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An apparatus for the study of intestinal transfer of drugs

SIR,—An *in vitro* method for the study of intestinal transfer suitable for use with radioactive labelled compounds is described. An outline of the apparatus is illustrated in Fig. 1. A segment of small intestine is tied to a cannula at each end and suspended vertically in an organ bath with its oral end upwards so that any peristaltic contractions propel fluid in the direction of the circulation. Fluid to be perfused through the intestinal lumen (mucosal fluid) is added to the reservoir and its circulation is started by raising the reservoir initially to allow filling of the connecting tubings, and is maintained by a continuous stream of 5% carbon dioxide in oxygen. This gas mixture also serves to keep the pH constant at 7·4 and supply the oxygen requirements of the mucosal layer of the intestine. The serosal aspect of the intestine is bathed in fluid (serosal fluid) of composition similar to the mucosal fluid except that it contains the substance being studied. The serosal fluid is aerated with the same gas mixture introduced through a thin polythene tubing. The apparatus as illustrated is immersed in a thermostatic tank at 38°.

To mount the intestine, the lower cannula, consisting of a silicone tubing held by a rubber bung, is lightly coated with silicone fluid to facilitate its sliding within the lumen of the bung. The anal end of the intestine is then tied to the silicone tubing and the oral end of the intestine "threaded" into the organ bath. The silicone tubing is pushed into the organ bath until the oral end of the intestine protrudes from the bath and can be tied to the glass cannula. The silicone tubing is then retracted sufficiently to allow the preparation to be totally immersed in fluid. The reservoir can be raised or lowered to provide the desired intraluminal pressure as measured by the difference in level of the mucosal and serosal fluids (see Fig. 1). An intraluminal pressure of 2 cm or more will initiate peristaltic contractions (with guinea-pig small intestine). If peristaltic contractions are not required, the intraluminal pressure should be just high enough to allow a constant circulation of mucosal fluid to be maintained. The rate of the circulation can be adjusted by regulating the flow of the gas mixture and circulation time can be varied from 20 to 60 sec. A thin smear of silicone antifoam prevents frothing at the serosal and mucosal fluid surfaces in the organ bath and reservoir respectively.

This simple method permits the economical use of labelled compounds and also provides for efficient oxygenation which is vital in *in vitro* studies. We

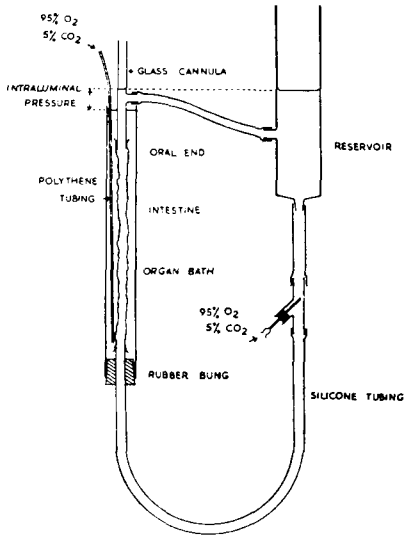

FIG. 1. Apparatus for the study of intestinal transfer by isolated pieces of intestine.

have used this apparatus to study the transfer of ^{14}C-labelled glucose by guinea-pig small intestine. Both the mucosal and serosal fluids have the following composition: (in g/litre) NaCl, 6·92; KCl, 0·35; $CaCl_2$, 0·28; $MgSO_4$, 0·14; KH_2PO_4, 0·16; $NaHCO_3$, 2·0. Labelled glucose containing 1 μc ^{14}C activity and 40 mg glucose were added to 20 ml of mucosal fluid. Glucose 0·01 g/litre was added to the serosal fluid. Glucose was estimated by the photometric method as described by Nelson (1944). ^{14}C activity was assayed in a liquid scintillation counter using a xylene scintillation mixture (Lambie, 1964). Serosal samples of 0·25 ml were used for the chemical and radioactive assay. The results from 6 experiments showed glucose appeared in the serosal fluid at 450 $\pm$39 μg/cm intestine/hr and ^{14}C activity at 15·4 $\pm$2·1 nc/cm intestine/hr. When the total amounts appearing in the serosal fluid are expressed as a percentage of the initial amounts in the mucosal fluid the values are 10·2 $\pm$0·9% for glucose and 11·1 $\pm$1·3% for ^{14}C activity. Application of the *t* test indicates the difference to be not significant. Isolation of the ^{14}C activity in the serosal fluid by chromatographic and autoradiographic techniques indicates that the activity is due to labelled glucose. Since labelled glucose can only have been transferred from the lumen of the intestine it is likely that glucose chemically assayed has the same origin.

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