucose brought to the intestine in the arterial

mesenteric blood compared with the amounts absorbed from the intestine, and the occurrence of glycolysis in the intestinal mucosa even when no glucose was being absorbed.

To avoid these difficulties, a study was undertaken of the fate of radioactive glucose introduced into the lumen of the intestine. The glucose was uniformly labelled with ^{14}C and the ^{14}C was traced in the absorption products. A preliminary account of the work has been given by Parsons & Smyth (1956), and since the work was completed an account of some similar experiments, with results consistent with our own, has been given by Kiyasu, Katz & Chaikoff (1956).

METHODS

Operative procedure

The principle of the method used was that of Matthews & Smyth (1954) in which the blood draining a loop of intestine is collected, so that none of the products of absorption entering the blood stream are returned to the animal. In this method, the blood pressure was maintained at a low level in order to reduce the rate of intestinal blood flow. In the present experiments it was particularly desired to avoid anoxia of the intestine, as this might easily interfere with carbohydrate metabolism, and the technique of Matthews & Smyth (1954) was therefore modified in a number of ways. The details of the procedure were as follows. Male and female dogs weighing from 10 to 20 kg were anaesthetized with intravenous pentobarbitone sodium. The abdomen was opened and a loop of intestine about 20 cm long was selected. The vein draining the loop was prepared for cannulation, and the animal was heparinized. The vein was now cannulated, but during the cannulation the loop was not tied off from the rest of the intestine, in order to maintain as much circulation as possible through the loop by means of anastomoses from adjacent parts of the intestine. With this same object of maintaining a good circulation, the blood pressure was not reduced at any stage during the experiment, and a blood flow of 10–20 ml./min was maintained throughout. The loop was then washed out with warm saline and tied off from the rest of the intestine.

The experimental arrangement is shown in Fig. 1. The cannula draining the vein from the loop was attached to a polythene tube, and this tube was brought out through a small hole in the abdominal wall. Immediately outside the abdomen it formed a coil which was immersed in ice-cold water. It then passed through the small dropping chamber (for observing the rate of flow), and from this it passed to the bottom of a measuring cylinder containing liquid paraffin: the cylinder was also immersed in ice-cold water. In this way glycolysis was inhibited and loss of carbon dioxide from the venous mesenteric blood was prevented. In some experiments sodium fluoride was added to the blood being collected, as an additional precaution to prevent glycolysis. When the collection of venous blood from the intestine was satisfactorily established, a solution of glucose was injected into the lumen of the selected loop. The loop of intestine was now replaced in the abdomen, and its position adjusted so as to permit uninterrupted blood flow; this was facilitated by maintenance of slight tension on the venous cannula. The dropping chamber referred to above was considered to be of great importance in permitting continuous observation of the venous flow. In its absence unobserved stoppages of flow due to movement of the venous cannula with the respiratory movements might lead to anoxia, which could increase lactic acid production.

After the injection of the glucose into the intestinal lumen the venous blood was collected for a period varying from 15 to 60 min. At the end of this period the contents of the loop were washed out and collected. In a few experiments a trichloroacetic acid extract of the intestinal loop was made. The residual fluid from the loop, the mesenteric venous blood draining the loop, and in some cases the extract of the loop, were then examined for various constituents and for radioactivity.

Chemical investigation

Samples of the mesenteric venous blood, loop contents and loop extract, were taken for the determination of glucose and lactic acid, and in some experiments of pyruvic acid, alanine and carbon dioxide. True glucose was estimated by the method of Shaffer & Somogyi (1933) coupled with an adaptation of the microfermentation method of Reinecke (1943), lactic acid by the method of Elsden & Gibson (1954), pyruvic acid colorimetrically as described by Umbreit, Burris & Stauffer (1945), alanine by the method of Alexander & Seligman (1945) as modified by Christensen, Riggs & Ray (1952), and carbon dioxide by the Haldane method.

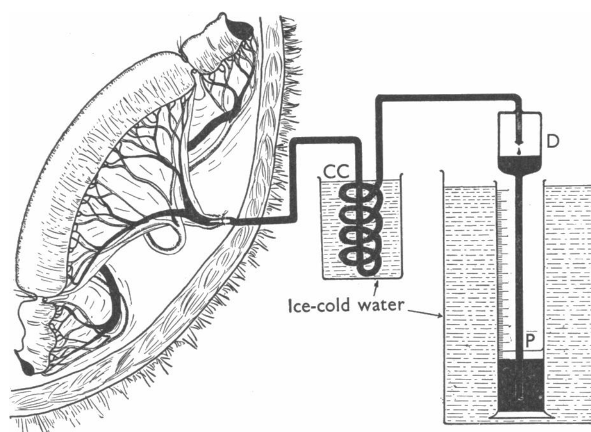

Fig. 1. Arrangement used for collection of venous blood from a loop of intestine. The loop of intestine has an intact arterial blood supply, but the venous blood leaving the loop passes through a cooling coil CC, a dropping chamber D, and is collected in a cylinder under a layer of liquid paraffin, P.

Estimation of specific activity

The samples investigated were treated by various procedures outlined below so that the ^{14}C was incorporated either into barium carbonate or into a 2:4-dinitrophenylhydrazone. These substances were plated and counted, and after the usual corrections the relative specific activity expressed as counts/min at infinite thickness. The term relative specific activity (R.S.A.) is used because counting at infinite thickness gives a value which is proportional to the specific radioactivity, or concentration of ^{14}C/unit wt. of the material counted, and not to the total amount of ^{14}C in the sample counted. The hydrazone was formed by addition of a saturated solution of 2:4-dinitrophenylhydrazine in 2N-HCl, after preliminary stages had led to the production of an aldehyde or ketone. The details of the procedures with different substances were as follows:

Carbon dioxide. A small sample of blood (10–20 ml.) was acidified with 20% tartaric acid *in vacuo*. The carbon dioxide was collected in baryta water to form barium carbonate.

Pyruvic acid. To a small sample (about 20 ml.) of blood 50 mg sodium pyruvate was added as carrier. The blood was then deproteinized by the barium hydroxide–zinc sulphate method of Nelson (1944). The filtrate was concentrated *in vacuo* to 10–20 ml. After acidification and centrifuging the pyruvate was precipitated from the supernatant by addition of 2:4-dinitrophenylhydrazine, to form the corresponding hydrazone.

Glucose, alanine and lactic acid. After samples had been taken for the chemical estimations and for the determination of ^{14}C in carbon dioxide and pyruvic acid, the residue (about 200–250 ml.) of the collected blood was used for determination of ^{14}C in glucose, lactic acid and alanine. Lithium lactate and DL-alanine were added as carriers. The blood was then deproteinized with

barium hydroxide and zinc sulphate. The filtrate was passed through a cation exchange resin column (Zeo-Karb 215) in the hydrogen form. The alanine was retained on the column, and the glucose and lactic acid were present in the effluent. The alanine was eluted from the column with 0·16 N ammonia. This was concentrated *in vacuo*, adjusted to pH 5·5 with phosphate buffer, oxidized to acetaldehyde with ninhydrin under the conditions described by Alexander & Seligman (1945), and the acetaldehyde precipitated with 2:4-dinitrophenylhydrazine to form the corresponding hydrazone.

The solution containing glucose and lactic acid was neutralized and concentrated *in vacuo* at 40° C to a volume of 50 ml. This was then acidified and extracted with ether in a Kutscher–Steudel extractor for 8 hr. The ether-soluble fraction (containing lactic acid) was evaporated to dryness and dissolved in 50 ml. water. It was then treated with copper lime which removed traces of glucose, together with a certain amount of other organic material. The filtrate was then oxidized with ceric sulphate in the apparatus of Elsden & Gibson (1954) for determination of lactic acid. Instead of collecting the acetaldehyde in metabisulphite, it was collected in 2:4-dinitrophenylhydrazine, thus forming the hydrazone.

The solution remaining after ether extraction (containing glucose) was treated in one or other of two ways. In the first the principle of Drury, Wick & MacKay (1950) was used. The solution was adjusted to pH 5·5 with phosphate buffer and fermented with washed baker's yeast at 37° C under anaerobic conditions; the carbon dioxide evolved was absorbed in baryta water to form barium carbonate. In the second method the solution was de-ionized by passage through Zeo-Karb 215 and Amberlite IR. 4B (OH). The effluent was concentrated to about 3 ml. and impurities were removed by passing first through a cellulose column, using butanol–acetic acid or propanol–water as solvents. The glucose-containing fraction was then passed through a celite–charcoal column to remove polysaccharides. The effluent was combusted by the technique described by Newey *et al.* (1955) to form CO_2, which was converted to $BaCO_3$.

Calculation of the amount of radioactive material in the blood. If the radioactivity of a substance in the intestinal lumen is A counts/min, the concentration in the blood B mg per 100 ml., the total volume of the blood C ml., and the radioactivity of the substance in the blood D counts/min, then the absorbed labelled substance (or compound derived from it) in the mesenteric blood has been diluted by similar but unlabelled substance in the ratio A/D. The total amount of absorbed substance (or derivative) in the mesenteric blood is therefore $CB/100 \times D/A$ mg. This calculation is quite straight-forward in the case of glucose and carbon dioxide as both the radioactive glucose absorbed and the glucose and carbon dioxide recovered from the blood are counted as barium carbonate. In the cases of lactic acid, alanine and pyruvate, there is a complicating factor. The radioactivity of these substances is measured as a phenylhydrazone, and cannot be directly compared with the radioactivity of the glucose absorbed, measured as barium carbonate. In order to compare directly the radioactivities of lactic acid and glucose, the radioactivity of the hydrazone obtained from lactic acid must be multiplied by a factor of 0·57 to allow for the different proportions of radioactive carbon in the barium carbonate and in the acetaldehyde phenylhydrazone molecules. The same factor is used for alanine. In the case of pyruvate the factor will be 0·45. No further correction will be required provided the self-absorption curves of barium carbonate and the phenylhydrazones are not widely different, and in fact the determination of the self-absorption curves of $BaCO_3$ and the acetaldehyde phenylhydrazone indicated that they were nearly the same.

In order to provide a further check on the comparison of the radioactivities of barium carbonate and acetaldehyde 2:4-dinitrophenylhydrazone, the following experiment was also carried out. Some intestinal mucosa was incubated in Krebs's bicarbonate saline for 30 min to remove any glucose present. The suspension medium was then discarded, the mucosa washed in saline and then resuspended in bicarbonate saline containing radioactive glucose. In this way, the formation of lactic acid from non-radioactive glucose was eliminated. After one hour's incubation of the radioactive glucose with the washed mucosa, the final solution was run as a strip chromatogram using butanol–acetic acid as solvent. The lactic acid spot was

identified, eluted and the lactic acid estimated. Non-radioactive lactate was added as carrier. A fraction of the lactic acid was now combusted according to the method of Newey *et al.* (1955) and another fraction was converted to the hydrazone as described above. The ratio of the radioactivity of the barium carbonate to that of the phenylhydrazone was 0·63. This compares with the theoretical value of 0·57 for the ratio of the proportion of radioactive carbon in the barium carbonate and in the hydrazone molecules. It is thus evident that the procedure of comparing the radioactivities of barium carbonate and the acetaldehyde hydrazone is justified, and if we use a factor of 0·6 the error will be small.

RESULTS

The results of the experiments can be expressed as the percentage of ^{14}C in the glucose disappearing from the intestine which can be accounted for in radioactive compounds in the venous mesenteric blood. The details of one typical experiment were as follows.

A male dog was used, weighing 17·5 kg. The intestinal loop, made from the jejunum, was 19 cm long, and after the completion of the experiment the wet weight of the loop was 21·04 g. Glucose labelled uniformly with ^{14}C was injected into the intestinal lumen, and the venous blood collected from the loop for 35 min. The total volume collected was 336 ml., and hence the rate of flow was 9·6 ml./min, or 45·2 ml./100 g/min. Lawson & Ambrose (1942) gave figures for intestinal blood flow in dogs varying from 26 to 97 ml./100 g/min. In a series of thirteen experiments, six animals had a flow of less than 45 ml./100 g/min, so that our figure would appear to fall within the normal range, and is therefore regarded as providing an adequate rate of oxygen supply to the intestinal loop. At the end of the experiment the intestinal contents and washings were collected. The venous blood and final intestinal contents were analysed as described in the previous section. The results are shown in Table 1. This table shows that 35 mg of glucose was absorbed during the experimental period, and also shows the analysis of the venous blood. It is evident that about 90 % of the radioactive glucose-C disappearing from the lumen of the intestine can be accounted for in the venous blood. About 70 % of it is accounted for in glucose, 13 % in lactic acid and very small amounts in carbon dioxide, pyruvic acid and alanine. The radioactivity of pyruvic acid is scarcely inside the range of experimental error.

A number of experiments of this kind were done, and Table 2 gives a summary of the results. Only in a few of these have alanine, pyruvic acid and carbon dioxide been studied. It is seen that the experiments in Table 2 support the one experiment described in detail, and in general it can be concluded that 80–90 % of the glucose-C disappearing from the intestine can be accounted for in glucose and lactic acid, the lactic acid fraction varying from 7 to 17 % of the glucose-C disappearing from the intestine.

In some of the earlier experiments, not included in Table 2, the condition of the intestine was not considered satisfactory, either because of small blood flow, congested appearance of the mucosa at the end of the experiment or for

some other reason. In these experiments it was found that the percentage of absorbed glucose-C accounted for in lactic acid was increased, and values of 30, 40, 36 and 35 were obtained. These values are significantly higher than those obtained in the experiments included in Table 2, when the experimental conditions were considered satisfactory.

TABLE 1. Analysis of venous blood collected from the intestinal loop during absorption of ^{14}C-labelled glucose

Vol. of blood collected = 336 ml. ^{14}C-glucose absorbed = 35 mg* (i.e. 14 mg labelled carbon) of relative specific activity (R.S.A.) 4600 counts/min.†

	Glucose	Lactic acid	Carbon dioxide	Pyruvic acid	Alanine
Concentration (mg/100 ml.)					
in venous blood	89	27	125	5	9
after addition of carrier	89	71	125	265	57
R.S.A. as hydrazone (counts/min)	—	142	—	6	22
R.S.A. as $BaCO_3$ (counts/min)	377	(85)	17	(3)	(13)
Amount of labelled compound in 336 ml. blood	24·5	4·4	1·5	0·6	0·5
C in compound (%)	40	40	27	41	41
Amount of absorbed glucose-C in this form (mg)	9·8	1·8	0·4	0·2	0·2
Absorbed glucose-C accounted for (%)	70	13	3	2	2

The radioactivity of glucose and carbon dioxide was determined as $BaCO_3$. The radioactivity of lactic acid, pyruvic acid and alanine was determined as the hydrazone, and converted to the corresponding values for $BaCO_3$ by multiplying by factors as described in the text.

* Of 36 mg ^{14}C-labelled glucose injected into the intestinal loop, 1 mg glucose, with an R.S.A. of 4200 counts/min, remained at the end of the experiment.

† All radioactivity measurements were made by the 'infinite thickness' counting technique, and the actual counts observed therefore express the R.S.A. of the materials counted.

TABLE 2. The recovery and distribution of absorbed ^{14}C in mesenteric venous blood collected from intestinal loop after introduction of ^{14}C-glucose

				Percentage of absorbed ^{14}C recovered in					
Expt. no.	Initial concn. of glucose in intestine (mg/100 ml.)	Absorption period (min)	^{14}C-glucose absorbed (mg)	Glucose	Lactic acid	Alanine	Pyruvic acid	CO_2	Total recovery of absorbed ^{14}C (%)
1	390	60	39	75	13	—	—	—	88
2	350	35	35	75	9	—	—	—	84
3	390	51	37	65	7	—	—	—	72
4	360	40	36	69	11	—	—	—	80
5	260	30	24	68	11	—	—	—	79
6	173	30	25	80	13	—	—	—	93
7	350	30	35	66	17	3	1	3	90
8*	376	35	36	70	13	2	2	3	90

* This experiment reported in detail in Table 1.

In a few experiments the residual fluid in the intestinal lumen was examined for lactic acid, pyruvic acid and carbon dioxide. In all cases the radioactivity of these substances was negligible. Extracts of the intestine in a few experiments showed the presence of ^{14}C-labelled glucose, lactic acid and traces of alanine. The amounts were small in all cases and the investigation of the intestinal extracts was not followed further.

We have not included in Table 2 figures for the concentration of glucose in the blood. In most cases the glucose concentration in the blood was around 100 mg/100 ml. so that at the beginning of the experiment there was a small gradient for diffusion. As absorption proceeded almost to completion (usually less than 1 mg of glucose remained in a volume of 1–3 ml. fluid) the glucose concentration in the intestine in the latter part of the experiment must have been lower than that of the blood, and absorption must have occurred against a concentration gradient. In one experiment (no. 6, Table 2) the blood glucose concentration was artificially raised above that in the intestine (venous blood concentration = 254 mg/100 ml.) and in this case absorption of glucose presumably took place against a concentration gradient throughout the whole experiment. There is thus reason to believe that our results on the fate of glucose apply to the glucose which is actively absorbed.

DISCUSSION

The results show that up to 90% of the glucose disappearing from the lumen of the intestine can be accounted for in the mesenteric venous blood as either glucose or lactic acid. They do not therefore agree with the claim of Hestrin-Lerner & Shapiro (1953, 1954) that glucose is absorbed in the form of some unknown substance. In the case of the experiments *in vitro*, Wilson & Wiseman (1954) suggested that the unknown substance might be lactic acid, and this suggestion was supported by Newey *et al.* (1955). It seems likely that this same suggestion is applicable to the *in vivo* results of Hestrin-Lerner & Shapiro (1954), as our results showed that if the experimental conditions were not good, nearly half of the glucose disappearing from the lumen was accounted for as lactic acid. It is of considerable interest that our results on the disappearance of glucose from the intestine of the dog agree so closely with those of Kiyasu *et al.* (1956) carried out on rats, and with very different methods of studying the fate of the absorbed glucose.

A comparison of the present results with the *in vitro* results of Newey *et al.* (1955) shows that *in vivo* a greater fraction of the absorbed glucose is accounted for as glucose, and less accounted for as lactic acid and CO_2. This does not necessarily imply that the intestine behaves *in vitro* differently from *in vivo*. Under conditions *in vitro* the only supply of glucose to the mucosa is that in the lumen of the intestine, and this must serve as substrate for both the oxidative and glycolytic mechanisms. Under conditions *in vivo* a rich supply of glucose is made available through the arterial blood for both oxidation and glycolysis, and it is not therefore surprising that much of the carbon dioxide and lactic acid produced by the intestine came from this source of glucose, and not from the glucose in the lumen of the intestine.

The results answer fairly definitely the question raised by Wilson & Wiseman (1954) as to whether some glucose is absorbed as lactic acid. It seems likely

that it is, but only to the extent of accounting for 10–15% of the glucose absorbed.

Barany & Sperber (1939) first showed that glucose disappeared from the intestine even when in a concentration below that in the blood, and this is always regarded as one of the main pieces of evidence in favour of active absorption of glucose. Our experiments go further in showing that the glucose which disappears from the intestine against a concentration gradient does in fact appear in the mesenteric blood.

A matter of some interest is the fact that the specific activity of the glucose remaining in the intestine at the end of the experiment is almost the same as that at the beginning. This shows that the movement of glucose is in one direction only, and is not the net result of a two-way movement with increased rate in one direction.

SUMMARY

1. Techniques are described for studying the fate of ^{14}C-labelled glucose absorbed from a loop of dog's intestine.

2. It was found that 70–80% of the glucose disappearing from the intestine is accounted for as glucose in the mesenteric venous blood, 7–17% as lactic acid, and insignificant amounts as carbon dioxide, alanine and pyruvic acid.

3. The results are discussed in relation to theories of glucose absorption and compared with those obtained with intestinal preparations *in vitro*.

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REFERENCES

ALEXANDER, B. & SELIGMAN, A. M. (1945). A colorimeter method for the microdetermination of α-alanine in blood. *J. biol. Chem.* **159**, 9–19.

BARANY, E. & SPERBER, E. (1939). Absorption of glucose against a concentration gradient by the small intestine of the rabbit. *Skand. Arch. Physiol.* **81**, 290–299.

CHRISTENSEN, H. N., RIGGS, T. R. & RAY, N. E. (1952). Concentrative uptake of amino-acids by erythrocytes *in vitro*. *J. biol. Chem.* **194**, 41–51.

DRURY, D. R., WICK, A. N. & MACKAY, E. M. (1950). Formation of glucose by the kidney. *Amer. J. Physiol.* **163**, 655–661.

ELSDEN, S. R. & GIBSON, Q. H. (1954). The estimation of lactic acid using ceric sulphate. *Biochem. J.* **58**, 154–158.

HESTRIN-LERNER, S. & SHAPIRO, B. (1953). Absorption of glucose from the intestine. I. *In vitro* studies. *Biochim. biophys. acta*, **12**, 533–541.

HESTRIN-LERNER, S. & SHAPIRO, B. (1954). Absorption of glucose from the intestine. II. *In vivo* and perfusion studies. *Biochim. biophys. acta*, **13**, 54–60.

KIYASU, J. Y., KATZ, J. & CHAIKOFF, I. L. (1956). Nature of C^{14} compounds recovered in portal plasma after enteral administration of C^{14} labelled glucose. *Biochim. biophys. acta*, **21**, 286–290.

LAWSON, H. & AMBROSE, A. M. (1942). The utilisation of blood oxygen by the distended intestine. *Amer. J. Physiol.* **135**, 650–659.

MAGEE, H. E. & REID, E. (1931). The absorption of glucose from the alimentary canal. *J. Physiol.* **73**, 163–183.

MATTHEWS, D. M. & SMYTH, D. H. (1954). The intestinal absorption of amino-acid enantiomorphs. *J. Physiol.* **126**, 96–100.

NELSON, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *J. biol. Chem.* **153**, 375–380.

NEWEY, H., SMYTH, D. H. & WHALER, B. C. (1955). The absorption of glucose by the *in vitro* intestinal preparation. *J. Physiol.* **129**, 1–11.

PARSONS, B. J. & SMYTH, D. H. (1956). The intestinal absorption of radioactive glucose. *J. Physiol.* **134**, 7–8*P*.

REINECKE, R. M. (1943). The kidney as a source of glucose in the eviscerated rat. *Amer. J. Physiol.* **140**, 276–285.

SHAFFER, P. A. & SOMOGYI, M. (1933). Copper-iodometric reagents for sugar determinations. *J. biol. Chem.* **100**, 695–713.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1945). *Manometric Techniques and Related Methods for the Study of Tissue Metabolism.* Minneapolis: Burgess.

WILSON, T. H. & WISEMAN, G. (1954). The use of sacs of everted small intestine for the study of transference of substances from the mucosal to the serosal surface. *J. Physiol.* **123**, 116–125.