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INTERACTION OF RETINOL AND INTESTINAL MICROVILLUS MEMBRANES STUDIED BY FLUORESCENCE POLARIZATION

DAVID SCHACHTER[a], URI COGAN[b] and MEIR SHINITZKY[c]

[a]*Department of Physiology, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032* (U.S.A.), [b]*Department of Food Engineering and Biotechnology, Technion-Israel Institute of Technology, Haifa (Israel) and* [c]*the Laboratory of Membranes and Bioregulation, Weizmann Institute of Science, Rehovot (Israel)*

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SUMMARY

The interaction of retinol and microvillus membranes prepared from the duodenum, jejunum and ileum of the rat can be studied readily by fluorescence and fluorescence polarization. A characteristic pattern of increased retinol anisotropy in membranes from more distal segments is demonstrated. Studies with trypsin indicate that the membrane proteins influence retinol fluorescence intensity and anisotropy.

Although vitamin A is well recognized as an essential nutrient absorbed by the mucosal cells of the small intestine [1], the mechanism of its transit across the microvillus membrane is not yet characterized. Since retinol is also a useful fluorescent probe for membrane studies [2], we explored the feasibility of studying the interactions of the vitamin with isolated microvillus membranes by measurements of fluorescence intensity and polarization. The results below demonstrate that such interactions can be observed readily and provide a useful approach to the study of the intestinal microvillus membrane in general, and the transport of vitamin A in particular.

Experiments were conducted initially at the Weizmann Institute and subsequently at Columbia University with similar results, despite the following differences in procedure. Albino rats of the CR/RAR strain were used at the Weizmann Institute and Sherman strain rats at Columbia. The fluorescence polarization instrument at the Weizmann Institute has been described [3]; that used at Columbia was obtained from SLM Instruments, Champaign, Ill. In all experiments groups of 4–5 rats weighing 200–300 g were fasted for 18 h, the small intestine resected and the mucosa scraped and pooled as previously described [4]. Microvillus membranes from duodenal (proximal 12 cm), jejunal (middle 25 cm) and ileal (distal 15 cm) segments were prepared by either of two methods. In the first procedure brush borders were prepared using hypotonic EDTA [4, 5] and microvillus membranes obtained from these by high-speed homogenization followed by differential centrifugation [6]. The second procedure utilized $CaCl_2$ to obtain a brush border fraction [7] which was then treated as described above to obtain the microvillus membranes. When assayed for maltase activity [4] the final membrane preparations from each intestinal region were

uniformly 15- to 18-fold purified as compared to the corresponding mucosal homogenates. Both procedures yielded similar results in studies with retinol and the data are therefore pooled below.

For fluorescence studies a fresh solution of $4 \cdot 10^{-4}$ M all transretinol (Sigma Chemical Co.) in ethanol was prepared and generally 5 μl added with rapid mixing to 2 ml of phosphate buffered saline [8] containing 50–100 μg of microvillus membrane protein [9]. After 15–20 s the fluorescence intensity and polarization were measured at 23 °C with exciting light of wavelengths 334 nm (Hg line) and passing the emitted light through 1 cm cut-off filters of 2 M $NaNO_2$. The fluorescence of each membrane sample minus retinol and that of retinol added to buffer alone were also determined and subtracted as corrections [10] which never exceeded 20 % of the total values. Fluorescence intensity, F, was defermined as $F = I_{||} + 2\,I_{\perp}$, where $I_{||}$ and $I_{\perp}$ are the fluorescence intensities polarized, respectively, parallel and perpendicular to the direction of polarization of the exciting beam. The polarization of fluorescence was expressed as the fluorescence anisotropy, $r = (I_{||} - I_{\perp})/(I_{||} + 2\,I_{\perp})$ and in terms of the microviscosity parameter $[(r_0/r) - 1]^{-1}$. The latter is proportional to the rotational relaxation time of the probe and provides a quantitative index of the microviscosity around it [11]. The term r_0 is the maximal limiting anisotropy and for retinol in propylene glycol at −50 °C we obtained an r_0 value of 0.367. This observed value, which approaches the upper theoretical limit of 0.40, was used in the calculations below. The microvillus membranes were also studied with the fluorescent hydrocarbon 1,6-diphenyl 1,3,5-hexatriene. This probe has been used to study a number of different membranes [13, 14] and provides information on the hydrophobic interior of the plasma membrane. To load with diphenylhexatriene, microvillus membranes were incubated with a suspension of $2 \cdot 10^{-6}$ M probe at 37 °C for 2 h, as previously described [13, 14]. Fluorescence was estimated as described for retinol, except that the excitation wavelength was 365 nm (Hg line). The r_0 value (maximal limiting anisotropy) used for diphenylhexatriene was 0.362 [13].

To test the effects of trypsin, a sample of microvillus membrane containing 200 μg protein was suspended in 0.4 ml of phosphate buffered saline in the presence or absence of 2 mg/ml of crystalline trypsin (Worthington). After incubation at 37 °C for 4 h the membranes were diluted with 10 volumes of phosphate buffered saline, harvested by centrifugation at 30 000 $\times g$ for 30 min in a Sorvall refrigerated centrifuge (5 °C) and resuspended in the saline for studies with the fluorescent probes.

The fluorescence intensities observed on treating a sample of ileal microvillus membranes with micromolar concentrations of retinol are illustrated in Fig. 1. Binding of retinol by the membranes resulted in a marked enhancement of fluorescence which greatly exceeded that of the probe in buffer alone. Similar results were obtained with preparations of whole brush borders. The polarization of the fluorescence was examined with microvillus membranes from each of 7 groups of rats. Inasmuch as different segments of the intestine are specialized for specific transport functions [1], we prepared and studied separately the duodenal, jejunal and ileal membranes. In every experiment the retinol anisotropy, r, was found to increase progressively in the more distal segments. The values of r (mean $\pm$S.E.) for the duodenal, jejunal and ileal membranes, respectively, were 0.272 ± 0.008, 0.292 ± 0.007 and 0.304 ± 0.009. By paired analysis the duodenal-jejunal, duodenal-ileal and jejunal-ileal differences were significant at the $P < 0.05$, $P = 0.02$ and $P < 0.02$ levels,

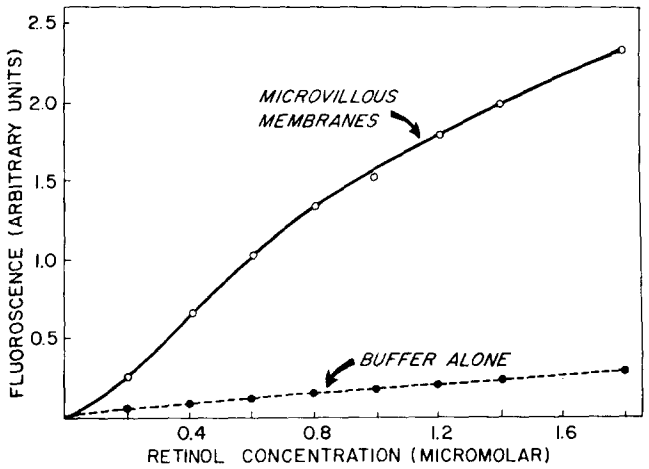

Fig. 1. Fluorescence intensity observed on adding retinol to ileal microvillus membranes (100 μg membrane protein per ml phosphate buffered saline; shown in upper curve) versus buffer alone (lower curve).

respectively. Mean values of $[(r_0/r)-1]^{-1}$ calculated for the duodenal, jejunal and ileal membranes, respectively, were 3.24, 4.57 and 6.23. In contrast, the corresponding mean values for fluorescence intensity were 7.76, 8.70 and 7.70, respectively, not significantly different. The results indicate that with increasing distance from the proximal end of the intestine the membranes offer a hydrophobic environment which is progressively more resistant to rotation of the probe, i.e., a less fluid milieu.

Retinol is transported in plasma via binding to a retinol binding protein and prealbumin [12], and it was of interest, therefore, to determine whether membrane proteins are involved in the binding to microvillus membranes. Accordingly, membranes prepared from 3 separate groups of rats were treated with trypsin and the effects on fluorescence examined with retinol and with diphenylhexatriene. The results are summarized in Table I. Trypsin treatment decreased the retinol fluorescence intensity by 12–20 % in each preparation tested ($P < 0.005$). Simultaneously, retinol anisotropy was decreased in each preparation ($P < 0.001$) and $[(r_0/r)-1]^{-1}$ was reduced by 8–18 %. In contrast, diphenylhexatriene fluorescence intensity and anisotropy were not significantly affected by trypsin. The results indicate that membrane proteins susceptible to tryptic digestion do influence retinol fluorescence, either directly by binding the probe or indirectly by influencing the lipid in the vicinity of the probe. The latter possibility would imply further that retinol and diphenylhexatriene localize in different regions of the membrane. The hydroxyl group of retinol would favor a more polar locus, one more susceptible to the influence of tryptic digestion of proteins, whereas the hydrocarbon diphenylhexatriene would seek more apolar regions in the lipid core of the membrane.

It is further noteworthy in Table I that the pattern of increased retinol anisotropy in ileal versus duodenal membranes was observed also for diphenylhexatriene anisotropy ($P < 0.001$). The results are in accord with more extensive studies with diphenylhexatriene to be reported elsewhere (Schachter, D. and Shinitzky, M., unpublished) and reinforce the conclusion that microvillus membranes from distal

TABLE I

EFFECTS OF TRYPSIN TREATMENT ON FLUORESCENCE OF RETINOL AND DIPHENYLHEXATRIENE IN MICROVILLUS MEMBRANES

Mean values for 3 groups of rats are shown. For the effect of trypsin on retinol fluorescence intensity, paired comparisons (all segments) give S.E. = 0.40, $P < 0.005$. For the effect of trypsin on retinol anisotropy, paired comparisons give S.E. = 0.0018, $P < 0.001$. Paired comparisons of the anisotropy values for diphenylhexatriene in duodenal versus ileal membranes give S.E. = 0.002, $P < 0.001$.

Probe	Segment	Fluorescence intensity (arbitrary units)		Anisotropy (r)		$[(r_0/r)^{-} - 1]^{-1}$	
		No trypsin	Trypsin	No trypsin	Trypsin	No trypsin	Trypsin
Retinol	Duodenal	9.6	8.0	0.265	0.252	2.61	2.20
	Jejunal	11.5	10.1	0.290	0.278	3.77	3.11
	Ileal	9.4	7.5	0.312	0.308	5.65	5.22
Diphenyl-hexatriene	Duodenal	5.5	5.7	0.286	0.282	3.74	3.52
	Jejunal	2.8	2.9	0.294	0.296	4.35	4.48
	Ileal	3.0	3.3	0.304	0.304	5.24	5.24

intestinal segments provide apolar environments which are less fluid and offer greater resistance to the rotation of lipid probes.

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