sometimes quickly killed by copulation (see discussion in ref.[9]) and the new finding that sensitivity is also increased by high growth temperature further complicates the interpretation. There is some evidence that sensitive females are sexually more receptive[12]. A recent observation[13] that oogenesis in sensitive female is only slightly stimulated by insemination could also provide a starting point for further analysis.

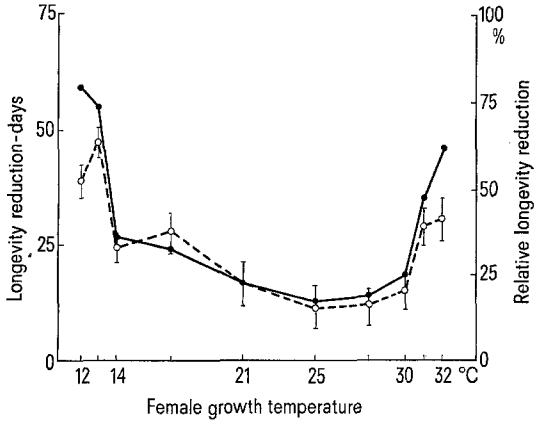

Fig. 3. Variation of female sensitivity to copulation with growth temperature.
○, reduction in longevity as compared to virgins; ●, relative reduction in % of virgin lifespan.

Behavior studies in *Drosophila*[14] showed that usually, in strains where males were sexually very active, the females had a low sexual receptivity and reciprocally. Our results present some analogies with such observations, although the variations are not genetic but epigenetic and reflect the influence of preimaginal environment upon adult physiology. Moreover, these epigenetic effects are much more extreme than those obtained by genetic factors since we demonstrate that males can be indeed harmful and that females can even be killed by copulation[8].

When both sexes are grown under the same thermal conditions, mating probably does not result in very harmful effects because the physiological variations are correlated: when males are the most aggressive, females have a maximum resistance. It is probable that the reproductive incompatibility observed when aggressive males are mated to sensitive females rarely occurs under natural conditions. The possible adaptative significance of these variations is still a matter of speculation, although they are probably parameters of the individual fitness and could be important for insect population dynamics. A better knowledge of these variations can be useful in managing pest control experiments, where laboratory reared males are released in nature for competition with wild individuals.

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Ultrastructural Changes of the Luminal Plasma Membrane of the Transitional Epithelium of the Rat Urinary Tract in Essential Fatty Acid Deficiency

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Summary. Rats fed an essential fatty acid deficient diet (EFAD) showed a statistically significant decrease in the thickness and ultrastructural asymmetry of the luminal membrane and cytoplasmic vesicles of transitional epithelium of the urinary tract, due to a marked thinning of the peculiar thick luminal leaflet. These changes were reversed by adding EFA to the diet. This indicates that the unusual EM appearance of urothelial membrane depends on its content in EFA.

We have been interested in producing in the rat an experimental condition by a deficient diet in certain basic nutrients which should cause changes in cell membranes, expecting that this may contribute to the understanding of their role in membrane organization and function. The most promizing model seemed to be the state of deficiency in the essential fatty acids: linoleic, linolenic and arachidonic (EFA)[2], since they are constituents of phospholipids and cholesterol esters, 2 constant components of biological membranes[3]. It could then be predicted that membrane changes might appear when rats were deprived of EFA. Furthermore, since the condition may occur in man, this could be of further clinical interest[4].

We wish to report that the characteristic thickness and ultrastructural asymmetry of the unit membrane of the luminal plasmalemma and the cytoplasmic vesicles of transitional epithelium of the ureter and bladder (urothelial membrane) was notably altered by feeding rats on a diet which was deficient in essential fatty acids (EFAD).

In the normal animal, the membrane is distinctly thick, showing an asymmetric unit membrane arranged in plaque zones which are bound by short segments of thinner and symmetric membrane. The luminal (outer) osmiophilic leaflet of the plasmalemma and the luminal (inner) one of the vesicles are about twice as thick as the leaflet adjacent to the cytoplasm (Figure 1). In these laminae or leaflets, subunits, which mainly contain

[1] Acknowledgment is due to Mrs. M. G. Iwakawa and N. T. Benítez for technical help and Mr. J. Hiza for animal care. D. Lis and A. Eynard are research fellows of Consejo Nacional de Investigaciones Científicas y Técnicas. Work supported in part by CONICET, Argentina.
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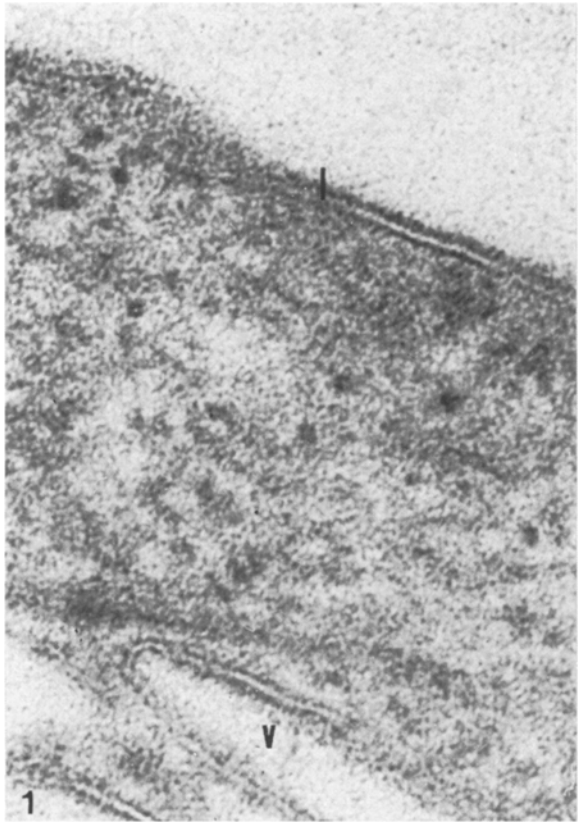
Thickness of lumenal and vesicle membrane of the urothelium in EFA deficient and control rats

	Lumenal plasmalemma				Cytoplasmic vesicle membrane			
	Controls	EFAD	Difference[a] (%)	P[b]	Controls	EFAD	Difference[a] (%)	P[b]
Total thickness	110.6 ± 2.43	100.8 ± 2.65	− 8.8	< 0.05	109.0 ± 2.80	99.2 ± 3.39	− 9.0	< 0.05
External leaflet	45.0 ± 1.83	36.2 ± 1.22	− 19.5	< 0.01	44.6 ± 1.92	35.4 ± 1.59	− 20.6	< 0.01
Middle leaflet	31.7 ± 1.32	31.1 ± 0.77	− 1.9	N.S.	31.7 ± 0.76	31.3 ± 1.11	− 1.3	N.S.
Internal leaflet	33.9 ± 0.93	33.5 ± 0.96	− 1.2	N.S.	32.7 ± 0.54	32.5 ± 1.25	− 0.6	N.S.
Difference (%)[c]	32.7	8.0	—	—	36.4	8.9	—	—
P[d]	< 0.001	N.S.	—	—	< 0.001	N.S.	—	—

Results are mean values ± SEM, expressed in angstrom. 6 experimental and 6 control animals were studied. Values for each animal were obtained averaging 15 measurements, made on 4 EM prints of the membrane total thickness and of each leaflet width. [a]Control values were considered 100%. [b]Statistical significance was estimated by the Student's t-test for non-correlated samples. [c]External and internal leaflet difference percentage. The mean of the internal leaflet values was taken as 100%. [d]Statistical significance of this difference. N.S., non-significant.

lipids, have been recognized[5–9]. On the free side of the plasma membrane, negatively charged sites have been made apparent by cationic dyes, in keeping with the demonstration of sialic acid residues of glycoproteins and glycolipids in isolated urothelial membranes[10].

Materials and methods. For producing the deficiency in EFA, pregnant rats from a Wistar strain were fed from the 1st day of pregnancy with a low-lipid diet. After weaning, the offspring received the same diet[11]. Controls were given the diet supplemented with corn oil. Skin changes in the tail, followed often by necrosis appeared in the deficient animals at about 30 days of age. Gas chromatography revealed the characteristic lipid pattern of EFAD. 6 of each lot of 75 deficient rats and 47 controls, were separated at random for the present study. Animals sacrificed were 140 to 300 days old. Samples of ureter and urinary bladder were fixed in 3% buffered glutaraldehyde and 1% osmium tetroxide, dehydrated and embedded in Epon. Sections were stained with uranyl acetate and lead citrate. On prints of the EM



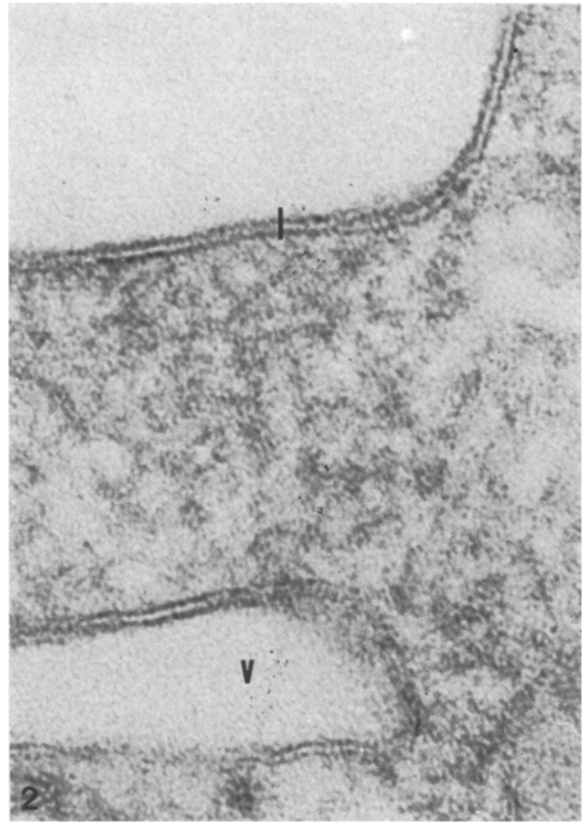


Fig. 1. Control rat. The characteristic asymmetric appearance of the urothelial membrane in both luminal surface (l) and cytoplasmic vesicles (v) is shown. Note the thick, luminal leaflet. × 204,000.

Fig. 2. EFAD rat. The urothelial membrane is thinner. The luminal leaflet is diminished in thickness in both luminal surface and cytoplasmic vesicles. The unit membrane is much less asymmetric. × 204,000.

photographs, estimations were made by 2 persons with a measuring magnifier. Data was processed in a Digital PD P8/e (Digital Equipment Co., Maynard, Mass.).

Results. In the control rats, the whole thickness of the urothelial membranes was about 110 A at the level of the plaque areas. The unit membrane was asymmetric, since there was a significant difference in width between outer and inner leaflets (Figure 1 and Table).

In the EFAD rats, the overall thickness of the membrane was reduced, measuring about 100 A, whereas the asymmetry was notably diminished due to a marked decrease in the width of the thick, luminal leaflet (Figure 2). Changes in the thickness of inner and middle leaflets were statistically not significant. Similar changes in width were observed in the interplaque areas of membrane. No significant difference was noted between luminal plasmalemma and vesicle membrane in control and experimental animals.

Preliminary observations indicated that the ultrastructural changes were reversed by adding to the diet a source of EFA, such as corn oil.

Discussion. Our observations are in keeping with data showing that polyunsaturated fatty acids made up on molar basis 52.3% of the total fatty acid content of the bladder luminal membrane[12]. A perusal of the Table indicated that the 3 EFA gave the high value of 46.6%.

We should like to propose that the unusual asymmetry of the urothelial membrane is primarily due to its content in EFA rather than to the membrane protein or mucoprotein[13]. Furthermore, it may be that EFA containing lipids may have an asymmetric arrangement in the membrane, as has been shown for phospholipid and glycolipid components in red blood cells[14].

The ultrastructural changes of urothelial membrane in the EFAD would support the view that the composition of the dietary fatty acids may affect the pattern of these lipids in subcellular fractions[15]. Furthermore, it would seem that the higher the content of EFA in a membrane, the greater the likelihood that changes will be induced by a deficient diet in these nutrients. Thus, liver mitochondria and plasmalemma[16–18], as well as red cell membrane[19], which are rich in EFA, showed chemical, ultrastructural and physical changes in the EFAD[20–22]. Yet, no variations in the thickness of the membrane of these organelles have been reported in the EFAD.

Since it has been indicated that the presence of lipids containing polyunsaturated fatty acids favours a loose by expanded arrangement of membrane constituents and controls, certain physical properties such as fluidity, as well as functional characteristics like permeability[23], it remains to establish the actual role of EFA in the structural and functional properties of the urothelial membrane which has been considered unusually rigid and impermeable[12–13].

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Characteristics of the Nucleolini Observed under the Electron Microscope

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Summary. Observations with the electron microscope permitted us to ascertain that in molluscs and echinoderms oocytes and in malignat tumour cells, the nucleolini, already seen with the phonton microscope, correspond to the 'clear fibrillar zones'. These present fibrils 40–60 Å thick, spread throughout a very clear matrix. All around these zones there are other closely thickened and interlacing fibrils.

In a previous article which was published in this journal[1], the characteristics of the nucleolini observed under the photon microscope were explained. These nucleolini could also be extracted by micromanipulator needles[2].

In a first series of investigations done by one of us[3], it appeared that under the electron microscope, corresponding to the nucleolini, there were found dense masses of granulations with a diameter not greater than 50 Å. However, the researches begun by Fabbri[4], and followed by other authors[5], have shown how 'clear fibrillar zones (or centres)' or simply 'clear zones (or centres)' correspond to the nucleolini.

Thus it seemed to us necessary to resume our investigations to see whether the previous results obtained by one of us could be confirmed or whether they should be suit-

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