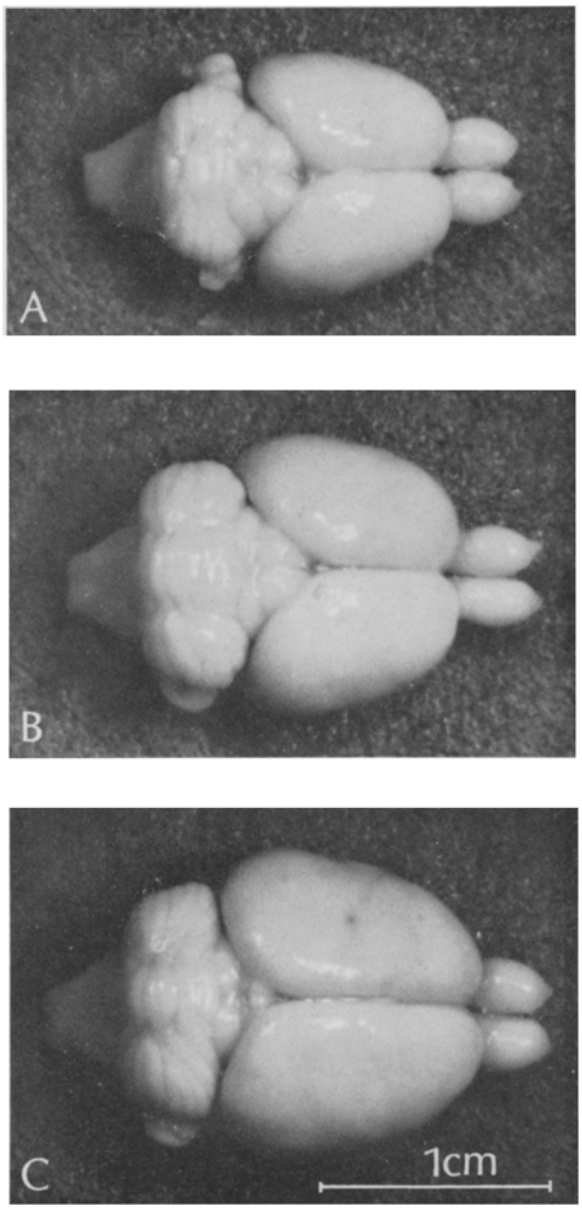

Brains of 21-day-old animals, that received ethylnitrosourea on days 14(A), 17(B) and 20(C) of gestation transplacentally.

compared, it was evident that the cerebral cortex and cerebellum had been affected in a graded fashion. In the animals that received ENU on day 14 of gestation cerebral cortex and cerebellum appeared very small in size (Figure A), and these two neural structures became larger and larger in graded manner as we approached the animals injected on higher stages of gestation (Figures B and C). In other words, ENU had severe effects on the brains of animals injected on earlier stages of embryonic development and these effects became less and less pronounced in the brains of animals injected at increasingly advanced stages of embryogenesis. Identical observations were made on the brains of animals that were sacrificed on days 15 and 75 of postnatal life, which suggested that these gross developmental defects could be detected during weaning period and that they were permanent in nature.

When these brains were dissected into blocks for histology it was found that even the internal structures such as basal ganglia, thalamus and hypothalamus, which could be compared under dissecting microscope, showed similar graded changes in their size. Gross changes in the brains of animals treated with ENU during their embryonic development reflect the possibility of massive changes in the histogenesis of the nervous system as well as in the differentiation of the existing stock of neurons. However, exact nature of these changes will be determined from histological evaluation of this material. It is worthwhile to comment that similar graded changes in the overall size of the brain have been produced by low level doses of X-ray irradiation of the rat embryos in our laboratory as well as at the hands of other investitigators[4,5].

Zusammenfassung. Wiederholte Applikation von Äthylnitroso-Harnstoff bei trächtigen Ratten bewirkt bei Föten eine Hemmung der Gehirnentwicklung, wobei vor allem die Hemisphären und das Cerebellum unterentwickelt bleiben. Diese Hemmung ist am ausgeprägtesteniw wenn die Behandlung am 14. Tag der Trächtigkeit einsetzt.

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[4] S. P. Hicks, Physiol. Rev. *38*, 337 (1958).
[5] R. Rugh, A. Rev. nucl. Sci. *3*, 271 (1953); *9*, 493 (1959).

Milk Microsomes, Viruses, and the Milk Fat Globule Membrane

In 1954 Morton[1] isolated lipoprotein particles from milk fat globules which he later demonstrated had considerable biochemical homology with microsomes derived from endoplasmic reticulum of mammary gland.

Recent examination of mammary tissue of cows and goats by conventional transmission microscopy has shown that the milk fat globule membrane (MFGM) rapidly changes its structure after release of the globule from the secretory epithelium[2,3] thus confirming the prediction of Knoop, Bargmann and their co-workers (for a review, see reference[4]). Immediately after release from the cell the fat globules have a continuous unit membrane plus a thin coat of cytoplasmic material (initial MFGM). This appears to rapidly bleb off in small vesicles or lift off in sheets into the milk serum leaving small blebs and plaques remaining on the 'secondary MFGM'[3], which is seen as a continuous single dense line around the globule[3]. This description has now been found true for milk fat globules

[1] R. K. Morton, Biochem. J. *57*, 231 (1954).
[2] F. B. P. Wooding, J. Cell Sci. *9*, 805 (1971a).
[3] F. B. P. Wooding, J. Ultrastruct. Res. *37*, 388 (1971b).
[4] W. Bargmann and U. Welsch, in *Lactogenesis* (Eds. M. Reynolds and S. J. Folley; University of Pennsylvania Press, Philadelphia 1969), p. 43.

from all the animals so far examined, i.e. human, horse, dog, wallaby, cow, goat, sheet, pig, mouse, rat and guinea-pig (Figure 1, a–d), using the electron microscope techniques detailed in previous papers[2,3].

The particles seen on the MFGM after freeze-etching[5] or shadowing[6] and characterized biochemically as lipoprotein (see reference[7] for a review) are identical in size range and location to the small blebs of unit membrane with electron-dense content shown in Figure 1, b–d. I have also examined the MFGM isolated by churning washed cream and ultracentrifuging the butter milk. Unit membrane bounded vesicles with electron-dense content (inset, Figure 2) were found throughout the pellet produced and the lower layers contained increasingly large profiles of unit membrane coated on one surface with electron-dense material – identical with the initial MFGM (Figure 2) and the MFGM preparations of KEENAN, OLSON and MOLLENHAUER[8]. This is the process used to isolate milk microsomes by MORTON[1] and since they are in the same size range (300–2000 A) it is reasonable to assume that these unit membrane bounded vesicles are the same as MORTON's milk microsomes. Microsomes are generally defined as products of the vesiculation of cellular endoplasmic reticulum. However, in this case the initial MFG unit membrane is partly derived from the plasma membrane (BARGMANN and WELSCH[4]) of the secretory cell and partly directly from Golgi vesicle membrane[2]. Considerable chemical and biochemical sim-

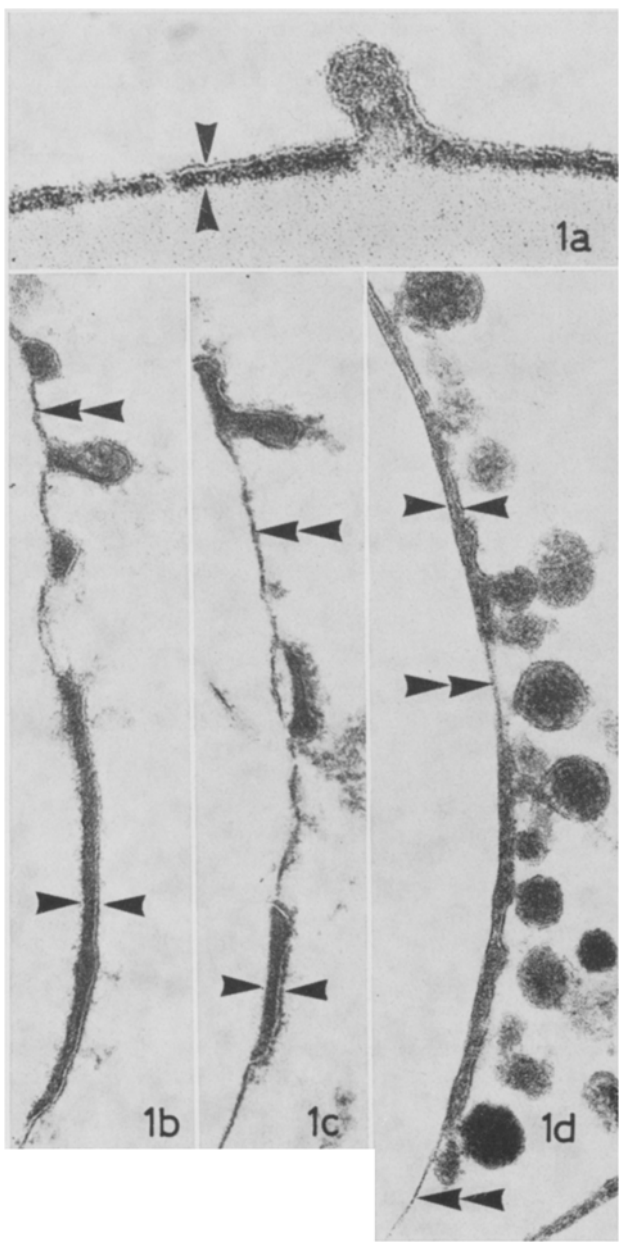

Fig. 1. Transverse sections through the membranes around milk fat globules from a) and b) rat; c) human and d) rabbit. The initial MFGM is shown between arrowheads, the secondary MFGM by double arrowheads. Blebbing is apparent in all three species. a) ×140,000. b) ×120,000. c) ×100,000. d) ×120,000.

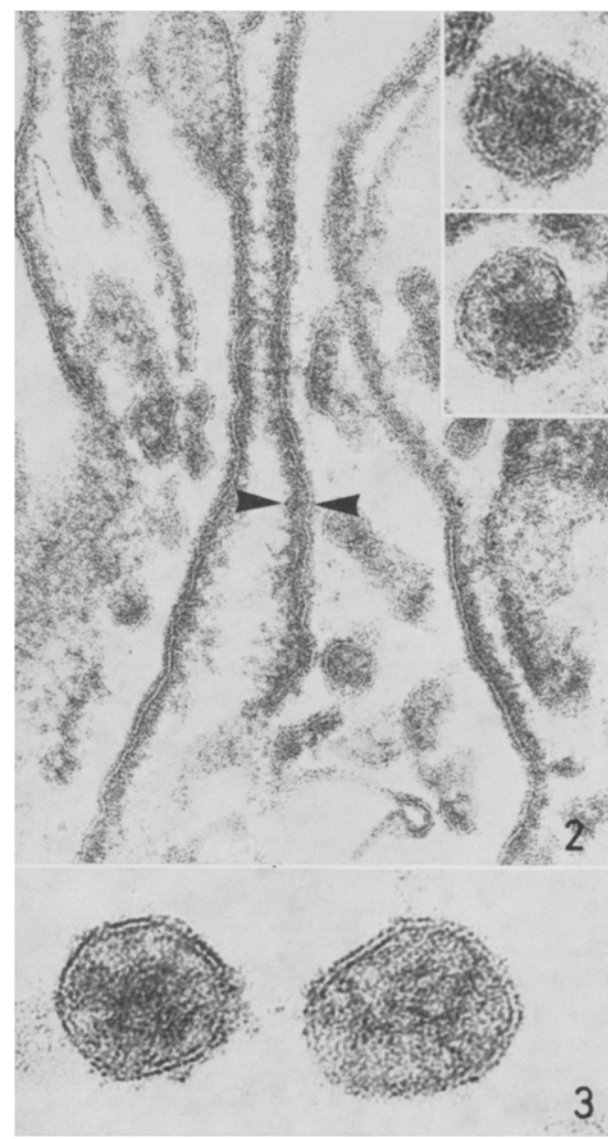

Fig. 2. Section of a pellet from washed cow cream subjected to 1 freeze and thaw cycle and centrifuged at 86,000 × *g* for 90 min. Long profiles of initial MFGM (between arrowheads) are found towards the bottom of the pellet. Inset are vesicles typical of those found throughout the pellet. They are bounded by a unit membrane and contain a dense core. ×36,000. Insets ×250,000.

Fig. 3. Two vesicles from a pellet from cow skim milk centrifuged at 86,000 × *g* for 30 min. ×250,000.

ilarities have recently been demonstrated between MFGM preparations and plasma membrane fractions from mammary cells[9]. The description 'milk microsomes' is thus misleading since the endoplasmic reticulum membrane, although believed to be related dynamically to the Golgi and plasma membranes, has quite different chemical and enzymic components[9].

Reports of cellular rupture which released endoplasmic reticulum (which would produce milk microsomes) during milk secretion[10,11] have been shown by more recent studies[2,4] to be due to deficiencies in fixation and/or embedding technique. It has been shown[12] that milk normally contains a small percentage of milk fat globules with a cytoplasmic crescent attached, but this is always bounded by a unit membrane. Such crescents are too infrequent for their occasional content of endoplasmic reticulum to make a significant contribution to a milk microsome fraction such as that isolated by MORTON.

The work described in this report demonstrates the uniformity of the behaviour and structure of the MFGM in all species so far examined. In the cow the various categories of milk microsomes and lipoprotein particles can thus now be related directly to the initial MFGM. Since no evidence for any significant direct contribution from the cellular endoplasmic reticulum has been observed, 'plasmalemmasome' would be a more accurate descriptive term than milk microsome for the blebs produced by breakdown of the initial MFGM. The blebbing of the initial MFGM (Figure 1, a–d) is similar to the budding off of virus particles from infected cells, and such blebs are equivalent in size and structure to C type particles[13] isolated from the milk of bovine and human leukemia patients. These particles had been tentatively identified as virus particles[14–16]. However, similar particles were found, though fewer in number, in milk from healthy individuals[16].

Examination of the upper layers of pellets from an MFGM fraction isolated from cream (inset, Figure 2) or from a high speed centrifugation of skim milk (Figure 3) show particles which are indistinguishable from the micrographs of virions in the DUTCHER et al.[15] or DMOCHOWSKI[14] papers. This similarity in both size and appearance of some MFGM vesicles and the virions makes identification of the latter in milk fractions very dubious on morphological grounds alone. DE HARVEN[13] has summarized the morphology of the murine viruses and pointed out the dangers of assuming that any membrane bounded vesicle is the correct size range with dense cored contents may be a virus. The profiles of milk microsomes shown (inset, Figure 2 and Figure 3) look identical to some of the less characteristic (C type) virus particles in DE HARVEN's excellent micrographs or the extra-cellular virus particles from cultured leukemic cow cells illustrated in a recent paper[17]. This emphasizes the danger of any attempt to identify such a virus on purely morphological grounds in milk with its natural content of plasmalemmasomes (in all 11 species so far examined) originating from breakdown of the initial MFGM.

Zusammenfassung. Membranen, welche die Fettkügelchen der Milch umhüllen, sind identisch mit dem Plasmalemm der sezernierenden Zellen und nicht mit Überresten von endoplasmatischen Cysternenmembranen. Das Plasmalemm erfährt nach der Sekretion eine charakteristische Veränderung.

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[5] W. BUCHHEIM, Naturwissenschaften *57*, 672 (1970).
[6] F. C. SWOPE and J. R. BRUNNER, J. Dairy Sci. *53*, 691 (1970).
[7] J. R. BRUNNER, in *Structure and Functional Aspects of Lipoproteins in Living Systems* (Eds. E. TRIA and A. SCANU; Academic Press, New York 1969), chapter C7.
[8] T. W. KEENAN, D. E. OLSON and H. H. MOLLENHAUER, J. Dairy Sci. *54*, 195 (1971).
[9] T. W. KEENAN, D. J. MORRÉ, D. E. OLSON, W. N. YUNGHANS and S. PATTON, J. Cell Biol. *44*, 80 (1970).
[10] J. D. FELDMAN, Lab. Invest. *10*, 238 (1961).
[11] O. STEIN and Y. STEIN, J. Cell Biol. *34*, 251 (1967).
[12] F. B. P. WOODING, M. PEAKER and J. L. LINZELL, Nature, Lond. *226*, 762 (1970).
[13] E. DE HARVEN, in *Experimental Leukemia* (Ed. M. A. RICH; Appleton-Century-Crofts, New York 1968), chapter 4.
[14] L. DMOCHOWSKI, in *Current Research in Leukemia* (Ed. B. HAYHOE; Cambridge University Press 1965).
[15] R. M. DUTCHER, E. P. LARKIN and R. R. MARSHAK, J. natn. Cancer Inst. *33*, 1055 (1964).
[16] R. R. MARSHAK and A. A. ABT, in *Experimental Leukemia* (Ed. M. A. RICH; Appleton-Century-Crofts, New York 1968), chapter 8.
[17] J. F. FERRER, N. D. STOCK and P. LIN, J. natn. Cancer Inst. *47*, 613 (1971).

The Ultrastructure of Thiosomes of the Mouse Brain

The brains of mammals contain periventricularly localized glial cells characterized by the presence of cytoplasmic granulations strongly staining with Gomori's chrome haematoxylin and aldehyde fuchsin following acid permanganate oxidation[1]. These granulations were shown to contain large amounts of cysteine[2,3]. The aim of the present paper was to study the ultrastructure of the cysteine/sulphur/-rich glial granulations.

Small fragments of periventricular brain tissue from adult mice were fixed in buffered glutaraldehyde, post-fixed in osmium tetroxide, and embedded in Epon. Ultrathin sections were contrasted with lead hydroxide after KARNOVSKY[4] and examined in a Tesla BS 613 electron microscope.

The cytoplasmic granules of the periventricular glia of the mouse brain show in the electron microscope features distinguishing them from other known cell organelles. Taking this into account, as well as the fact that they contain very much sulphur, they will be called 'thiosomes'.

The thiosomes are large cytoplasmic organelles, 0.5 to 3 μm in diameter. The shape is generally round or oval (Figures 1, 3, 4), sometimes irregular (Figure 2). A single external limiting membrane is present. The matrix of the thiosome is amorphous or granular (Figure 1), showing sometimes 1 or 2 clear vacuoles. The most characteristic

[1] Z. SREBRO, Folia biol., Krakow *17*, 177 (1969).
[2] Z. SREBRO, Experientia *27*, 945 (1971).
[3] Z. SREBRO and T. CICHOCKI, Acta histochem. *41*, 108 (1971).
[4] M. J. KARNOVSKY, J. biophys. biochem. Cytol. *11*, 729 (1961).