fusion of adjacent golgi vesicles by which a significant amount of the cytoplasm is released from the apex of the secretory cell as well as the lipid droplet. This would be an understandable extension of one mechanism already suggested for milk fat globule release[10]. It could also explain the origin of the small pieces of cytoplasm with and without included lipid droplets previously shown to be present in goat's milk[11]. PATTON's[12] hypothetical mechanism for milk fat globule release, depending solely on the specific attraction between the lipids of the secretory cell plasmalemma and those of the cytoplasmic fat globule, cannot so easily be extended to explain the presence of cytoplasmic particles in milk. The decrease in biosynthetic activity in milk on storage[1,2] can be accounted for by a rapid breakdown in the organized structure of the cytoplasmic particles that occurs after they are released from the secretory epithelium.

Since most of the cytoplasmic droplets contained a great number of vesicles or sheets of the rough endoplasmic reticulum it is not surprising that they actively synthesized triglyceride since this is the site of that synthesis in vivo[12]. The preparation had almost identical biosynthetic properties so that of the skim milk and gave a similar pattern of products with respect to time as was found earlier[2]. Stearic acid desaturase activity was present as were the enzymes of the monoglyceride pathway. No stimulation of the biosynthetic reaction was obtained on adding the normal range of cofactors and, unlike skim milk, addition of free glycerol to the milk had no effect. The system is, therefore, more akin to mammary epithelial cells used in vitro[13] than to subcellular homogenates[14]. Also, the particles, like isolated mammary cells, had up to twice the biosynthetic activity when prepared in Krebs-Ringer bicarbonate buffer than in a phosphate buffer although oxidation of (^{14}C)-glucose to CO_2 could not be confirmed unequivocally.

The ability of freshly-secreted milk from goats to sustain triglyceride biosynthesis was not due then to tissue microsomes released into the milk by some process of tissue breakdown. The organized cytoplasmic structure of the cytoplasmic droplets provides a much more understandable system and one which has been shown in this report to incorporate fatty acids by the standard autoradiographic techniques[16].

Summary. The triglyceride biosynthesis which occurs in freshly secreted goat skim milk can be localized predominantly to large (1–8 μm) pieces of membrane-bounded cytoplasm. These contain the usual cytoplasmic organelles (but no nucleus) plus one or more large lipid droplets.

W. W. CHRISTIE and F. B. P. WOODING[17]

Department of Biochemistry, The Hannah Research Institute, Ayr (Scotland KA6 5HL), and ARC Institute of Animal Physiology, Babraham, Cambridge (England), 28 July 1975.

[10] F. B. P. WOODING, J. Cell Sci. *9*, 805 (1971).
[11] F. B. P. WOODING, M. PEAKER and J. L. L. LINZELL, Nature, Lond. *226*, 762 (1970).
[12] S. PATTON, J. Am. Oil Chem. Soc. *50*, 178 (1973).
[13] J. E. KINSELLA, Lipids *5*, 892 (1970).
[14] E. W. ASKEW, R. S. EMERY and J. W. THOMAS, Lipids *6*, 326 (1971).
[15] O. STEIN and Y. STEIN, Adv. Lipid Res. *9*, 1 (1971).
[16] Mr. L. J. JARVIS, Mrs. P. PATERSON and Miss J. V. WILSON gave skilled technical assistance.
[17] ARC Institute of Animal Physiology, Babraham, Cambridge, England.

Dense Core Vesicles in Cerebral Cortex of the Human Fetus

Preliminary studies on the fetal human cortex indicate that synaptogenic activity is initiated by the 8th week of gestation[1]. The initial synaptic contacts were described as being axo-dendritic with all presynaptic terminals containing translucent spheroidal vesicles. Though such contacts with their associated translucent spheroidal vesicles were noted to increase in frequency with advancing age, nothing was ascertained concerning the appearance of other vesicle populations; particularly those of the dense core variety. In view of this, the concern of the present report is to investigate the synaptic development of the human fetal cortex in order to determine when dense core vesicles first appear.

Materials and methods. To this end cortical tissue from 10 human fetuses ranging in age from 10 to 22 weeks of gestation was studied. All fetuses were obtained through hysterectomy abortion under those provisions established by the committee on human experimentation of the Medical College of the Virginia Commonwealth University. Tissue blocks cut from the mid-lateral telencephalon just dorsal to the primordial corpus striatum were immersed in a fixative composed of 2% glutaraldehyde, 2% paraformaldehyde in a 0.1 *M* sodium cacodylate buffer with 0.01% $CaCl_2$. Postfixation was achieved in 1% osmic acid in buffer, and then the tissue was processed in ascending grades of ethanol and embedded in Epon 812. Thick and thin sections were cut on glass and diamond knives respectively. Thick sections were used for orientation and thin sections were mounted on grids, stained with uranyl acetate, lead citrate and viewed and photographed on a Hitachi HU-12 electron microscope.

Results and discussion. Within the cortex of the fetus of 10 weeks gestation occasional synaptic contacts were observed. These contacts appeared to be confined to zones immediately superficial and deep to the developing cortical plate. As noted by MOLLIVER et al.[1], the axon presynaptic terminals contained only translucent spheroidal vesicles. There was no evidence of any vesicles of the dense core variety. By the 15th week of gestation, synaptogenic activity was still confined to the inner and outermost aspects of the cortical plate. However, the number of synaptic contacts has significantly increased and now in addition to translucent spheroidal vesicles (Figure 1), vesicles of the dense core variety are present. Such vesicles are approximately 700–1,000 Å in diameter and possess an electron dense core of 400–500 Å in diameter. These vesicles occur singularly or may be multiple in number and are widespread in their distribution. Some are found in axon cylinders either together with translucent spheroidal vesicles (Figure 2) or in close association with numerous neurotubules (Figure 3). Others lie within distal, dilated axon segments which contain a fine

[1] M. E. MOLLIVER, I. KOSTOVIC and H. VAN DER LOOS, Brain Res. *50*, 403 (1973).

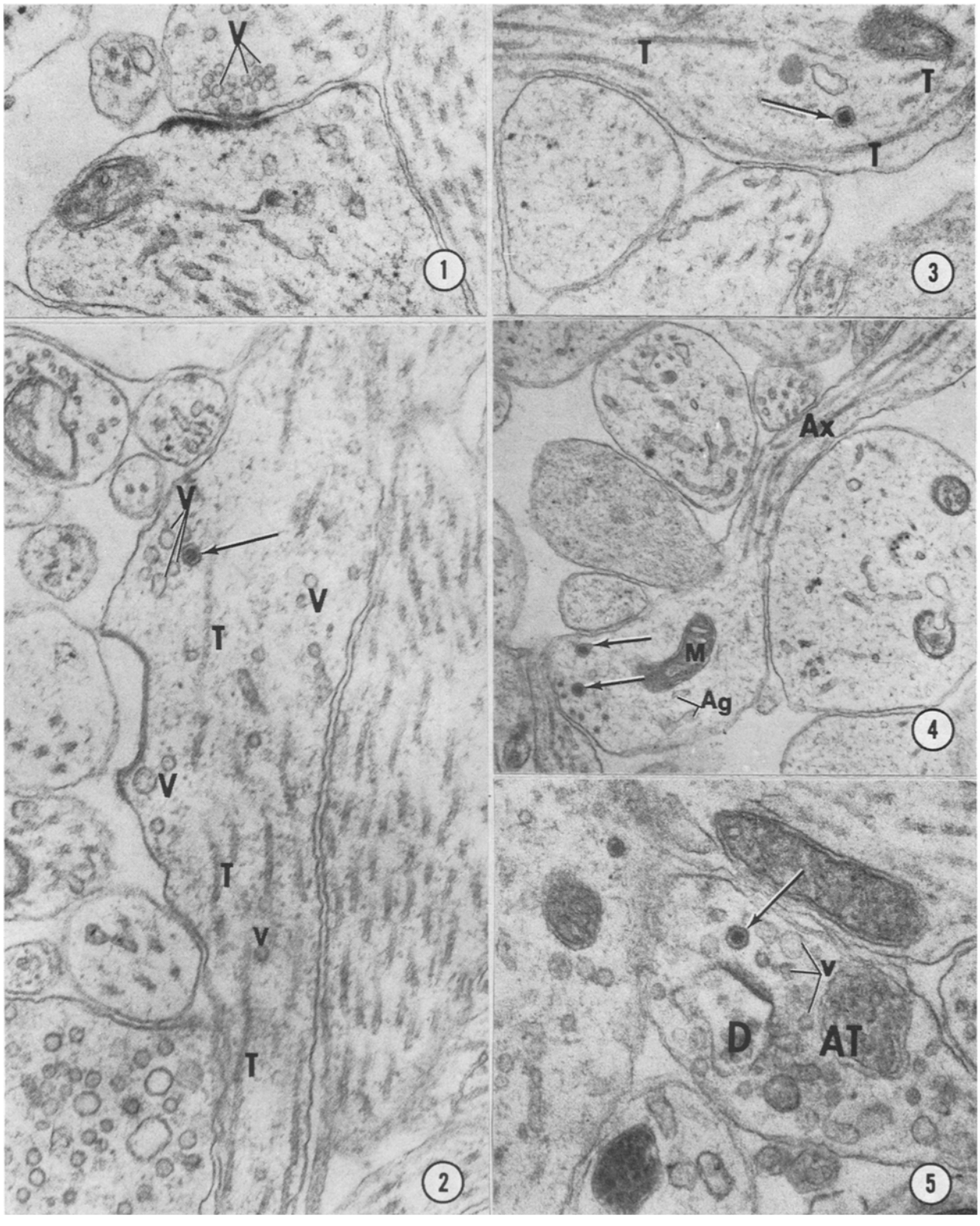

Fig. 1. An axo-dendritic synapse from the zone immediately superficial to the cortical plate. Note that the presynaptic axon terminal contains numerous translucent spheroidal vesicles (V). 15 weeks of gestation, × 45,000.

Fig. 2. This section cut parallel to the long axis of the axon cylinder demonstrates a typical dense core vesicle (arrow) lying among several translucent spheroidal vesicles (V). Also note within the axon cylinder, scattered translucent spheroidal vesicles (V) and neurotubules (T). Zone superficial to the cortical plate, 15 weeks of gestation, × 60,000.

Fig. 3. Within this axon cylinder a dense core vesicle (arrow) is seen in close proximity to several neurotubules (T). Zone deep to the cortical plate, 15 weeks of gestation, × 60,000.

Fig. 4. An axon (Ax) is here seen to terminate in a bulbous expansion, characterized as a growth cone. Note that within the growth cone 2 dense core vesicles (arrows) a mitochondrion (M) and profiles of agranular endoplasmic reticulum (Ag) are seen interspersed in a fine filamentous substance. Zone superficial to the cortical plate, 15 weeks of gestation, × 45,000.

Fig. 5. An axon terminal (AT) forms synaptic contact with a dendritic spine (D). Note that this terminal contains a dense core vesicle (arrow) as well as numerous translucent spheroidal vesicles (V). Zone deep to the cortical plate, 15 weeks of gestation, × 50,000.

filamentous material, mitochondria and agranular endoplasmic reticulum (Figure 4). Lastly, other dense core vesicles are found within presynaptic axon terminals (Figure 5). When within these terminals, the dense core vesicles are frequently found in conjunction with translucent spheroidal vesicles. By the 17th and 22nd weeks of gestation, the pattern of dense core vesicle distribution remains unchanged. However, they are now more frequently seen, as by this period there has occurred a significant increase in synaptic development.

The results of the present study indicate that in the human cerebral cortex by the 15th week of gestation, dense core vesicles are found within axon cylinders, distal, dilated axon segments and within presynaptic axon terminals. Those vesicles within the axon cylinders are probably representative of synaptic vesicles in transit[2]. On the basis of morphological criteria established in other species[3–5], those dilated, distal axon segments containing dense core vesicles appear to be axon growth cones, and thus, the presence of dense core vesicles therein demonstrates that this vesicular component is present in the actively growing axon processes. Lastly, the identification of dense core vesicles within the presynaptic axon terminals seems of particular significance, for it suggests that these vesicles may be involved in the process of neural transmission. Dense core vesicles comparable to those described have been identified in the rodent cerebral cortex and such vesicles were identified as storage sites for the monoamine, norepinephrine[6,7]. Though it is attractive to speculate that the dense core vesicles of the present study are also linked with the storage of norepinephrine, it is impossible on the basis of purely morphological data to make such an assumption. Additionally, the fact that even cholinergic terminals contain occasional dense core vesicles[8], makes the correlation of these dense core vesicles with the monoamine, norepinephrine, all the more equivocal. Though the neurotransmitter linked with these dense core vesicles remains to be identified, the fact that these vesicles are present at all must be considered significant. The presence of dense core vesicles here at the 15th week of gestation indicates that in terms of the synaptic vesicle population, i.e., translucent and dense core vesicles, the typical synaptic complexes of the human cerebral cortex appear quite mature particularly in light of this very early stage of fetal development.

Summary. The present study clearly demonstrates that by the 15th week of gestation dense core vesicles appear within the human cerebral cortex. These vesicles can be identified within axon cylinders, axon growth cones, and axon synaptic terminals. The role of these vesicles is speculative, yet, their very presence at this early fetal stage seems to reflect an advanced state of synaptic vesicle development.

J. T. Povlishock

Department of Anatomy, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond (Virginia 23298, USA),
26 May 1975.

[2] E. G. Gray, J. Cell Sci. *7*, 189 (1970).
[3] J. T. Povlishock, Brain Res. *82*, 272 (1974).
[4] V. M. Tennyson, J. Cell Biol. *44*, 62 (1970).
[5] R. P. Skoff and V. Hamburger, J. comp. Neurol. *153*, 107 (1974).
[6] K. Fuxe, B. Hamberger and T. Hökfelt, Brain Res. *8*, 125 (1968).
[7] T. Hökfelt, Z. Zellforsch. *79*, 110 (1967).
[8] G. D. Pappas, in *Structure and Function of Synapses* (Ed. G. D. Pappas and D. P. Purpura; Raven Press, New York 1972), p. 4.

Primary Neural Induction as Studied by Scanning Electron Microscopy

Primary neural induction has been studied extensively by light and transmission electron microscopy[1,2] and recent studies have demonstrated two morphological markers of primary neural induction in the early stage-5 embryo.

In the light microscope a band of nuclei can be seen immediately anterior to Hensen's node[3] and in the transmission electron microscope a ribosome free area has been observed[4].

The present study investigates the scanning electron microscopic (SEM) appearances during primary neural induction in the chick embryo, paying particular attention to the mesoderm cells and their relationship to the ectoderm layer.

Materials and methods. Stereoscanning electron microscopy (SEM). White leghorn chick embryos were incubated at 37.5 °C until early stage-5[5]. The eggs were opened and some of the albumen poured away. Karnovsky's fixative[6] was then gently injected below and above each embryo. The specimens were cut off the yolk and placed in Karnovsky's fixative for several h. They were then buffered in Cacodylate buffer[7] for several h, followed by 2% OsO_4 for 1 h and dehydrated in a graded series of acetone/water. In 70% acetone/water a narrow fracture was directed across the area pellucida through the region immediately anterior to Hensen's node. It was then possible to study mesoderm cell contacts in the area pellucida and anterior to the node. In some specimens, the endoderm was peeled off in the Cacodylate buffer to study the ventral ectoderm in this region.

The specimens were critical point dried by replacing the acetone with liquid CO_2 and mounted on Cambridge stubs with colloidal silver paint. They were then coated with gold and palladium approximately 40 nm thick and viewed on a Cambridge S4-10 scanning electron microscope.

Transmission electron microscopy (TEM). Specimens for study by TEM were fixed and buffered as described for SEM specimens. They were then dehydrated in a graded series of ethanol/water and embedded in Araldite[8]. Following ultrathin sectioning they were viewed on a Siemens Elmiskop 1B (80 KV) transmission electron microscope.

[1] L. Saxen, Devl. Biol. *3*, 140 (1961).
[2] J. Gallera, G. Nicolet and M. Baumann, J. Embryol. exp. Morph. *19*, 439 (1968).
[3] M. A. England, Experientia *29*, 1267 (1973).
[4] M. A. England, Experientia *30*, 808 (1974).
[5] V. Hamburger and H. L. Hamilton, J. Morph. *88*, 49 (1951).
[6] M. J. Karnovsky, J. Cell Biol. *27*, 137A (1965).
[7] M. Plumel, Bull. Soc. Chim. biol., Paris *30*, 129 (1948).
[8] A. M. Glauert and R. H. Glauert, J. biophys. biochem. Cytol. *4*, 191 (1958).