AUTORADIOGRAPHIC STUDY OF SUGAR AND AMINO ACID ABSORPTION BY EVERTED SACS OF HAMSTER INTESTINE

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ABSTRACT

Autoradiographs were prepared from frozen sections of everted sacs of hamster jejunum which had been incubated *in vitro* with C^{14}- or H^3-labeled sugars and amino acids. When such tissue was incubated in 1 mM solutions of L-valine or L-methionine, columnar absorptive cells at tips of villi accumulated these amino acids to concentrations ranging from 5 to 50 millimoles per liter of cells. Quantitative data were obtained by microdensitometry of C^{14} autoradiographs. Similar, though less striking, results were obtained with the sugars: galactose, 3-0-methylglucose, α-methylglucoside, and 6-deoxyglucose. In all cases the marked "step-up" in concentration occurred near the brush border of the cell, and a "step-down" in concentration occurred at the basal pole of the cell. Known inhibitors of intestinal absorption, *e.g.*, phlorizin in the case of sugars, blocked the concentrative step at the luminal border of the absorptive cell. It is inferred from these data that active transport systems for sugars and amino acids reside in the brush border region of the cell. Additional evidence suggests that the basal membrane of the cell may be the site of both a diffusion barrier and a weak transport system directed into the cell.

INTRODUCTION

Columnar absorptive cells lining the small intestine have the capacity to absorb sugars and amino acids from the lumen of the intestine against a concentration gradient. In traversing the epithelial cell these substances must cross at least two cell membranes, *i.e.* enter at the brush border and exit at the base of the cell. It is of interest to inquire whether one or the other of these membranes is primarily responsible for the energy-requiring step in the transport process.

It has been known for a number of years that when intestinal segments are incubated *in vitro* in the presence of sugars or amino acids, the tissue accumulates these substances to concentrations higher than those in the incubation medium (1, 2). Although the exact locus of accumulation within the tissue was not directly determined in these early studies, subsequent work (13) provided indirect evidence for accumulation within the columnar epithelial cell. It was postulated (1, 13) that intestinal absorption of these compounds results from uphill transport into the cell at the brush border followed by diffusion through the cell and exit across the basal membrane. More direct evidence for this hypothesis was obtained from the microdissection experiments of Mc-

Dougal *et al.* (12) and from the preliminary autoradiographs prepared by Kinter (6).

The present work extends the autoradiographic approach to localization of absorption mechanisms for sugars and amino acids in everted sacs of hamster jejunum. Both carbon-14 and preliminary hydrogen-3 autoradiographs are presented along with certain quantitative measurements from the former. These results substantiate the concept of a separate brush border entrance pump and basal membrane exit barrier. For each class of compounds the entrance pump is not only a site of uphill transport, but also a site of inhibition, *e.g.*, by phlorizin in the case of sugars. The exit barrier may be composed not only of a passive element but also of a weak opposing pump.

After the freed intestine had been everted over a long stainless steel probe, segments of jejunum were filled with approximately 1 ml of fluid medium and tied off at each end to form individual sacs 2 to 4 cm long and 0.5 to 1 cm in diameter. Sacs were incubated singly or by pairs in 50-ml Erlenmeyer flasks containing additional medium. Being in contact with the mucosal epithelium, this medium in the flask is termed mucosal fluid; that in the lumen of the everted sac is termed serosal fluid. Incubation of a given sac was terminated abruptly by opening the flask, lifting out the sac, and plunging it into 100 to 200 ml of isopentane or dichlorodifluoromethane cooled to −160°C with liquid nitrogen. With practice these maneuvers took but a few seconds, and the surface of the sac acquired the whitish, opaque appearance of frozen tissue within a second of being plunged into the freezing fluid. Longitudinal splits often appeared at this moment. To ensure complete freezing of deeper regions, the sac was kept immersed for several minutes. It was then removed, wrapped in aluminum foil, and stored at temperatures below −40°C to await autoradiographic procedures. For certain experiments we modified the above procedure in order to freeze the sac along with a thin layer of the mucosal fluid, which normally drained off as the sac was lifted out of the flask. At this stage, therefore, the sac was placed in a cylinder of aluminum foil having a diameter only 1 to 2 mm larger than that of the sac. The space around the sac was quickly filled with mucosal fluid from the flask, and the entire cylinder was plunged into the freezing fluid.

TABLE I

Effect of 12 per Cent Albumin on Galactose Transport

Everted sacs of hamster jejunum and ileum were filled with 1 ml of Krebs' solution containing 12 per cent human serum albumin and 10 mM galactose and incubated in 50-ml Erlenmeyer flasks containing 4 ml of the same solution. Control experiments were performed in a similar fashion without albumin. Incubation was for 1 hour at 37°C in an atmosphere of 5 per cent CO_2 in O_2. Values represent means for 5 sacs.

Incubation medium (mucosal and serosal fluid)	Final galactose conc.		Net transport
	Mucosal side	Serosal side	
	mM	*mM*	μmoles /100 mg tissue/hr.
Krebs' solution	1.5	28.6	15.2 (±0.4 SE)
12% albumin in Krebs' solution	2.9	26.2	14.0 (±1.0 SE)

METHODS AND MATERIALS

Everted Sacs of Hamster Jejunum

PREPARATION AND FREEZING: We used adult golden hamsters maintained on a normal laboratory diet. Everted sacs from the jejunal portion of the small intestine were prepared according to the original method of Wilson and Wiseman (19). Briefly, an animal was killed by a blow on the head, the abdomen opened, and the intestine washed out *in situ* with 0.15 M NaCl. The next step, in which the small intestine was stripped of its mesentery while being pulled from the abdomen, requires special comment. Heretofore, it was assumed that only the mesentery tore away, leaving the intestinal wall intact. Actually our histological data reveal that as much as one-half of the intestinal circumference is denuded of both serosa and underlying tunica muscularis, *i.e.*, longitudinal and circular muscle coats. The importance of this finding for studies with everted sacs is shown by our results.

INCUBATION MEDIA: The mucosal and serosal fluids consisted of Krebs-Henseleit bicarbonate-saline (8) with or without 12 to 15 gm human serum albumin per 100 ml. When frozen, fluid containing this much albumin cuts like tissue instead of shattering like ice. To facilitate cutting frozen sections of sacs for autoradiography, albumin was always added to

serosal fluid. When a surface layer of mucosal fluid was intentionally frozen along with the sac, protein was also added to that fluid. Control experiments of two types established the fact that albumin did not interfere with transport of test compounds. First, no hydrolysis of protein to the amino acid level during incubation could be detected with paper chromatography. A significant increase in free amino acid content of mucosal or serosal fluid might have inhibited transport of test amino acids. Second, transport of test sugars and amino acids occurred at normal rates in the presence of albumin. Data for experiments with galactose are given in Table I.

LABELED COMPOUNDS: We studied two amino acids and four sugars known to be transported by sacs. In addition, inulin was used as an example of a non-transported compound. Generally the radioactive label[1] was carbon-14, but preliminary experiments were performed with tritium-labeled 6-deoxyglucose and L-methionine. The labeled compound was added either to mucosal or to serosal fluid, but never to both. Initial chemical concentrations ranged from 1 to 6 mM. Specific activities of all compounds, whether labeled with C^{14} or H^3, were such that initial isotope concentrations ranged from 0.1 to 10 μc/ml mucosal or serosal fluid. We also studied inhibitory actions of unlabeled 0.5 mM phlorizin and 10 mM L-methionine on sugar and amino acid transport, respectively.

INCUBATION PROCEDURES: In experiments in which labeled compound was added to mucosal fluid, incubation periods were so brief, 0.5 to 20 minutes, that we adopted a procedure of prior incubation to allow the tissue to reach a steady state before actually adding labeled compound. Each flask, containing 1 or 2 sacs, an atmosphere of 5 per cent CO_2 in O_2, and a measured volume of 3 to 5 ml of mucosal fluid, was shaken in a 37°C bath for 15 minutes. Then the shaker was stopped long enough to unstopper the flask and to pipette in 0.1 to 0.2 ml of stock solution containing a precise quantity of labeled compound. Within 1 minute the labeled compound was uniformly distributed in all mucosal fluid, even that in the deepest interstices between villi (inulin-C^{14} results). Thus, the entire mucosal surface of the sac was abruptly exposed to a readily computed concentration of test substance under conditions judged to provide a steady state with respect to capacity for absorptive transport. Incubation was continued for periods measured from the time the labeled compound was added to the flask, and it was terminated for a given sac by withdrawal and rapid freezing. In general, these periods were so short that the initial mucosal fluid concentration of test substance was not decreased appreciably by absorptive transport into serosal fluid. Inhibitors, when used, were present in both mucosal and serosal fluid at the start of the initial 15-minute incubation period. In serosal experiments, sacs were filled with a serosal fluid to which the labeled compound had already been added, then were placed singly in flasks and incubated for 20 to 60 minutes. To prevent secondary absorptive transport of any labeled material which passed through the sac wall, these flasks contained sufficient mucosal fluid, 10 to 20 ml, to keep the concentration of such material at a negligible level.

[1] Inulin-carboxyl-C^{14}, D-galactose-C^{14} (uniformly labeled), α-methyl-D-glucoside-C^{14} (u.l.), L-valine-C^{14} (u.l.), L-methionine-C^{14} (u.l.), and L-methionine-methyl-H^3 were purchased from New England Nuclear Corp., Boston, Mass. 3-0-Methyl(C^{14})-D-glucose was obtained from Isotopes Specialties Co., Burbank, Calif.

6-Deoxy-D-glucose was synthesized by Dr. Bernard Landau and tritiated with the Wilzbach procedure by the New England Nuclear Corp. The sugar was lyophilized repeatedly to remove easily exchangeable H^3 and then purified by paper chromatography.

Direct Contact Autoradiographs from Frozen Sections

GENERAL METHOD: Our method is based on the principle of keeping the tissue and adjacent fluid solidly frozen from the time incubation is terminated until exposure to autoradiographic emulsion is completed. Otherwise, small, water-soluble test molecules rapidly redistribute by diffusion in all available water. We have verified this fact with control autoradiographs in which the pronounced intracellular accumulation normally observed in mucosal cells was obliterated by thawing sections of sacs for 1 to 2 seconds before exposure to emulsion (Fig. 5). As the basic autoradiographic method has been published (7), only modifications will be described in detail. Briefly, we used a cryostat in which to cut frozen sections and place them directly on Nuclear Track Plates (Eastman Kodak Company). Details of improved techniques for establishing and maintaining tissue-emulsion contact are given in the following section. The plates generally used had a 10 μ thick layer of type NTB emulsion without the 1 μ thick, antiabrasion coating of gelatin. Occasionally plates with this protective coating were used, and, as one would predict, it interfered with H^3 but not C^{14} autoradiographs. The frozen sections were either 6 or 8 μ thick, cut transversely with respect to the long axis of the sac. For optimal sectioning the cryostat was maintained at -17 ± 2°C. To minimize diffusion of test molecules, all tissue awaiting sectioning and all sections being exposed to the emulsion were stored at temperatures below -40°C in an insulated box partly filled with solid CO_2. In one series of experiments with galactose-C^{14}, sacs were stored for a period of 3 weeks and no significant diffusion

could be detected by comparing autoradiographs made at the beginning and end of this period. Because of a high C^{14} content in sections from these particular sacs, the autoradiographic exposure took only a few days. In most experiments, including those with H^3, the exposure time was less than 2 weeks (see Fig. 1 for typical C^{14} data). As expected, H^3 required about 10 times more exposure than C^{14} to produce an equally dense image for a given section content in μc/ml. Exposure was terminated by warming the Nuclear Track Plate to 20°C and developing the autoradiographic image for 4 to 16 min in Eastman Kodak D-19.

TISSUE-EMULSION CONTACT: In the course of this study we used the following three procedures for placing frozen sections in contact with emulsion: (i) "sandwich" technique (Figs. 3 to 5); (ii) "modified sandwich" technique (Fig. 11); and (iii) "surface tension" technique (Figs. 6 to 10, 12 to 16). Each technique was an improvement on the preceding, and the last was good enough to produce images with H^3 as well as with C^{14}. With the latter two procedures, the tissue portion of the section could usually be retained in position on the emulsion, thus greatly facilitating interpretation of the underlying autoradiograph. Throughout the entire study, we never observed chemical fogging of the emulsion due to contact with intestinal tissue.

i. The sandwich technique has been described with the basic method (7). Briefly, it consisted of pressing frozen sections between a clean 1 x 3-inch glass slide and a Nuclear Track Plate of similar dimensions. Pressure was maintained with spring clamps throughout exposure. The major difficulty was that the frozen sections adhered to both slide and emulsion, with the result that when the sandwich was opened to develop the emulsion, the section disintegrated into useless fragments. To obtain tissue with which to compare autoradiographs, alternate sections were fixed and stained with standard histological procedures.

ii. The modified sandwich technique was initially designed for use with mammalian kidney by Dr. Jordan Cohen (personal communication). It included three additional steps. First, the glass slide was coated with Saran F-120 (Dow Chemical Company) as follows: Four drops of solution containing 4 gm of this material per 100 ml methyl-ethylketone were allowed to spread between two slides, which were then separated and dried. The Saran coating prevented frozen sections from adhering to the slide. Thus, the sections remained intact on the emulsion when the sandwich was opened upon completion of exposure. Second, to secure sections so that they did not wash away during development of the emulsion, the entire Nuclear Track Plate with sections in place was coated with gelatin by being dipped in a 1 per cent aqueous solution at 20°C. The sections were, of course, thawed by

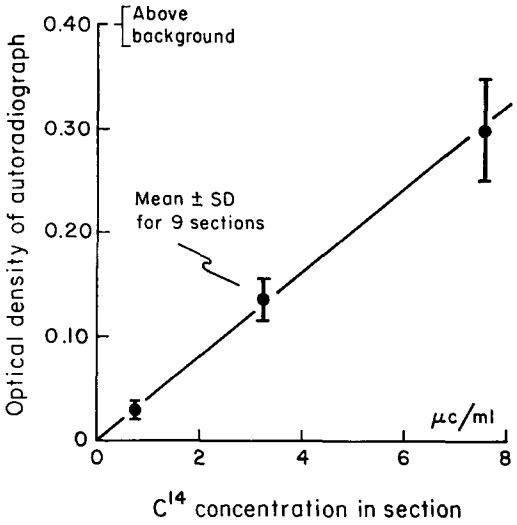


FIGURE 1 Relation between concentration of C^{14} in frozen sections of standard solutions and optical density of autoradiographs prepared on a single Kodak Nuclear Track Plate. Technical data: 6 μ thick frozen sections of solutions containing 3-0-methylglucose-C^{14} and about 15 g human serum albumin per 100 ml; 9 sections of each C^{14} concentration placed in contact with emulsion using technique iii; type NTB emulsion 10 μ thick without antiabrasion coating; exposed for 10 days at approxinately −60°C; developed for 16 minutes in D-19 at 20°C; optical density measured with microdensitometer from circular areas of emulsion 4 μ in diameter.

this procedure but not displaced. This step was carried out with the aid of a red safelight (Wratten No. 2 filter). The gelatin was dried for 30 minutes in a gentle stream of air to form a thin, insoluble, but water-permeable layer which bound the tissue sections in place in a relatively satisfactory manner. The emulsion was then processed, the developer and hypo diffusing through gelatin and tissue to reach the underlying emulsion. In each case removal of the chemicals was effected by prolonged rinsing in running water, 10 minutes after developer and 30 minutes after hypo. Third, the tissue was fixed with formaldehyde by immersing the whole preparation in a 10 per cent aqueous solution for 10 minutes. The formaldehyde did not visibly alter autoradiographic images in the emulsion. After a further 20-minute water rinse, the preparation was dehydrated by passage through graded alcohols, cleared in xylol, and mounted in Permount under a single coverslip large enough to cover most of the emulsion side of the 1 x 3-inch Nuclear Track Plate. After the gelatin was cleaned off the glass side of the plate, the now permanent preparation was ready for viewing and photographing. For the unstained tissue we used

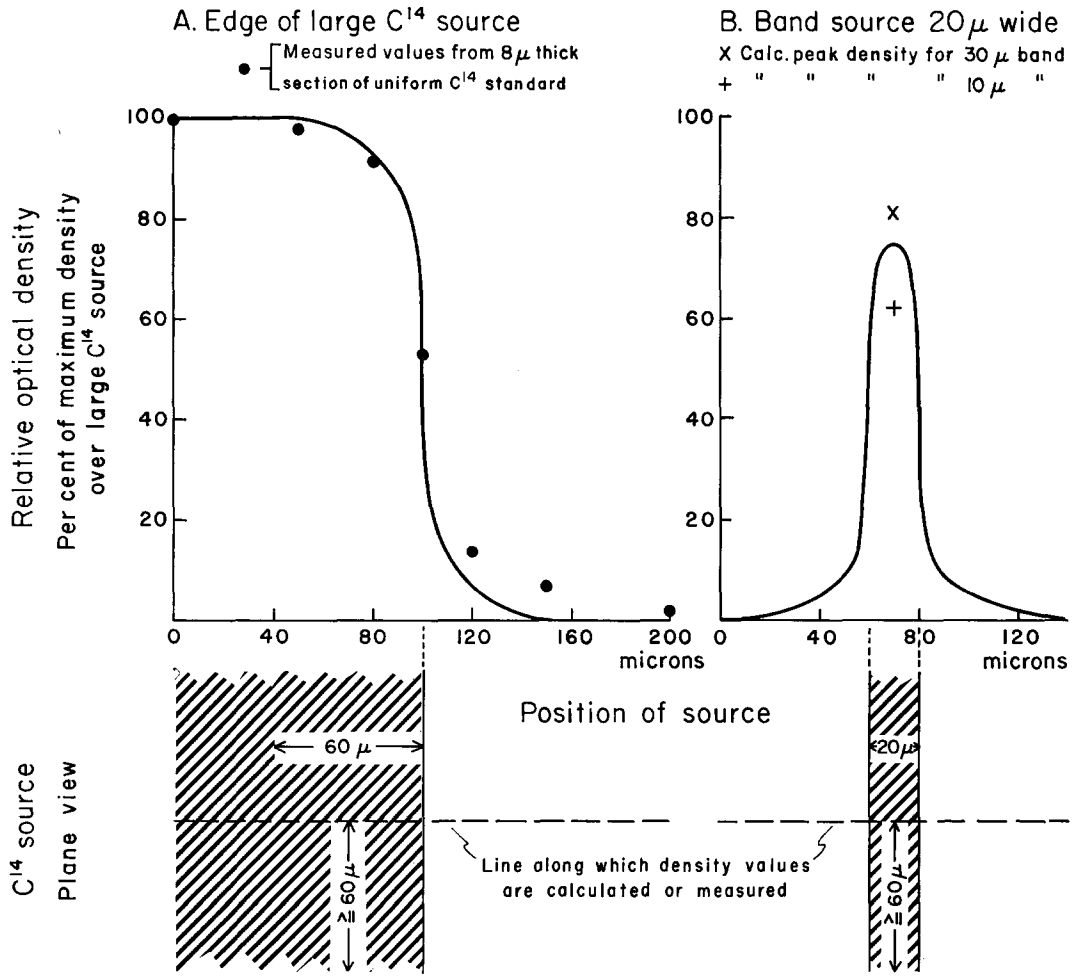


FIGURE 2 Optical density profiles of autoradiographs from thin C^{14} sources differing only in plane configuration. Smooth curves are profiles calculated (7) for beta particles having a mean effective path of 60 μ.

phase contrast microscopy, and for the autoradiographic images we used conventional light microscopy with either bright or dark field illumination.

iii. The surface tension technique represents a different approach to the critical problem of tissue-emulsion contact. The basic idea of using the surface tension of an evaporating organic solvent containing a trace of "glue," *e.g.*, hexane with mineral oil, to flatten and hold a frozen section against emulsion was developed by Taugner and Wagenmann (18). The mineral oil, although a good glue at low temperatures, was impermeable to water and had to be removed along with the tissue section in order to develop the emulsion. Consequently, we sought a water-permeable glue which would also retain the tissue in position during development just as the gelatin did in technique ii. At the same time our proposed glue had to be soluble in an organic solvent which did not leach the labeled sugar or amino acid from the frozen section during establishment of contact. To date, our most successful combination has been anhydrous ether and the acrylic resin from a commercial spray coating (Crystal-Clear, No. 1301, Krylon Inc., Norristown, Pennsylvania). Both section retention and water permeability were satisfactory when the spray was sufficiently diluted with the ether to give 0.005 per cent acrylic resin.

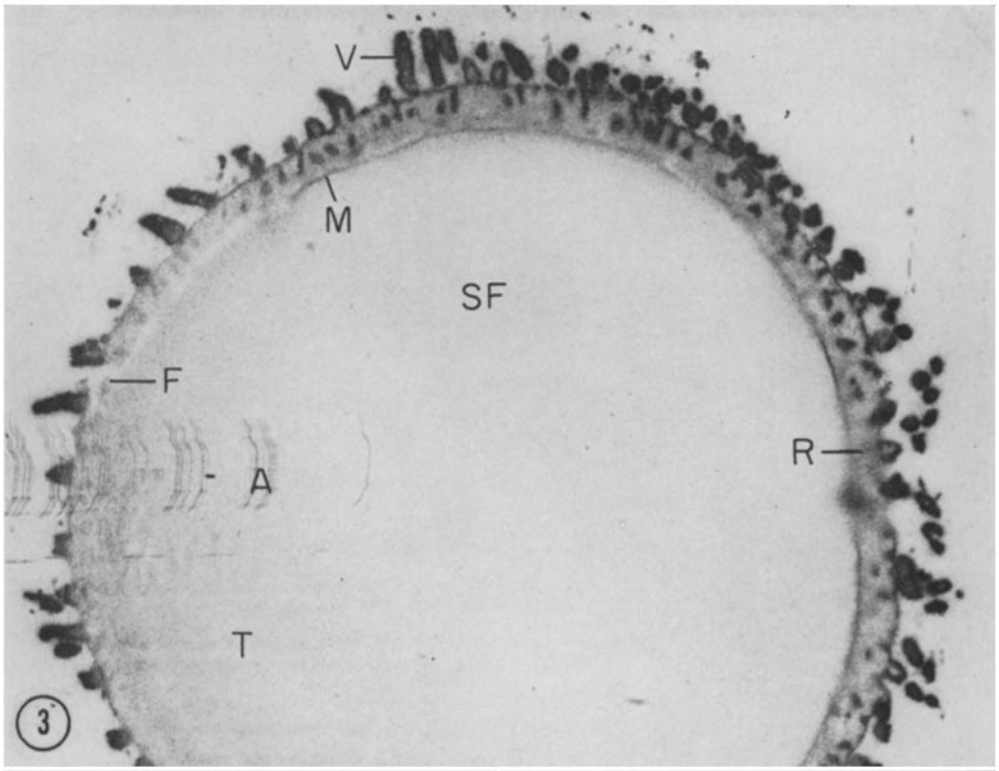
The first step in the surface tension technique was to cut about a dozen frozen sections in the cryostat at approximately −17°C. To avoid thawing, everything with which the frozen sections came into contact was precooled to this temperature. The cut sections were stored temporarily on a Saran-coated slide, to which frozen sections do not adhere (tech-

nique ii). At this point all light was excluded from the cryostat except that from a safelight, and, working with a sharp-pointed instrument, we transferred the frozen sections individually to a Nuclear Track Plate positioned horizontally with emulsion side up. Next, each section was flooded with a single drop of acrylic resin in ether. The drops were delivered from a 1-ml syringe fitted with a 21-gauge needle. In order to deliver just one drop at a time, it was necessary to reduce the vapor tension of the ether by cooling the syringe to nearly −70°C with dry ice. Once on the emulsion at −17°C, the ether evaporated in less than a minute, leaving the sections bound down with acrylic resin. To absorb the vaporized ether and minimize the hazard of an explosion, a Petri dish of activated charcoal was kept in the cryostat. Then the Nuclear Track Plate with attached sections was put in a light-tight box and stored at below −40°C for the duration of the exposure period. At the end of this period, the preparation was removed from the box and placed directly in a gentle stream of 20°C air for 30 minutes in order to thaw and thoroughly dry the tissue sections. After such treatment, the acrylic resin held the tissue in place throughout all subsequent procedures beginning with development of the emulsion. The details of these procedures are identical with those described for technique ii.

MICRODENSITOMETRIC ANALYSIS OF EPITHELIAL CELL ACCUMULATION FROM C^{14} AUTOGRAPHS: Details of the basic procedures pertaining to microdensitometry are reported in a previous publication (7). These include: (*a*) calibration of individual autoradiographic plates with standard frozen sections of albumin solutions containing known concentrations of isotope, (*b*) measurement of optical density in circular areas of emulsion 4 μ in diameter, and (*c*) calculation of optical density profiles for specific beta particle sources in order to correct results for source geometry. Since these procedures were developed for use with I^{131}, we undertook to show that they are also applicable to C^{14}. First, the quantitative nature of C^{14} images was verified experimentally. As shown in Fig. 1, a strictly linear relation exists between C^{14} concentration of standard sections and optical density of autoradiographic images. The standard deviation for image density amounted to less than ±15 per cent of the mean value at each concentration. Second, C^{14} beta particles have enough energy and, therefore, a long enough linear path in frozen tissue and emulsion (mean effective path 60 μ) so that some correction must be made for source geometry in determining the true C^{14} content of a band of epithelial cells only 20 μ wide. The optical density profiles used for this correction are shown in Fig. 2. In the case of a large uniform source, the calculated profile agrees well with density values measured directly from the autoradiograph of a standard C^{14} section, the edge of which was located precisely by fragments of denatured albumin adhering to the emulsion. Even the discrepancy existing at the terminal end (right side of Fig. 2 *A*) is an expected result of simplifying assumptions incorporated into the calculation procedure (7). Close agreement in the case of the large source validates use of this procedure and a mean effective beta path of 60 μ to calculate profiles for C^{14} sources of other geometry, *e.g.*, a band corresponding to columnar cells near the tip of a villus (Fig. 2 *B*). On the basis of these profiles, the following generalized source geometry corrections were applied to all columnar cell data before conversion of density to intracellular content: (*a*) assuming a typical 20-μ band of cells, maximal

FIGURE 3 Galactose-C^{14} autoradiograph from mucosal experiment with 10-minute incubation (see Table II for details). In this low power, bright field photomicrograph, silver grains indicating the presence of C^{14} label appear as dark regions. The autoradiograph was prepared from a cross-section of everted sac in which villi (*V*) projected outward and serosal fluid (*SF*) filled the lumen. Muscularis (*M*) was absent on the left side, and muscosal fluid, which originally bathed the villi, fell away during sectioning. Artifacts found in frozen section autoradiographs include: fracture (*F*) and uneven thickness (*T*) of the section, abrasion (*A*) of the emulsion, and fuzzy resolution (*R*) due to poor tissue-emulsion contact. × 20.

FIGURE 4 L-Methionine-C^{14}, mucosal experiment with 1-minute incubation (Table II). Fig. 4 *a*, bright field view of alternate tissue section stained with hematoxylin and eosin. Fig. 4 *b*, bright field view of autoradiograph prepared from an adjacent frozen section. Neither muscularis (*M*) nor crypts of Lieberkühn (*L*) accumulated detectable amounts of radioactivity. Major accumulation, indicated by numerous black grains, was restricted to villi and particularly to columnar absorptive cells (*C*). A unique ulcerated notch (*U*) appears on the side of one villus. × 110.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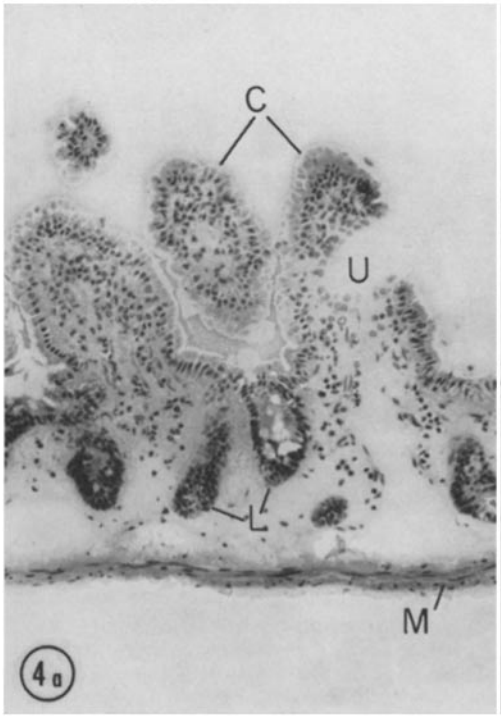
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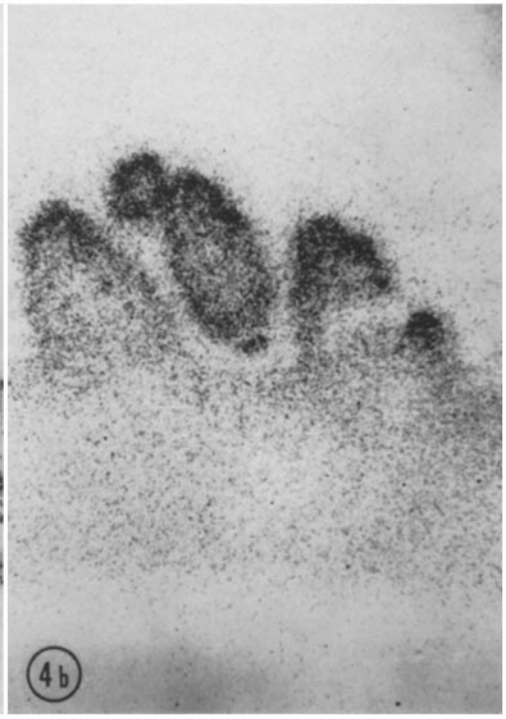
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measured density was multiplied by a factor of 1.3 to correct for its being only 75 per cent of true density owing to the straying of beta paths outside the limits of the band (Fig. 2 *B*); (*b*) when a high density source existed next to the cell band, 15 per cent of this density was subtracted from the maximal measured value to correct for stray density from the high source before multiplying by 1.3 (Fig. 2 *A*).

RESULTS

Autoradiographs from Mucosal Experiments

GENERAL APPEARANCE WITH TRANSPORTED SUGARS AND AMINO ACIDS: When the mucosal surface of an everted sac of hamster jejunum was exposed to galactose-C^{14} for 10 minutes, the tissue was found to accumulate radioactivity. Fig. 3 is a low-power photomicrograph of the autoradiographic image from a frozen cross-section of the sac. Dark areas represent exposed silver grains in the autoradiographic emulsion and, accordingly, indicate the presence of C^{14} label. The most marked accumulation is associated with the villi which project from the outer surface of the tissue ring. These appear either as black fingers or as dots, depending on the angle of sectioning. The mucosal fluid itself

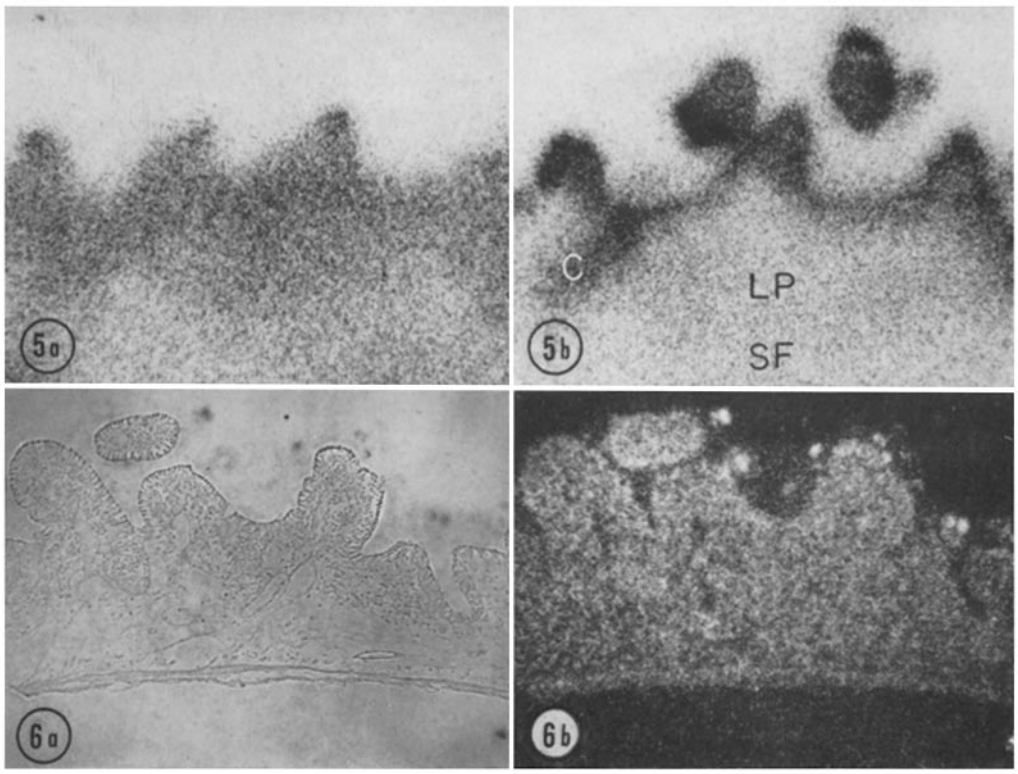


FIGURE 5 3-0-Methylglucose-C^{14} autoradiographs (bright field views) from mucosal experiment with 10-minute incubation (Table II). Fig. 5 *a*, autoradiograph from a tissue section thawed for 1 to 2 seconds before being placed in contact with the autoradiographic plate (technique i, Methods). Fig. 5 *b*, normal autoradiograph from an unthawed section on same plate. Both views depict the sector of the sac wall with muscularis detached, and no line demarcates lamina propria (*LP*) from serosal fluid (*SF*). Each view includes a crypt (*C*) on the left side. × 80.

FIGURE 6 6-Deoxyglucose-H^3, mucosal experiment with 10-minute incubation and 1 mM of the sugar in mucosal fluid initially. Fig. 6 *a*, phase contrast photomicrograph of unstained tissue with muscularis intact (section retained on autoradiographic plate). Fig. 6 *b*, dark field view of underlying autoradiograph (silver grains appear white). Autoradiographic resolution does not equal that in the other H^3 experiment (Figs. 7 and 8). × 60.

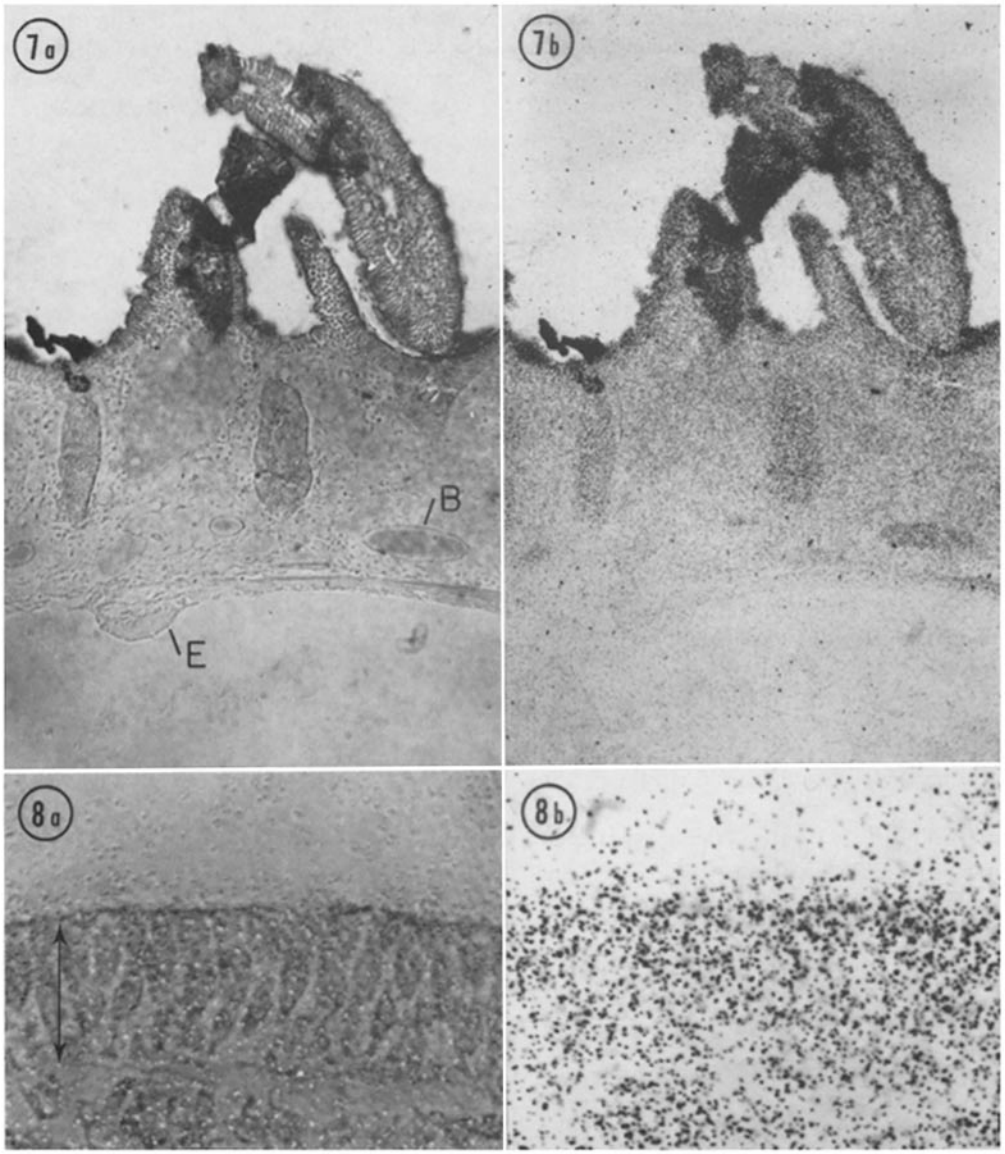


FIGURE 7 L-Methionine-H^3, mucosal experiment with 20-minute incubation and 4.8 mM of the amino acid in mucosal fluid initially (autoradiographic plate 322). Fig. 7 *a*, phase contrast view of tissue with muscularis detached on left side. Fig. 7 *b*, bright field view of underlying autoradiograph. The torn and contracted edge (*E*) of the muscularis shows clearly. Several somewhat folded sections of villi slipped against and over the main section of the sac wall, and a large blood vessel (*B*), contained more radioactivity than did the surrounding tissue. Mucosal fluid did not survive sectioning. The autoradiographic resolution represents the finest obtained and is suitable for observing features of columnar cell accumulation under higher magnification (Fig. 8). × 80.

FIGURE 8 Highly magnified photomicrographs from L-methionine-H^3 experiment (autoradiographic plate 330; see Fig. 7 for other details). Fig. 8 *a*, phase contrast view of a strip of columnar absorptive cells near the tip of a villus. Fig. 8 *b*, bright field view of underlying autoradiograph. Arrow (Fig. 8 *a*) marks total width of the columnar epithelium from brush border surface (top) to basal ends of cells in contact with connective tissue (bottom). Most of the mucosal fluid disappeared during sectioning, and the majority of grains in the autoradiograph above the band of cell accumulation represent background in the emulsion. Individual grains, which can be identified in the phase contrast view (Fig. 8 *a*), verify the nearly perfect coincidence of bursh border surface and line of greatest autoradiographic grain density. × 600.

shattered during sectioning and is generally missing even between villi. The distribution of labeled compound in the remaining parts of the tissue wall as well as in serosal fluid depends on which sector of the sac one considers. On the left side of Fig. 3 the serosa and muscularis were stripped away during preparation of the sac (see Methods). Where these tissue layers are missing, the galactose-C^{14} content decreases uniformly, starting from just beyond the villi and extending well into the serosal fluid. This gradual decrease has the distinct appearance of a simple diffusion gradient passing across the lamina propria and into the serosal fluid. Since the sac was filled with serosal fluid to the point of being somewhat turgid, a diffusion gradient could exist in this fluid, as mixing by bulk flow would be minimal. The appearance of the autoradiograph is markedly different in the other sector of this sac, where serosa and muscularis are intact (top and right side of Fig. 3). The intact muscularis reveals its presence by accumulating sufficient radioactivity to appear as a thin dark line on the inner border of the tissue ring. In this sector radioactivity has accumulated in connective tissue spaces and very little has passed into serosal fluid. Thus it appears that the muscularis forms a significant barrier restraining diffusion from the sac wall into the serosal fluid. Another distinctive feature of this sector is the accumulation by the epithelium both between bases of villi and in the crypts of Lieberkühn.

Findings relating to the mucosal epithelium itself are best seen in more highly magnified photomicrographs such as those in Fig. 4, which depict only a small segment of the sac wall. The stained tissue (Fig. 4 *a*) is typical of our frozen sections of hamster jejunum showing the columnar absorptive cells and crypts of Lieberkühn. The thin tunica muscularis, consisting of only a few circular and longitudinal fibers, is intact in this sector of the wall. The serosa is not distinguishable. The autoradiograph of an adjacent tissue section is shown in Fig. 4 *b*. During the 1 minute that this sac was incubated with L-methionine-C^{14}, sufficient radioactivity accumulated to produce in the emulsion a dense band of silver grains corresponding to the epithelial surface of the villi. Moreover, a gradient of accumulation clearly exists along the length of the villi, being greatest in the cells near the tips and almost undetectable at the bases near the mouths of the crypts. Although no distinct anatomical regions of the sac wall such as crypts or muscularis exhibit specific accumulation, the general diffuse pattern of silver grains throughout the lamina propria clearly indicates that some transepithelial movement of L-methionine-C^{14} had occurred during the 1-minute incubation period. Similar autoradiographs from other short incubation experiments, *e.g.*, 0.5 minute with L-methionine-C^{14} and 1 minute with galactose-C^{14}, are published in a preliminary report (6). In mucosal experiments with longer incubation periods, 10 to 20 minutes, larger amounts of transported compounds were present in the lamina propria (Figs. 3, 5 to 7, and 10). Included among these figures is at least one example of each sugar and amino acid tested except α-methylglucoside and L-valine. Dark field illumination makes silver grains appear a brilliant white and is often a great help in enhancing contrast in autoradiographs. The advantages of viewing the actual tissue in contact with the emulsion are obvious, and phase contrast photomicrography reveals sufficient detail in unstained tissue to identify the main structures of the sac wall. Some cellular detail is lost when unfixed tissue is exposed to photographic solutions (see Methods). Thus the lamina propria often appears "washed out." On the other hand, the presence or absence of the tunica muscularis can always be ascertained. For example, Fig. 7 *a* shows the junctional region between the sector of the sac wall on which the muscle layer was still intact and the

FIGURE 9 Phlorizin inhibition of galactose-C^{14} absorption in mucosal experiment with 20-minute incubation (Table II). Fig. 9 *a*, phase contrast view of tissue with muscularis intact. Fig. 9 *b*, dark field view of underlying autoradiograph with some mucosal fluid (*MF*). See Fig. 10 for control experiment with another sac from same animal. × 160.

FIGURE 10 Normal galactose-C^{14} absorption in mucosal experiment with 20-minute incubation (Table II). Fig. 10 *a*, phase contrast view of tissue with muscularis. Fig. 10 *b*, dark field view of autoradiograph with mucosal fluid (*MF*). × 160.

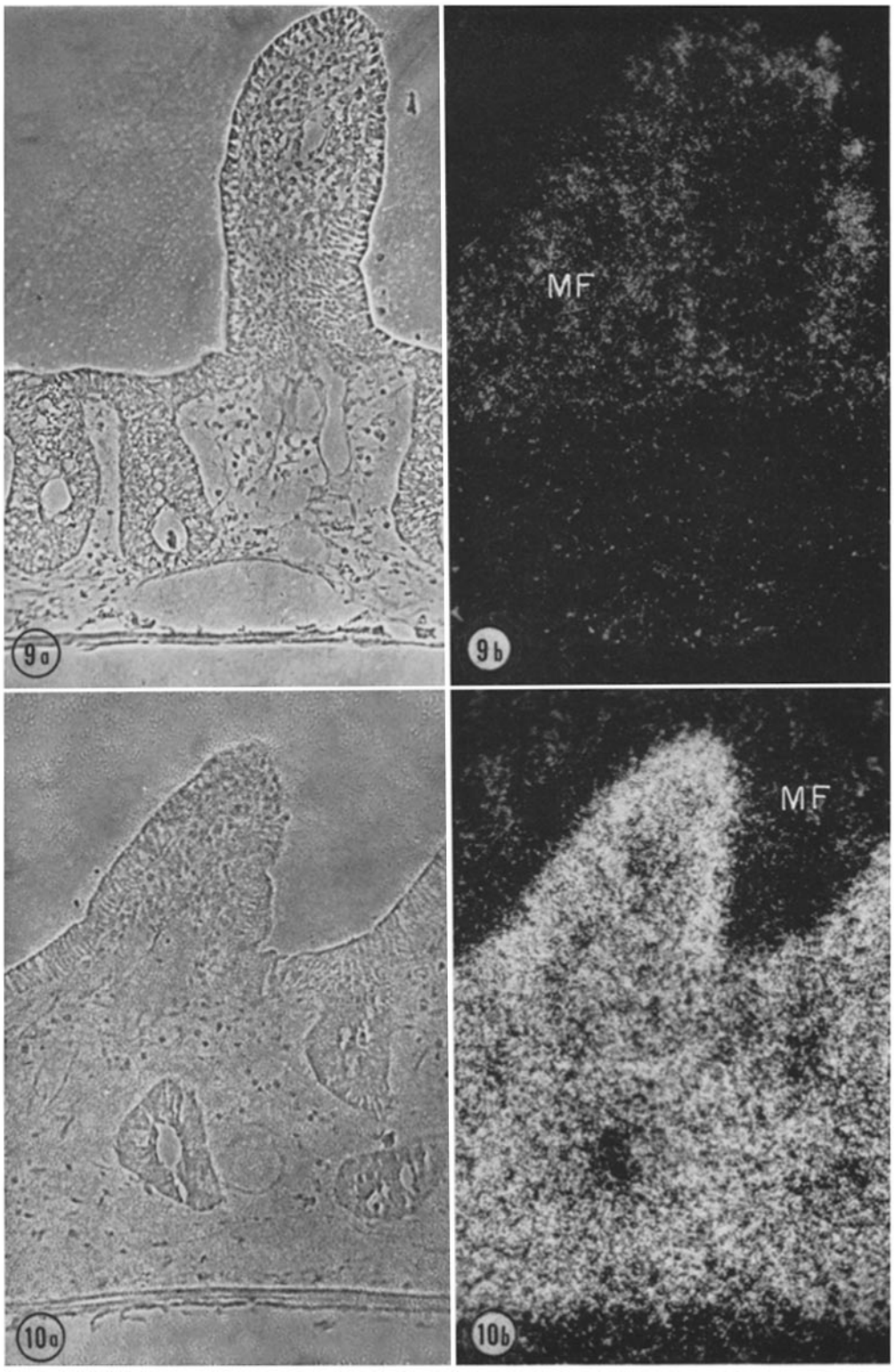

MF
9a
9b
MF
10a
10b

TABLE II

Columnar Cell Accumulation of C^{14}-Labeled Compounds from Mucosal Fluid

Test compound (inhibitor*)	Incubation period	Animal (capital letter), sac (small letter), and autoradiographic plate (no.)	Mucosal fluid concentration		Cell/mucosal fluid concentration ratio§ range for tip cells from 5 to 10 villi
			Initial	Autoradiograph‡	
	min.		*mM*		
Galactose-C^{14}	1	Ea 35	5.5		5–7
	1	Ea 36	5.5		5–7
	10	Eb 42 (Fig. 3)	5.5		4–5
	10	Eb 43‖	5.5		4–7
	10	Eb 44	5.5		4–5
	20	Ma 405‖ (Fig. 10)	4.3	3.3	8–13
(0.5 mM phlorizin)	20	Mb 399‖ (Fig. 9)	4.3	6.5	0.6–2
3-0-Methylglucose-C^{14}	1	Fa 71	2.7		1.3–2
	1	Ha 108	4.9		1.3–1.7
	10	Fb 72	2.7		3–5
	10	Hb 98 (Fig. 5)	4.9		2–3
	10	Na 426‖	4.8¶	5.0	3–4
(0.5 mM phlorizin)	10	Nb 430‖ (Fig. 12)	4.8¶	4.6	<0.6
α-Methylglucoside-C^{14}	10	Ia 169‖	5.0		1.0–2
Inulin-C^{14}	1	Fc 59‖	1.0¶	1.2	<0.2
	30	Fd 65‖ (Fig. 11)	1.0¶	1.1	<0.2
L-Methionine-C^{14}	0.5	Ca 27	1.1		5–8
	1	Ba 18 (Fig. 4)	1.1		5–7
	5	Cb 30	1.1		13–17
L-Valine-C^{14}	1	Pa 492‖	1.0		12–15
	1	Pa 493	1.0		15–20
	10	Pb 515	1.0		28–50
	10	Pe 517‖	1.0¶	1.4	8–16
(10 mM L-methionine)	10	Pf 500‖	1.0¶	1.2	1.1–2

* Added to serosal as well as mucosal fluid.

‡ Microdensitometric data where mucosal fluid was retained by chance or because albumin was present.

§ Cell content (millimoles per liter of cells) divided by initial mucosal fluid concentration (mM); cell content obtained by microdensitometry of autoradiograph with appropriate correction for source geometry (see Methods).

‖ Tissue sections retained on autoradiographic plate.

¶ Albumin in mucosal fluid (approx. 15 gm/100 ml).

other sector from which it had become detached. Consistently the torn edge of muscle, demarcating the exact limit of detachment, is contracted into a rounded mass. Sometimes the content of labeled sugar or amino acid is greater in the muscle layer than in the adjacent connective tissue of the sac wall (Figs. 3, 6, and 7).

ACCUMULATION IN COLUMNAR ABSORPTIVE CELLS: Autoradiographs from mucosal experiments in which compounds are actually being transported consistently demonstrate that the site of greatest radioactivity is the columnar epithelium on the outer portions and tips of villi. There is always a dense band of silver grains corresponding to these cells (Figs. 4 to 7 and 10). Thus there is both a marked step-up in content of radioactivity on going from the mucosal fluid bathing the free or brush border surface of the epithelium to the intracellular compartment, and a smaller step-down near the basal side of the epithelium, which is in contact with underlying tissue. Examination of autoradiographs in which mucosal fluid survived sectioning, either by chance or because albumin was added as a binder, leaves

no doubt that the step-up is real. One such autoradiograph from a galactose-C^{14} experiment is shown in Fig. 10. Another feature of accumulation within columnar cells is the rapidity with which the intracellular material can redistribute in all available tissue water. Fig. 5 illustrates the redistribution of 3-0-methylglucose-C^{14} which occurred when one of a series of frozen sections was thawed for 1 to 2 seconds and then refrozen before autoradiography. Movement into the lamina propria was so extensive (Fig. 5 *a*) that the original accumulation in columnar absorptive as well as in crypt cells (Fig. 5 *b*) was almost obliterated. Rapid redistribution was also demonstrated with L-methionine-C^{14}.

MICRODENSITOMETRIC ANALYSIS OF COLUMNAR CELL ACCUMULATION: Quantitative autoradiographic data for all mucosal experiments with C^{14}-labeled compounds are given in Table II. These data consistently show the columnar cell content of test compounds undergoing transport to be elevated above that of mucosal fluid, again confirming the existence of a step-up in content at the brush border side of the epithelium. Since the step-up in concentration is usually greater at the tip than at the base of a villus, the most meaningful datum for comparing experiments is the concentration ratio (cell/mucosal fluid) for columnar cells close to or at the tip. Tip cell ratios were measured for 5 to 10 full length villi on a given autoradiographic plate. Villi were selected by eye as being typical of those on the plate and as having good autoradiographic quality. Tip cell ratios did not differ significantly for villi from the two sectors of the sac wall, *i.e.*, the one with the tunica muscularis intact and the other denuded. Agreement was excellent among duplicate or triplicate plates from a given sac experiment: Ea 35 and 36, Eb 42 to 44, Pa 492 and 493. Retention of tissue sections on plates Eb 43 and Pa 492 did not affect results significantly, *i.e.*, the presence of tissue did not interfere with photographic development of the autoradiographic image. In general, transported sugars and amino acids were accumulated within tip cells so rapidly that after 1 minute of incubation the magnitude of the brush border step-up was already half of that reached after 5 to 20 minutes. The highest ratio was observed with L-valine-C^{14} (Pb 515), but in absolute terms an equally high intracellular content, about 50 millimoles per liter of cells, was observed with galactose-C^{14} (Ma 405).

In addition to cell content, the concentration of labeled compound in mucosal fluid was determined from autoradiographs in eight experiments in which that fluid remained an integral part of the frozen sections (Table II). For these experiments the ratio of the autoradiographic value to the initial concentration in mucosal fluid averaged 1.1 SD $\pm$ 0.24. Reduction of mucosal fluid concentration, even by normal absorptive transport (Ma 405, Na 426, and Pe 517), was so slight as to be undetectable.

EFFECTS OF INHIBITORS: Autoradiographs from experiments demonstrating the action of phlorizin on galactose-C^{14} and 3-0-methylglucose-C^{14} absorption from mucosal fluid are shown in Figs. 9 and 12. For comparison, control sacs from the same animals were incubated under identical conditions except for the absence of phlorizin (Table II). Fig. 10 depicts the control sac with galactose-C^{14} alone. In these experiments mucosal fluid sectioned well and the C^{14} sugars outside the tissue may be seen clearly in the autoradiographs. With phlorizin present almost no C^{14} sugar could be detected in the tissue, and even after 10 to 20 minutes of incubation a steep downhill gradient still existed across the columnar epithelium from the mucosal fluid to the lamina propria. Also the usual step-up in columnar cell content was nearly or completely abolished (Figs. 9 and 12; Table II). Likewise, in an experiment with amino acids, unlabeled L-methionine strongly inhibited both transepithelial movement and columnar cell accumulation of L-valine-C^{14}. As shown in Table II, the step-up in cell content of L-valine-C^{14}, which ranged from 8 to 16 in the control sac (Pe 517), was reduced to 1.1 to 2 in the presence of L-methionine (Pf 500). Thus, with both types of inhibition, the site of action appears to be near the brush border of columnar cells where the step-up in content of transported compounds normally occurs.

PRELIMINARY H^3 AUTORADIOGRAPHS: Limited resolution in present C^{14} autoradiographs precluded the obtaining of more precise information concerning either the loci of the step-up and step-down or the distribution of transported molecules within the intracellular compartment. Accordingly, two experiments with tritium-labeled compounds were undertaken with the hope of achieving significant higher resolution (Figs.

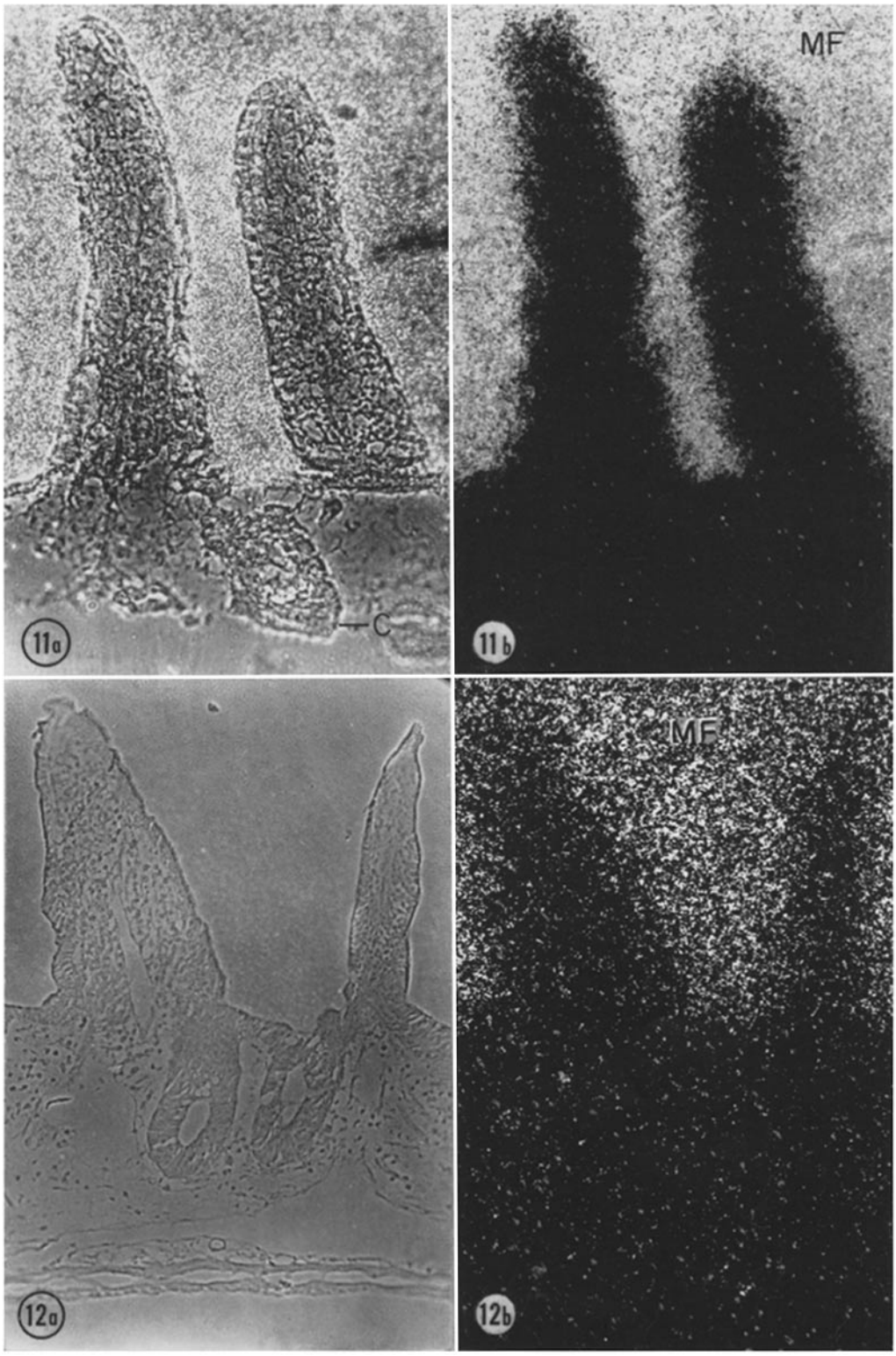
6, 7, and 8). We succeeded in the second experiment with L-methionine-H^3. A highly magnified view of a short strip of columnar cells from this experiment is shown in Fig. 8. The corresponding band of grains in the autoradiograph exhibits three important features. First, the upper edge of the band coincides so closely with the free surface of the columnar epithelium that the step-up in L-methionine-H^3 content is localized to within a few micra of the brush border. Second, passing across the width of the epithelium there is a sufficiently distinct gradient of decreasing grain density to suggest a diffusion gradient of methionine across the columnar cells from brush border region to basal end (see also Fig. 7). Third, the step-down, although somewhat obscured by a high underlying tissue content, can be localized to near the basal ends of the columnar cells. Judgment concerning these three features, and particularly the latter two, must be qualified owing to the preliminary nature of our tritium experiments. It is fair to note, however, that these features are exhibited by every one of several dozen autoradiographs prepared from sections of the sac incubated with L-methionine-H^3.

INULIN-C^{14} EXPERIMENTS: In autoradiographs with transported compounds the bands of grains corresponding to columnar cells are most dense near the tips of villi, progressively less dense down the sides of villi, and least dense at the bases and between villi. This gradient in accumulative capacity is conspicuous after short periods of incubation because the underlying lamina propria is as yet relatively free of labeled compound (Fig. 4). However, the gradient is still discernible even after 20 minutes of incubation (Figs. 7 and 10). Initially we supposed that mucosal fluid might not mix rapidly in the interstices between villi and that failure of columnar cells at bases of villi to accumulate to the same degree as those near tips might simply reflect limited access to transported compounds. To answer this question, we investigated the rate of mixing with the classical nontransported compound inulin-C^{14}, which should distribute only in mucosal fluid and not penetrate the sac wall. One sac was frozen with a surrounding shell of mucosal fluid just 1 minute after labeled inulin had been added to the incubation flask; a second sac was frozen in a like manner after 30 minutes of incubation. The distribution of inulin-C^{14} was absolutely identical in autoradiographs from the two sacs; an example, selected from the 30-minute sac, is shown in Fig. 11. Mucosal fluid sectioned well and shows clearly in the autoradiograph. The even grain density indicates perfect mixing of the large inulin molecules even at the bases of villi, thus ruling out our initial supposition of poor mixing. As expected, inulin-C^{14} showed little tendency to penetrate the columnar epithelium. Most of the grains underlying the tissue undoubtedly represent the tracks of high energy beta particles from C^{14} present in the adjacent mucosal fluid (Fig. 2). Upon applying the necessary source geometry correction for these grains, the measured columnar cell content was found to be less than 20 per cent of that in mucosal fluid even after 30 minutes of incubation (Table II).

CRYPT CELL ACCUMULATION: An unexpected finding in certain mucosal experiments with 10- to 20-minute incubation periods was the accumulation of sugars and amino acids by epithelial cells of the crypts of Lieberkühn (Figs. 3, 5, and 7). At first sight, the simplest explanation was the penetration of substances into the lumens of the crypts and transport into the cells across

FIGURE 11 Inulin-C^{14}, mucosal experiment with 30-minute incubation (Table II). Fig. 11 *a*, phase contrast view of tissue without muscularis. Fig. 11 *b*, dark field view of underlying autoradiograph. Mucosal fluid (*MF*) contained albumin and was purposely frozen with the sac (see Methods). A single crypt (*C*, Fig. 11 *a*) with open lumen is present in tissue (prepared with technique ii, quality not equal to that obtained with iii, *e.g.* Fig. 12 *a*). Distribution of inulin-C^{14} was identical with that in experiment with only 1-minute incubation (Table II and text). × 150.

FIGURE 12 Phlorizin inhibition of 3-0-methylglucose-C^{14} absorption in mucosal experiment with 10-minute incubation (Table II). Fig. 12 *a*, phase contrast view of tissue with muscularis. Fig. 12 *b*, dark field view of underlying autoradiograph with mucosal fluid (*MF*). See Fig. 5 *b* for normal 3-0-methylglucose absorption. × 140.


MF
C
11a
11b
MF
12a
12b

the brush border in the same manner as that seen with cells on the villi. This explanation, however, appears to be untenable for several reasons. First, in histological sections the necks of the crypts appear to be very narrow and plugged with mucus (Fig. 4). Second, when tissue was incubated for 30 minutes in the presence of inulin-C^{14}, none appeared in crypt lumens (Fig. 11). Likewise, when tissue was incubated with labeled sugars in the presence of phlorizin, which largely blocks uptake from mucosal fluid, no radioactivity entered the crypts (Figs. 9 and 12). It therefore appears that the crypts of Lieberkühn do not communicate with the mucosal fluid, and that sugars and amino acids accumulated within cells lining the crypts must have entered from the lamina propria, *i.e.*, across the basal ends of the cells. If this hypothesis is correct, then the addition of radioactive compounds on the serosal side of the intestinal sac should lead to accumulation in crypt cells via the basal route. Not only was this prediction verified in the following experiments, but basal accumulation by columnar cells on villi was also demonstrated.

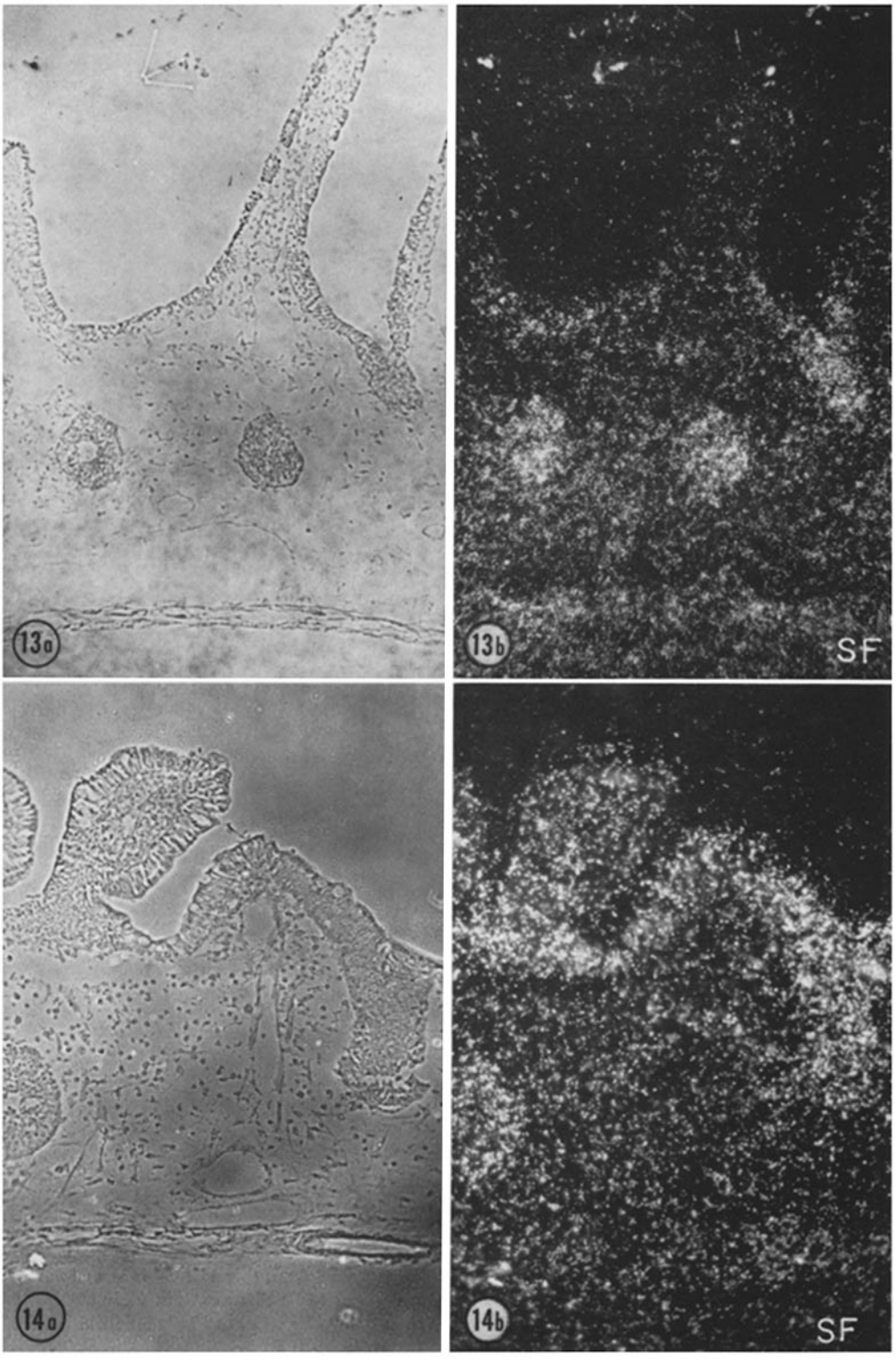
Autoradiographs from Serosal Experiments

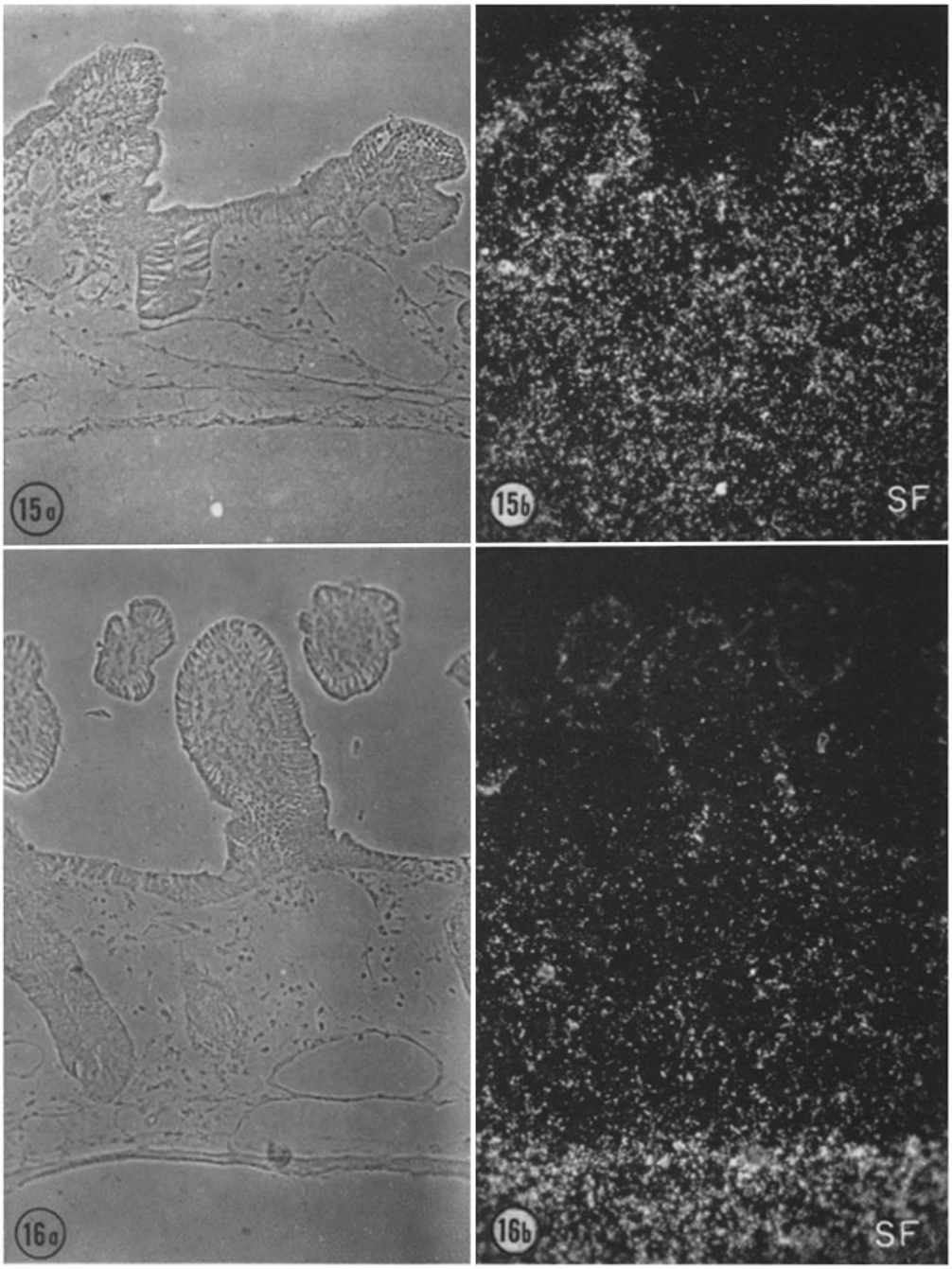
BASAL ACCUMULATION BY CRYPT AND COLUMNAR CELLS: Labeled sugars or amino acids added to serosal fluid diffuse through muscularis, where present, and lamina propria toward epithelial cells both in crypts and on villi. When sufficient material reaches the bases of these cells, after about 60 minutes with muscle present, uptake into the cells establishes an intracellular content distinctly higher than that in adjacent connective tissue. These findings are clearly illustrated in Figs. 13 and 14, which depict 60-minute serosal experiments with 3-0-methylglucose-C^{14} and L-valine-C^{14}, respectively. Identical autoradiographic results were obtained from a third sac incubated for 40 minutes with galactose-C^{14}. The phase views of tissue sections show intact muscularis, crypts in the lamina propria, and columnar cells on villi. The dark field views of autoradiographs show the content of labeled compound in serosal fluid, a lower content in connective tissue beyond the muscularis (clearly seen in Fig. 13), and accumulation by crypt and columnar cells. Although mucosal fluid did not survive sectioning and does not appear in these autoradiographs, there is strong evidence that all columnar cell accumulation occurred via the basal route and not from mucosal fluid via the brush border. First, we purposely used large volumes of mucosal fluid in these experiments to prevent any appreciable build-up of labeled compound in that fluid. Second, the only columnar cells to show accumulation were those with appreciable amounts of labeled compound at their basal ends. This fact is clearly illustrated in Fig. 13, where, owing to the goodly length of the villi, no 3-0-methylglucose-C^{14} had as yet diffused to the bases of the tip cells. Another aspect of basal accumulation is that when connective tissue content was very high, as in Fig. 15, further elevation of intracellular content did not occur in either crypt or columnar cells. In fact, the same observation holds for crypt cell accumulation in mucosal experiments (Figs. 6 and 10).

Since the above results suggested the existence of weak entrance pumps located at the basal end of both crypt and columnar cells, we tested the action of inhibitors: 0.5 mM phlorizin on 3-0-methylglucose-C^{14} and 10 mM L-methionine on L-valine-C^{14}. In both experiments, basal accumulation was identical with that in control sacs with-

FIGURE 13 Serosal experiment, 60-minute incubation with 4.8 mM 3-0-methylglucose-C^{14} in serosal fluid initially and 0.5 mM phlorizin in both serosal and mucosal fluid. Fig. 13 *a*, phase contrast view of tissue showing muscularis. Fig. 13 *b*, dark field view of underlying autoradiograph showing serosal fluid (*SF*) content of C^{14} sugar. Identical autoradiographic results were obtained in control experiment with 3-0-methylglucose-C^{14} alone (no phlorizin). × 100.

FIGURE 14 Serosal experiment, 60-minute incubation with 1 mM L-valine-C^{14} in serosal fluid initially. Fig. 14 *a*, phase contrast view of tissue showing muscularis. Fig. 14 *b*, dark field view of underlying autoradiograph showing serosal fluid (*SF*) content of C^{14} amino acid. Identical autoradiographic results were obtained in a comparable L-valine-C^{14} experiment with 10 mM L-methionine in both serosal and mucosal fluid initially. × 140.


13a
13b
SF
14a
14b
SF



FIGURES 15 AND 16 Serosal experiment, 20-minute incubation with 4.8 mM galactose-C^{14} in serosal fluid initially (animal M, sac c, autoradiographic plate 402). Figs. 15 *a* and 16 *a*, phase contrast views of tissue from sectors of the sac wall without and with muscularis, respectively. Figs. 15 *b* and 16 *b*, dark field views of the underlying autoradiographs showing serosal (*SF*) but not mucosal fluid. × 140.

out inhibitors. Indeed, the 3-0-methylglucose-C^{14} experiment shown in Fig. 13 is the one with phlorizin present. The question of a basal pump is treated in the Discussion.

TUNICA MUSCULARIS AS A DIFFUSION BARRIER: Results from mucosal experiments (Figs. 3, 6, and 10) have already implicated the tunica muscularis as a barrier impeding diffusion between lamina propria and serosal fluid. Autoradiographs from a sac incubated for 20 minutes with galactose-C^{14} in serosal fluid establish, beyond a doubt, that the muscle layer is a major diffusion barrier. Figs. 15 and 16 depict sectors of this sac without and with muscularis, respectively. In the former case, 20 minutes was sufficient for complete equilibration of galactose-C^{14} throughout the lamina propria, whereas in the latter, very little galactose-C^{14} had as yet penetrated the muscle barrier. Finally, it is noted that the content of sugars and amino acids may be greater in muscularis than in serosal fluid (Figs. 13, 14, and 16).

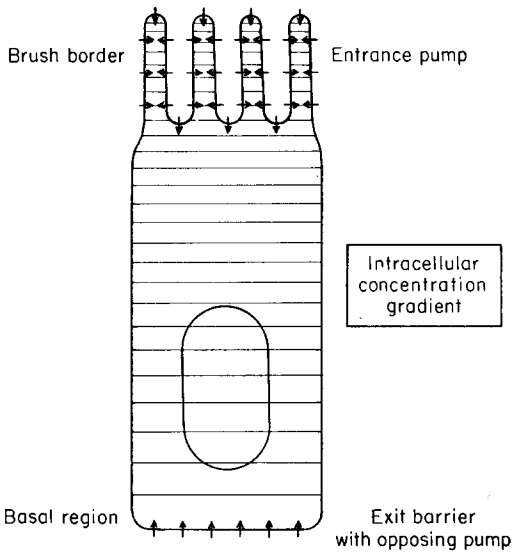


FIGURE 17 Schema for sugar and amino acid transport in columnar absorptive cell of small intestine (details in text).

DISCUSSION

Interpretation of the present autoradiographs requires a precise knowledge of the chemical form of the radioactive compounds within the epithelial cells. Although hamster jejunum is capable of a slow metabolism of galactose (10), considerable accumulation of the free sugar is known to occur as measured by direct chemical methods (5, 12). The other three sugars used in this study, 3-0-methylglucose, 6-deoxyglucose, and α-methylglucoside, undergo little or no chemical alteration in the intestine (2, 3, 9). It is inferred that, at least in the case of these three non-metabolizable sugars, it is exclusively the free sugar which accumulates within the tissue during transport. In the case of the amino acids L-valine and L-methionine, Finch and Hird (4) have shown that virtually all of the added amino acid can be recovered in the tissue or in the incubation medium at the end of an experiment under these conditions. With regard to the possibility of intracellular binding, a factor which is difficult to exclude, several experiments may be mentioned. Thawing of a tissue section for only 1 second, followed by refreezing, before exposure to photographic emulsion caused such extensive loss of radioactive 3-0-methylglucose from both mucosal and crypt cells that specific cellular accumulation could scarcely be detected (Fig. 5). Thus, the major fraction of the intracellular content of radioactive compound was diffusible and apparently not bound within the cell. McDougal *et al.* (12) demonstrated tissue accumulation of galactose by isolating the sugar from the tissue under very mild conditions which would not be expected to rupture covalent bands. Although weak binding of sugars and amino acids by intracellular components cannot be specifically excluded, the working hypothesis used in the subsequent discussion will be that the major fraction of the tissue radioactivity represents labeled test compound in the unbound state.

Our hypothesis for the subcellular localization of the sugar and amino acid transport machinery in the columnar absorptive cell of the hamster small intestine is given in Fig. 17. The first step in absorption is the entrance into the cell via an active transport system in the brush border region, perhaps across the plasma membrane of the microvilli. In the case of sugars, the entrance pump appears to be the site of phlorizin inhibition. Similarly, this region is the site of competition for transport between L-valine and L-methionine. The second step is the diffusion of the free sugar or amino acid from the region of high concentration below the brush border to a region of lower concentration in the basal part of the cell. The third step is the escape from the basal end of the cell across some type of diffusion barrier, probably the plasma membrane. This is inferred from the

definite fall in concentration between epithelial cell and lamina propria.

The postulation of a simple diffusion barrier at the basal pole of the absorptive cell is not entirely consistent with the experimental finding of accumulation within epithelial cells exposed to sugar or amino acid from the serosal side. It might be argued that under these conditions sugars or amino acids diffuse back into the mucosal solution, from which the cells recover them by active transport. This explanation appears untenable, however, as recovery was prevented by dilution in the large mucosal volume and by blocking the brush border transport system with phlorizin (in the case of the sugar pump) and L-methionine (in the case of the amino acid pump). If accumulation in these experiments cannot be via the brush border pump, we are forced to consider the possibility that the plasma membrane of the basal region of the cell contains a transport system capable of active transport from the lamina propria into the cell. The hypothesis of a strong brush border pump opposed by a weaker basal pump was originally postulated by Oxender and Christensen on entirely theoretical grounds (15). It was recently pointed out by Parsons (17), however, that to obtain net transport across the columnar absorptive cell the ratio of pump to leak must be different in the membranes at the two poles of the cell.

An unexpected finding in everted sacs with the serosal side exposed to 3-0-methylglucose was that the accumulation via the basal pump in both the crypt and villus cells did not appear to be affected by phlorizin at concentrations which would have completely inhibited uptake from the brush border side. This observation is consistent with the findings of Newey *et al.* (13), who found that phlorizin did not inhibit glucose uptake from the serosal side of the intestine. Likewise, our failure to obtain inhibition of L-valine accumulation from the serosal side by L-methionine parallels the findings of Newey and Smyth (14) that suggest a basal exit mechanism for glycine which does not involve competition with L-methionine.

The route of transfer of sugars and amino acids across the lamina propria and muscularis in the *in vitro* preparation deserves brief comment. Lee (11) found that with *in vitro* preparations of rat intestine most of the fluid traversed the tissue in lymphatic vessels or blood vessels while only a small fraction diffused across the tissue layers. In the present experiments with hamster intestine, it was found that the smooth muscle was partially detached in every everted sac examined, and, as shown in Fig. 3, that the concentration of radioactivity in the lamina propria was considerably less in the sector lacking the muscular coat. It was in this region that the radioactive substances appeared to diffuse most rapidly into the interior of the sac. Fig. 3 provides excellent evidence that in our *in vitro* preparation the muscular wall provides an appreciable barrier to diffusion. In areas with the muscularis intact, large vessels close to the serosal surface occasionally contain more labeled compound than does the surrounding tissue (*e.g.*, Fig. 7), a fact which suggests flow of the type demonstrated by Lee.

A final point of interest is the gradient of intracellular accumulation along the villus, with the greatest uptake in the cells at the tip and the least in those at the base. The relatively poor uptake in cells between villi is apparently not due to poor contact with the incubation medium, since inulin-C^{14} and other radioactive compounds reach the bases of the villi in less than a minute. The gradient, although most prominent during the first few minutes, is still visible in most preparations after longer incubations of 10 and 20 minutes. This phenomenon could be due to greater transport activity in the cells at the tips of the villi or to greater rate of exit from the cells closer to the crypts. It is well known that the cells at the bases of the villi are immature in many of their enzymatic functions and that these functions develop during the migration up the sides of the villi. Padykula (16) has recently emphasized the fact that during fat absorption the cells at the tips of the villi accumulate lipid most rapidly and in greatest abundance. It is therefore possible that, parallel with the development of enzymes, the transport capacity for lipids, sugars, and amino acids also matures, reaching maximum activity in cells at the tips of the villi.

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