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Report

A Correlation of Permeabilities for Passively Transported Compounds in Monkey and Rabbit Jejunum

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Permeability measurements were conducted for a series of compounds using *in vitro* tissue sections from monkey and rabbit jejunum. Jejunal segments were stripped of serosal musculature and mounted in a diffusion-cell system, using previously described methods and equipment. Permeability determinations of radiolabeled compounds ranging over two orders of magnitude in molecular weight were conducted. For the compounds examined, the permeability of the rabbit jejunum was approximately twice that of the monkey. This was in contrast to the relationship implied by the stripped tissue thickness measurements of 0.92 and 0.83 mm for rabbit and monkey, respectively. An investigation of the size of the paracellular space in the jejunum was undertaken to account for this apparent discrepancy in tissue permeability. Scanning electron micrographs of intestinal sections revealed a similar packing density of cells between species; however, a difference was noted in the shape and number of villi per unit area. Comparative measurements of the paracellular volume in both species using mannitol and methoxyinulin as extracellular space markers further suggests that the paracellular junctions are similar in size but more numerous per unit area of rabbit jejunum than that of the monkey. In contrast to passively transported compounds, the active transport of D-glucose was greater in monkey jejunum compared to rabbit tissue segments. When active transport was inhibited by blockade of the sodium pump with ouabain, the passive component of D-glucose transport for both rabbit and monkey tissue was in agreement with the relationship demonstrated above for compounds which are solely transported by passive processes.

KEY WORDS: *in vitro* permeability study; rabbit; monkey; intestine; species correlation; passive transport.

INTRODUCTION

A drug in solution, i.e., solute, may cross the gastrointestinal barrier and enter the blood by one of two basic pathways, transcellular or paracellular. Theoretically, transcellular passage of a molecule through the lipid portion of the membrane may involve a vast number of transport sites, influenced and limited by the physicochemical properties of the substances. Conversely, diffusion through the polar regions, or paracellular transport, may be considered to involve a relatively fewer number of transport sites governed by parameters such as molecular size (1).

Of particular interest in pharmaceutical development are the observed differences between species for the oral absorption of drug substances. Often, depending upon the specific compound, animal models do not accurately reflect the absorption kinetics in humans. Through *in vivo* experiments, it is difficult to discern the origin of these discrepancies, i.e., physiological or anatomical. *In vitro* experiments, however, allow investigation of the inherent properties of the membrane, which should be reflected in the permeabilities of passively transported compounds. These studies were initiated to determine differences in the passive diffusional transport properties of rabbit and monkey jejunum and to develop some correlations if differences were detected. It is anticipated that success in these efforts could lead to more accurate predictions in humans through future correlations with *in vivo* experiments.

EXPERIMENTAL

Materials

Radiolabeled mannitol, naproxen, RS-82856, progesterone, D-glucose, L-glucose, PEG 900, PEG 4000, and methoxyinulin were purified by vacuum distillation immediately prior to use. Radiolabeled methanol was used as received. Supplier, specific activity, and type of radiolabel for each compound are indicated in Table I. All other chemicals were either reagent or analytical grade and were used as received. Male albino New Zealand rabbits weighing between 2.5 and 3.5 kg were used throughout the studies. Animals were fasted overnight prior to use in an experiment. Jejunal sections from cynomologous monkeys of either sex were obtained from animals used as controls in various necropsy studies (Syntex Research, Department of Toxicology, Palo Alto, California).

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Table I. Radiolabel, Supplier, Specific Activity, and Concentration Applied to the Tissue for Compounds Used in *in Vitro* Permeability Determinations

Drug name	Label	Supplier	Specific activity (Ci/mmol)	Actual applied concentration ($\times 10^{-5}$ *M*)
Mannitol	C-14	Amersham[a]	0.055	0.02
Methanol	C-14	NEN[b]	0.058	5.0
Methoxy-inulin	H-3	NEN	0.93	0.04
D-Glucose	H-3	Amersham	6.44	0.004
L-Glucose	H-3	NEN	10.70	0.003
Naproxen	H-3	Syntex[c]	18.0	4.0
PEG 900	H-3	NEN	0.0061	8.21
PEG 4000	H-3	NEN	0.0080	0.42
Progesterone	H-3	Amersham	56	0.00076
RS-82856	C-14	Syntex	0.033	1.0

[a] Amersham, Arlington Heights, Ill.
[b] New England Nuclear, Boston, Mass.
[c] Syntex Research, Palo Alto, Calif.

Methods

Preparation of Drug Solutions

Drug solutions were prepared by the addition of tracer amounts of radiolabeled compound to oxygenated (O_2/CO_2,95/5) Kreb's Ringer bicarbonate buffer (pH 7.4) which was prepared daily. The actual concentrations of radiolabeled species applied in the donor chamber are given in Table I. To help maintain tissue viability, unlabeled D-glucose (40 m*M*) was added to the serosal medium. Unlabeled mannitol (40 m*M*) was added to the mucosal solution to provide an equivalent osmotic load between mucosal and serosal solutions, since even small amounts of glucose on the mucosal side markedly stimulate sodium and water absorption to the serosal side (2). In experiments to examine active transport 0.2 m*M* ouabain was added to the serosal solution.

Mounting of Jejunal Tissue

Rabbits were sacrificed by rapid injection of sodium pentobarbital through a marginal ear vein. Following a midline incision, 40 cm of small intestine extending from the duodenum was removed and placed in ice-cold oxygenated Kreb's ringer bicarbonate buffer (pH 7.4). Monkeys were sacrificed by an overdose of sodium pentobarbital and exsanguinated prior to surgery, and the entire intestine was removed within 10 min of death. Single jejunal segments from either species were cut beginning in the region 12 cm from the duodenal end of the excised tissue segment. Peyers patches could be easily identified visually and sections containing them were not used in these studies. The individual segments were opened along the mesenteric border to expose the epithelial surface.

Tissues were mounted in diffusion cells according to a previously described procedure (3). Briefly, the section was placed on a preheated, 37°C, acrylic half-cell (Precision Instrument Design, Los Altos, Calif.); the muscle fibers were carefully stripped from the serosal side of the tissue, and the matching half-cell was joined to seal the diffusion apparatus. Immediately after tissue mounting, the assembled cells were placed in an aluminum block heater which was capable of holding six cells and maintained a temperature of 37°C throughout the studies. The reservoirs were filled with warmed oxygenated Kreb's buffer which was circulated by gas lift (O_2/CO_2), controlled by valves (Precision Instrument Design, Los Altos, Calif.). The exposed surface area was 2.06 cm^2 and the volume of each half cell was 7 ml.

Samples of the donor phase (0.1 ml) were taken immediately at the beginning of each experiment and again at its conclusion. Receptor-phase samples (1 ml) were taken at indicated time points with replacement of the sampled volume by blank (non-drug-containing) buffer. Samples were placed in scintillation vials, scintillation cocktail (Aquasol, New England Nuclear, Boston, Mass.) added, and counted on a scintillation counter using an external standardization method. All experiments continued for 2 hr with the exception of those noted in Table III. The number of animals and experiments conducted is indicated in the tables. After each experiment the acrylic cells were sonicated in cleaning solution (Countoff, New England Nuclear, Boston, Mass.) and thoroughly rinsed with distilled water.

Tissue Thickness and Villus Density Determinations

Stripped and unstripped jejunal sections from both rabbit and monkey were fixed in formalin, embedded in parafin, sectioned, mounted, and stained in hemotoxylin and eosin. Sections were viewed by light microscope and photographed. Using a micrometer, measurements of tissue thickness were taken from the serosal edge to the tips of the villi and from the base of the villi to the villi tips. Fixed tissues for SEM studies were dehydrated in graded alcohols, sputter coated with gold, and viewed in a scanning electron microscope. A calculation of the number of villi per square centimeter was determined for both species. The values obtained were the average four or five measurements taken from at least six tissue samples.

Extracellular Space Measurements

Either mannitol or methoxyinulin was added to mucosal and serosal solutions, i.e., both solutions contained the same initial concentration. After a 2-hr incubation time, both so-

Table II. *In Vitro* Permeability Values Determined in Rabbit and Monkey Jejunum

Drug name	MW	Permeability in rabbit (cm/sec × 10^{-6})	N^a	Permeability in monkey (cm/sec × 10^{-6})	N
Methanol	34	44.40 (4.10)[b]	20/5	24.4 (2.16)	16/4
Mannitol	184	3.54 (0.19)	20/5	1.94 (0.17)	24/6
Naproxen	253	8.52 (0.84)	11/3	2.12 (0.38)	12/4
Progesterone	315	1.36 (0.18)	22/4	0.67 (0.14)	13/4
RS-82856	384.5	0.34 (0.04)	11/3	0.11 (0.01)	11/3
PEG 900	900	0.70 (0.07)	15/4	0.35 (0.04)	24/7
PEG 4000	4000	0.24 (0.04)	15/5	0.20 (0.05)	9/3
Methoxy-inulin	~5200	1.61 (0.14)	25/5	0.93 (0.05)	16/3

[a] Number of experiments/number of animals.
[b] Number in parentheses represents standard error of the mean.
[c] Not determined.

lutions were sampled, and the tissue was removed from the diffusion cell. The tissue segments were weighed and then dried under vacuum and reweighed. Tissue solubilizer (Protosol, New England Nuclear, Boston, Mass.) was added and, after dissolving, scintillation cocktail was added. The amount of compound in the tissue was determined by scintillation counting. A total of 9 to 12 tissue segments from three animals was used for each agent examined.

RESULTS

Permeabilities were determined for passively transported compounds in rabbit and cynomolgus monkey jejunum (Table II). These values were calculated from the change in concentration of the receiver (serosal) solutions per unit time as previously described (3). In all cases the measured permeabilities are greater in rabbit than in monkey tissue. An inverse relationship has been demonstrated between permeability and molecular weight, and this is consistent with previously published data (4). The permeability of methoxyinulin deviated significantly from this correlation. Lower molecular weight fragments have been previously described in the commercially available tritium-labeled material, and these can result in an overestimation of permeability (5,6). No purification except evaporation was undertaken for methoxyinulin in these studies.

Since the compounds examined constitute a range of more than two orders of magnitude in molecular weight, and approximately three orders of magnitude in permeability, a correlation of measured permeabilities between species was determined. This is shown in Fig. 1, and the fit (r^2 = 0.954) suggests that the relationship holds well over the entire range examined. A regression of the permeability values determined in rabbit versus those obtained in monkey gives a slope value of 1.80 (r^2 = 0.988), that is, the permeability of the rabbit tissue is approximately twice that of the monkey.

In contrast to these passively transported compounds, the measured permeability of the actively transported D-glucose was similar in monkey and rabbit (Table III). At time points greater than 2 hr, the permeability decreased in both species, however, the decrease was slightly greater in monkey than in rabbit, 34 and 45% of the original values, respectively. Ouabain, a known inhibitor of active glucose transport, was added to the serosal solution and reduced the measured permeabilities to 63 and 26% of the control value in monkey and rabbit, respectively. The permeability of L-glucose was considerably less than that obtained for D-glucose, 67% for rabbit and 65% for monkey.

Measurements of tissue thickness were conducted (Figs. 2 and 3, Table IV). In spite of the greater permeability determined in rabbit tissue, the stripped tissue thickness, representing the distance from the mucosal to serosal solutions, was slightly greater than that obtained for monkey (ratio of 1.11 rabbit/monkey). This finding was somewhat unexpected given that a greater diffusional path length should result in lower measured permeabilities. Conversely, the greater tissue permeability of the rabbit jejunum cannot be explained by a smaller tissue thickness compared to that of the monkey. The number of cells per unit area on the villi of monkey and rabbit jejunum did not differ significantly (Figs. 4 and 5, Table IV). The density of the villi, however, was significantly greater for the rabbit compared to the monkey (Figs. 6 and 7, Table IV).

Paracellular space measurements of jejunum from both rabbit and cynomolgous monkey were also estimated using mannitol and methoxyinulin (Table IV). The results suggest that the available free aqueous space for diffusion of these hydrophilic compounds is similar in both species and for each compound. This measurement, however, includes both the available paracellular space and the adjacent hydrogel layer of mucus. This total volume, for the sample size evaluated, can be estimated as approximately 37 μl in rabbit and

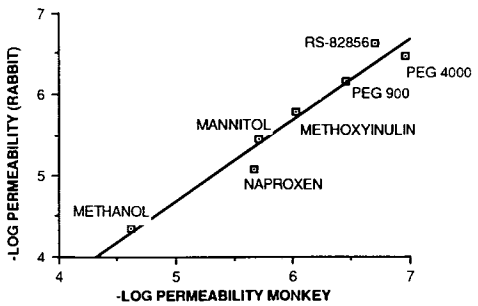

Fig. 1. −Log permeability in rabbit jejunum vs −log permeability in cynomologous monkey jejunum. $y = 0.335x + 1.003$ (R^2 = 0.954).

Table III. Measured Permeabilities of D- and L-Glucose in Rabbit and Cynomolgous Monkey Jejunum

Drug	Time (min)	Permeability in rabbit (cm/sec × 10^{-6})	N^a	Permeability in monkey (cm/sec × 10^{-6})	N
D-Glucose	<120	10.17 (0.75)[b]	42/9	12.12 (1.28)	19/5
D-Glucose	>120*	5.63 (0.34)	35/8	7.99 (2.46)	19/5
D-Glucose + 0.2 *M* ouabain	<120	7.50 (0.52)	15/5	4.48 (0.54)	17/5
L-Glucose	<120	3.35 (0.42)	12/3	4.20 (0.56)	12/3

[a] Number of experiments/number of animals.
[b] Number in parentheses represents standard error of the mean.
* Differences between D-glucose permeability measurements pre and post 120 min were significant to $P \leq 0.0005$.

34 μl in monkey. Total tissue water content was 84% for rabbit and 87.5% for monkey.

DISCUSSION

For the compounds examined in this study, it is possible to construct a correlation of jejunal permeabilities between rabbit and monkey. This correlation predicts that the permeability of a compound in rabbit jejunum will be greater by a factor of approximately 1.8 than that obtained for monkey. This is contrary to the relationship suggested by the stripped jejunal tissue thicknesses, with rabbit tissue being thicker than that of monkey (ratio of 1.11 rabbit/monkey). Most of the compounds examined are of a hydrophilic nature and are believed to be transported through the paracellular spaces (7). Therefore it seems plausible that some differences in the paracellular space must exist between rabbit and monkey jejunum. These may be comprised of either a difference in the size of the aqueous channel or a difference in the number of channels. Alternatively, both the size and the number could be affected.

Among the compounds chosen in this series, mannitol, PEG 900, PEG 4000, and methoxyinulin are known not to cross cell membranes and are restricted to movement through the paracellular shunt pathway. Since these paracellular markers vary over a wide range of molecular size (weight), and the linear correlation holds over this entire range, it seems reasonable to assume that the junctions are of a similar size between species. Size exclusion properties would predict that a deviation, dependent on molecular weight, would occur if the pathways differed in size.

The second factor that could conceivably influence permeability through the intestine is the number of channels, possibly the result of a difference in surface area or in the packing density (size) of the cells (8). Cell density counts on the intestinal villi of both rabbit and monkey jejunum revealed that, on average, there were no significant differences between species (Figs. 2 and 3, Table IV). However, a far greater number of villi per unit area exists in the rabbit jejunum (Figs. 4 and 5, Table IV), hence it appears that the micro surface area is greater for this tissue. To test this hypothesis, the ratios of the tissue thickness and villus density of the two species were used to calculate the known differences from permeability measurements:

$$\frac{\text{rabbit villus density}}{\text{monkey villus density}} \times \frac{\text{monkey stripped tissue thickness}}{\text{rabbit stripped tissue thickness}} = 1.97 \quad (1)$$

The calculated value only approximates the experimentally determined value (1.80), and this is most likely due to the different shape factors of the individual villi, which have not been accounted for in this calculation. The result indicates a difference in the actual surface area between tissues from these two species.

Further, consistent with this theory, one would expect to observe a correspondingly greater transport of D-glucose

Table IV. Tissue Measurements from Cynomolgous Monkey and Rabbit Jejunum

	Rabbit	Monkey
Unstripped (full thickness, mm)	1.08 (0.36) [33/6]	1.35 (0.27) [24/7]
Stripped (mm) 0.92 (0.03) [35/6]	0.83 (0.02) [58/7]	
Villus density (villi/cm^2)	3904 (580) [10/3]	1784 (181) [13/3]
Cell density (×10^6 cells/cm^2)	5.10 (0.61) [11/4]	5.22 (1.87) [4/2]
Tissue concentration [expressed as % 1-ml donor detected/g tissue (wet wt)]		
Mannitol	4.0	3.4
Methoxyinulin	3.4	3.4
Tissue weight (mg) [weight of exposed (2.0 cm^2) area]		
Dry	34.0 (7.5) [6/3]	41 (7.5) [4/2]
Wet	217.0 (28.0) [6/3]	328.2 (16.5) [4/2]

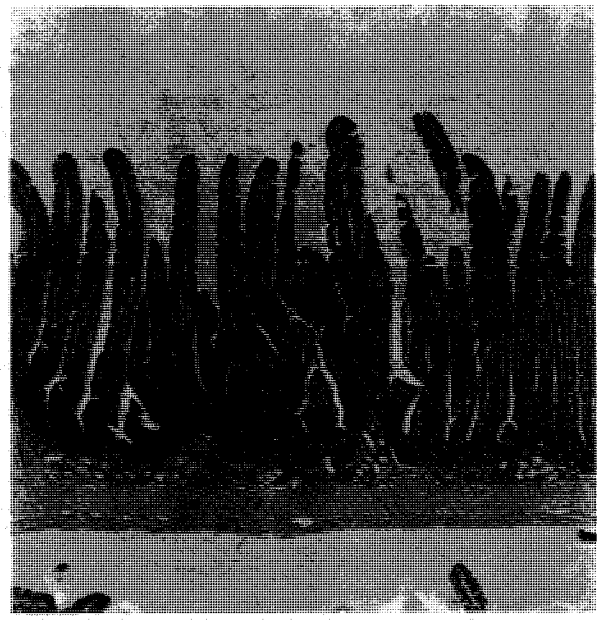

Fig. 2. Example of light micrograph of unstripped rabbit jejunum used to estimate tissue thicknesses. 33×.

because as the surface area increases, so does the area for the cellular route of transport. In contrast, however, to the relationship described for passively transported compounds, D-glucose transport was similar in monkey and rabbit. The facilitated transport of D-glucose is believed to occur in part by binding to cotransporters located in the brush border membrane of the intestinal epithelial cells. Species differences have been noted for the activity (binding capacity) of these active transporters (9,10), and it seems reasonable to assume that such a difference could exist between rabbit and

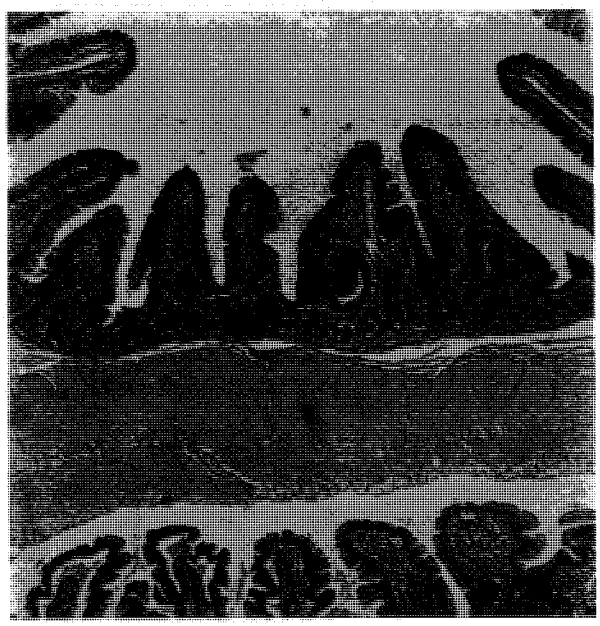

Fig. 3. Light micrograph of unstripped cynomolgus monkey jejunum used to estimate tissue thicknesses. 33×.

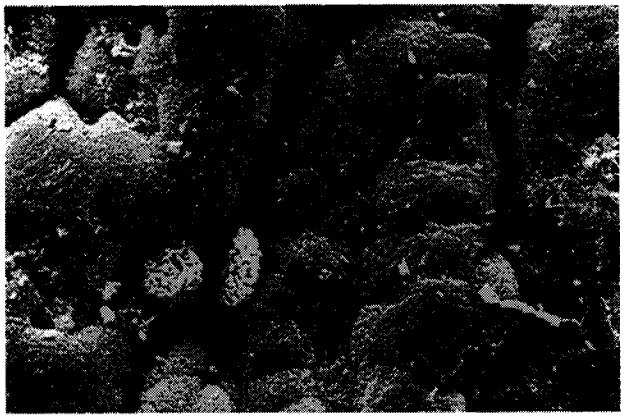

Fig. 4. Example of scanning electron micrograph of cynomolgus monkey jejunum used to estimate mean cell density. 2910×.

monkey. These constants were not estimated in these studies. When the active transport activity was diminished with ouabain, the ratio of the permeabilities observed approached that of the passively transported compounds (1.67 rabbit/monkey).

L-Glucose permeabilities were also not at the ratio predicted by this model. It was expected that the measured permeabilities should be similar to that obtained for mannitol, since these compounds have very similar physicochemical characteristics. In monkey, however, the determined permeability for L-glucose was somewhat higher than that obtained for mannitol. It is possible that L-glucose has some affinity for the glucose transporter, this affinity being much lower than that of D-glucose but still greater than that for mannitol. A low-affinity active transport mechanism has also been suggested for L-glucose in other species (9).

Initially, it was not expected that progesterone should fit the correlation observed for compounds transported via the paracellular pathway. Since progesterone is lipophilic, it is believed to partition through the membrane via the transcellular route. Therefore, the surface area of the lipid barrier should not be as critical to movement of progesterone as it is for hydrophilic entities. Progesterone has been previously shown to be limited in its movement across biological

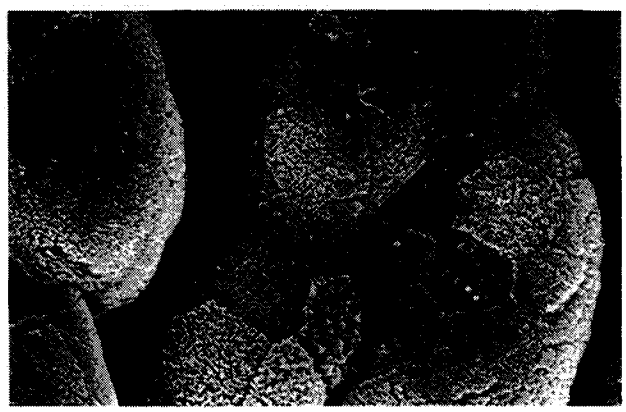

Fig. 5. Example of scanning electron micrograph of rabbit jejunum used to estimate mean cell density. 2940×.

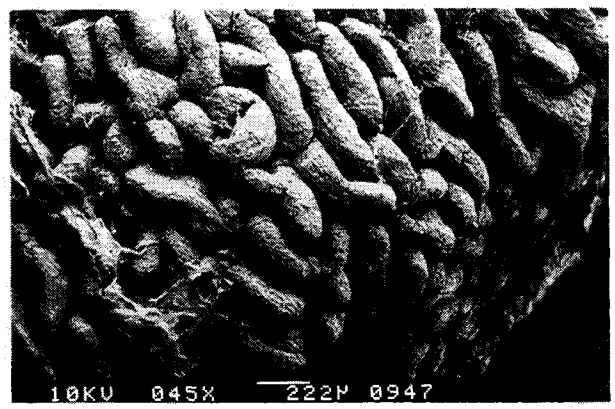

Fig. 6. Example of scanning electron micrograph of cynomolgus monkey jejunum used to estimate villus density. 45×.

membranes by diffusion through stagnant aqueous layers, following a model described by Flynn (11,12). The unstirred water layer associated with the intestinal surface can greatly influence the movement of various species, and considerable attention has been given to the interactions of these substrates and the magnitude of this mucus barrier (13). It is believed that the adherent mucus or barrier mucus rather than the soluble mucus would be most important physiologically in the inhibition of transport of hydrophobic compounds (14). Assuming that this adherent mucus layer associated with the surface of the villi restricts the rate of movement of the lipophilic progesterone molecule and varies in surface area with changes in area of the tissue, then the measured permeability is consistent with the species correlation dependent upon surface area. Since no metabolism studies were conducted, the contribution of potential metabolism to the measurement of progesterone permeability has not been examined.

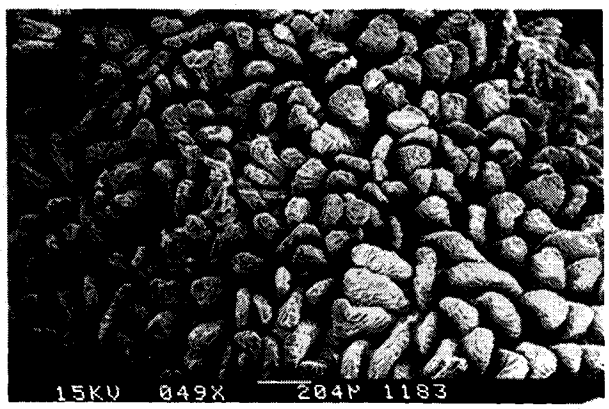

Fig. 7. Example of scanning electron micrograph of cynomolgus rabbit jejunum used to estimate mean villus density. 49×.

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