COMPARISON OF THE THICKNESSES OF THE LATERAL WALL MEMBRANE AND THE MICROVILLUS MEMBRANE OF INTESTINAL EPITHELIAL CELLS FROM RAT AND MOUSE

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The plasma membrane of intestinal columnar epithelial cells is a triple-layered structure which sometimes appears to be geometrically asymmetric and is substantially thicker around the microvilli than on the lateral wall of the cell (2–4, 6–8). However, there has been little agreement in the literature reporting the actual thickness of the microvillus membrane. Values can be found extending from 70 A (5) to 120 A. (7). These discrepancies have been attributed (6) to differences in resolution of the micrographs on which measurements were made and to variation in section thickness, both of which can contribute to a coarsening of the structural pattern and to a widening of the lines corresponding to stained layers in the specimen. Another factor frequently overlooked is that the quantity of stain which is deposited in the tissue during preparation may vary from one laboratory to another, rendering comparison of results dubious. Finally, it is possible that a species difference could account, at least in part, for some of these discrepancies.

In order to investigate this final supposition and at the same time to produce results having some relation to previous work, a preparative procedure based on that recently reported by Sjöstrand (6) was adopted. Both the mice (about 25 gm body weight) and the rats (between 100 and 200 gm body weight) used were starved for 24 hours prior to the commencement of the experiment but were allowed free access to water. The fixative used was a 1 per cent osmium tetroxide solution in veronal acetate buffer made isotonic by the addition of saline. The ice-cold fixative was injected into the jejunum by means of a hypodermic syringe within 30 seconds after killing the animal with a blow at the base of the skull. Two minutes after the injection, small pieces of the jejunum were transferred to a bulk solution of the ice-cold fixative, in which they were allowed to remain for a further 30 minutes. In order to minimise differences due to slight changes in preparative technique, such as small variations in times of fixation, the mice and rats were killed alternately. The specimens were dehydrated in ascending concentrations of alcohol and in dry acetone, and then embedded in Vestopal W. Sections were cut on a Cambridge Huxley-pattern ultramicrotome and, after mounting on carbon-covered grids, were stained in a dilute solution of lead monoxide (1) for 2 minutes.

Sections from specimens of both species were inspected alternately in a Siemens Elmiskop 1b and the image recorded at an electron optical magnification of × 40,000. All measurements were made, with the aid of a 0.1 mm scale viewed through a × 8 lens, on photographic prints at a final magnification of × 160,000. Where possible, these measurements of the thicknesses of the lateral wall membrane and microvillus membrane were obtained from prints taken from the same negative, but where two negatives were required these were recorded from the same cells.

The results obtained from rat tissue differed from those previously reported (2). The thickness of the microvillus membrane was found to be only 101 A (Table I). The decrease when compared with the previous estimate of 115 A may

be due to the change in staining procedure. The mean thickness of the lateral wall membrane was found to be 80 A (Table II), about 20 A less than that of the microvillus membrane.

Even though the plasma membrane on the lateral walls of the cell was asymmetric (Fig. 5), with the more dense component on the cytoplasmic side, the opaque components of the microvillus membrane were of approximately equal density although sometimes the peripheral surface did appear to be more dense. A typical preparation is shown in Fig. 1, and a detail of the microvillus membrane at a higher magnification in Fig. 2.

The studies on the mouse intestinal epithelial cells revealed the surface membrane on both the microvilli (Figs. 3 and 4) and the lateral walls to be an asymmetric triple layer with the more dense component on the cytoplasmic side. The mean thickness of the microvillus membrane was found to be 94 A and that of the lateral wall membrane 82 A (Tables I and II). These results agree with those reported recently by Sjöstrand (6).

From a comparison of these sets of results, it appears that there is little or no difference, either in appearance or thickness, in the lateral wall membrane of intestinal epithelial cells from mice and rats. This is compatible with the unit membrane concept (4). On the other hand, some differ-

TABLE I

The Thickness of the Plasma Membrane at the Surface of the Brush Border of the Intestinal Epithelial Cell

Mouse			Rat		
Micrograph No.	Membrane thickness mm	A	Micrograph No.	Membrane thickness mm	A
1300	1.48*	92.4*	1306	1.78	111.2
1301	1.51	94.4	1305	1.49	93.2
1302	1.50	93.7	1246	1.77	110.7
1239	1.56	97.4	1112	1.76	110
1297	1.59	99.3	1322	1.55	96.9
1298	1.51	94.4	1323	1.52	95.0
1318	1.50	93.7	1328	1.60	100
1307	1.40	87.5	1330	1.54	96.3
	Mean	94 A		Mean	102 A

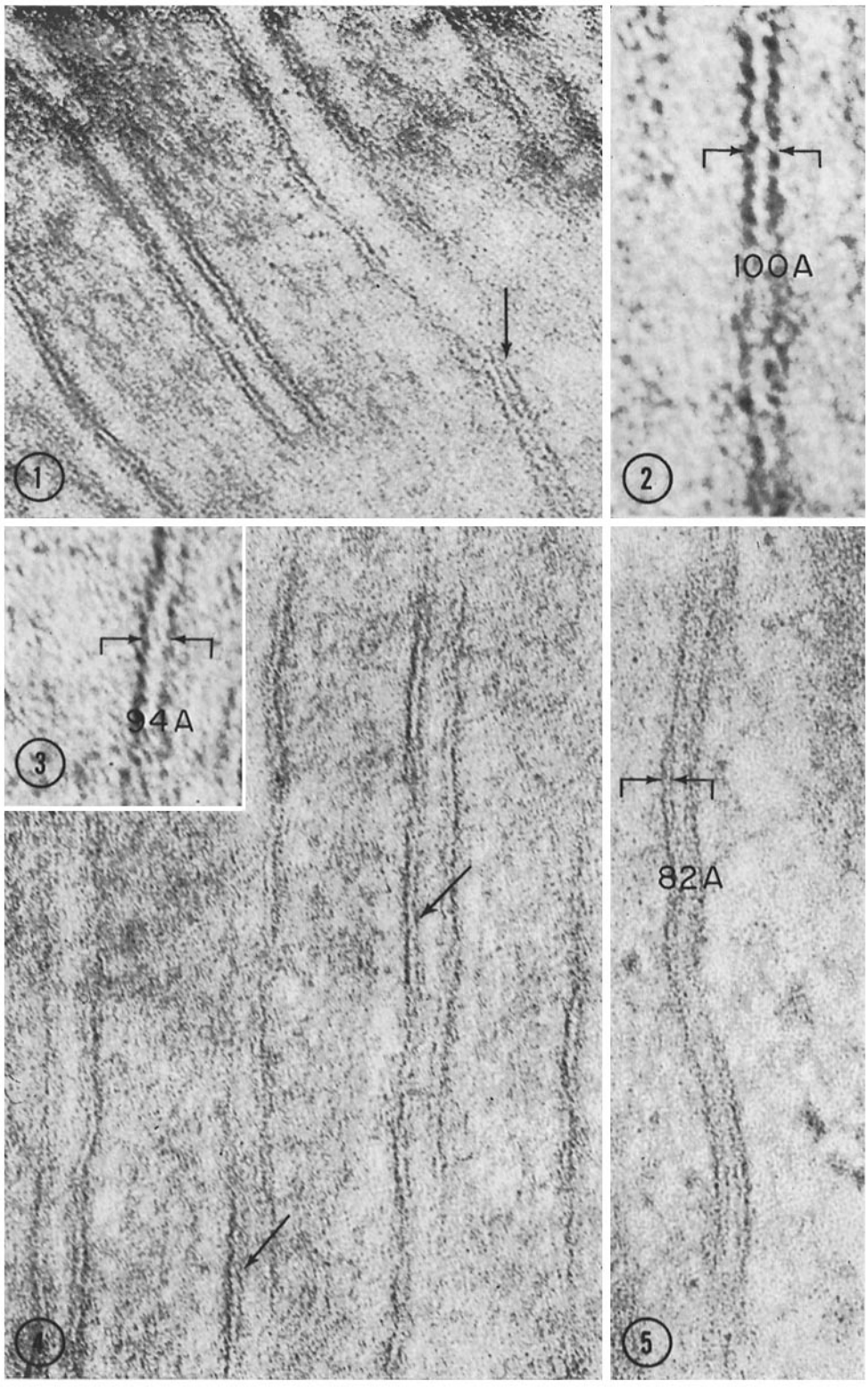
* Each estimate is the mean of ten randomly selected measurements.

TABLE II

The Thickness of the Plasma Membrane at the Lateral Surface of the Intestinal Epithelial Cell

Mouse			Rat		
Micrograph No.	Membrane thickness mm	A	Micrograph No.	Membrane thickness mm	A
1242	1.31*	81.9*	1326	1.25	78.2
1307	1.26	78.8	1327	1.35	84.3
1308	1.35	84.2	1324	1.24	77.5
1339	1.36	85.0	1325	1.28	80.0
	Mean	82 A		Mean	80 A

* Each estimate is the mean of ten randomly selected measurements.


1
100A
2
94A
3
82A
5

ences between the microvillus membranes in these two species are apparent. The mean thickness of the microvillus membrane in the rat exceeded that of the mouse by about 7 to 8 A. Now, variations due to section thickness had been controlled by inspecting the interference colours, using only grey or grey-silver sections in the microscope, and possible differences in resolution were minimised by inspecting the sections alternately. However, such a small difference in dimension is itself of doubtful significance. The peripheral surface of the microvillus membrane of the rat when compared with that of the mouse did appear to have a greater affinity for the lead monoxide stain, and this additional stain could account for the observed small difference in thickness, but such an explanation implies a species difference in the chemical structure of the membrane. It will be interesting to determine whether these small differences will prove significant with respect to the functions of the cell surfaces.

The author wishes to thank the British Cancer Research Fund for the provision of the ultramicrotome for studies on cell surfaces.

Received for publication, July 30, 1963.

REFERENCES

1. KARNOVSKY, M. J., *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 729.
2. MILLINGTON, P. F., and FINEAN, J. B., *J. Cell Biol.*, 1962, **14**, 125.
3. PALAY, S. L., and KARLIN, L. J., *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 363.
4. ROBERTSON, J. D., *Biochem. Soc. Symp.*, 1959, **16**, 3.
5. SUGANAMA, A., *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 292.
6. SJÖSTRAND, F. S., *J. Ultrastruct. Research*, 1963, **8**, 517.
7. SJÖSTRAND, F. S., and ZETTERQUIST, H., *in* Progress in the Chemistry of Fats and other Lipids, (P. T. Holman, W. O. Lindberg, and T. Malkin, editors), London and New York, Pergamon, 1955, **3**, 388.
8. ZETTERQUIST, H., Thesis Karolinska Institutet, Aktiebolaget Godvil, Stockholm, 1956.

FIGURE 1 Part of the brush border of a rat intestinal epithelial cell cut obliquely. The opaque layers of the microvillus membrane are of approximately equal density. The arrow indicates an attachment region, of the five-component type, where two adjacent cells come into contact. × 200,000.

FIGURE 2 A detail of the rat microvillus membrane taken from Fig. 1, clearly indicating a triple-layered structure about 100 A in thickness. × 480,000.

FIGURE 3 A detail of the microvillus membrane from mouse intestinal epithelium (enlarged from Fig. 4) showing the asymmetry of the membrane. Structure here is 94 A thick. × 480,000.

FIGURE 4 Part of the brush border of mouse intestinal epithelium. The denser layer lies on the cytoplasmic side of the membrane (arrows). × 220,000.

FIGURE 5 Cell boundary at the lateral surfaces of two adjacent intestinal epithelial cells of the rat. The membranes are asymmetric with the slightly more dense component on the cytoplasmic side. Region between the arrows measures 82 A in thickness. × 200,000.