vasopressin and oxytocin stores in hypothalamus and neurohypophysis of rats deprived of water up to 12 days was found either not to change or even to be more marked under reserpine treatment[23]. It may therefore be concluded that biosynthesis and release of neurohypophysial hormones in dehydrated animals seem to be influenced by reserpine each in a different way. The hypothetical assumption is that the existence of at least two kinds of reserpine-sensitive supraoptic and paraventricular afferents of monoaminergic origin cannot be excluded – one stimulating the rate of neurohormone synthesis and the other inhibiting its release[24, 25].

Summary. In rats dehydrated for 48 h and injected intraventricularly with L-cysteine-^{35}S-hydrochloride, the specific activity of TCA-precipitable material, both in the hypothalamus and neurohypophysis, was found to diminish under reserpine treatment.

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The Site of Triglyceride Biosynthesis in Milk

The study of triglyceride biosynthesis by the mammary gland using biopsy samples of mammary tissue in vitro is beset by numerous practical difficulties, not the least of which is the removal of the large amount of fibrous connective tissue in the gland. In 1964, McCarthy and Patton[1] found that freshly-secreted goat milk would actively incorporate isotopically-labelled fatty acids into lipids on incubation in vitro. Recently the potential of this simple system for the study of triglyceride biosynthesis in vitro was investigated and it was determined[2] that this activity was enzymatic and that the enzymes of both the glycerol-3-phosphate and monoglyceride pathways of triglyceride biosynthesis were present. Also, stearic acid was desaturated to oleic acid in milk and virtually all the oleic acid formed was found in esterified form. This, together with the finding that the reactions were not stimulated by additions of the relevant cofactors (ATP, glycerol-3-phosphate, coenzyme A and Mg^{++} for glyceride synthesis, together with NADH for desaturation) singly or in combination suggested that the enzymes concerned were contained within a membrane-bound particle in goat milk. Patton, Durdan and McCarthy[3] localized the active fraction in skim milk by centrifuging it after the incorporation of radioactivity from added fatty acids. Nearly all of the radioactivity was found in a fraction immediately above the casein pellet which contained a loose fluffy material. Freshly-secreted sow's milk (expressed after oxytocin administration) which had less than half the biosynthetic activity of goat milk, showed a similar localization[4]. The material was not characterized morphologically in these studies but it was suggested that it was the lipoprotein material known to be present in skim milk. This material had been called 'milk microsomes' by Bailie and Morton[5] because of its biochemical similarity to tissue microsomes. Recent work has indicated several possible sources for the lipoprotein fraction from skim milk, notably the milk fat globule membrane[6], the secretory cell plasmalemma[7], and debris from leucocytes[8]. In non-mastitic milk, the first two probably predominate. The present report describes a simple method for the isolation of the active fraction from goat skim milk and demonstrates that the material synthesizing triglyceride consists not of 'milk microsomes' but mainly if not exclusively of plasma membrane bounded pieces of cytoplasmic material from the secretory cell.

Freshly-secreted goat milk[2] was centrifuged at 40,000 *g* for 15 min in an angle-head rotor on a MSE High Speed 25 centrifuge at 0 °C. The skim milk fraction was removed from the sedimented material and added to 5 volumes of 0.08 *M* calcium chloride solution. A pellet, formed on centrifugation at 400 *g* or for 5 min at 4 °C was washed once with 10 ml of 0.15 *M* KCl solution buffered with 0.1 *M* phosphate (pH, 7.0, 4 °C) and was then suspended in this solution for incubation in vitro with (1-^{14}C)-palmitic acid as described earlier[2]. (Calcium chloride solutions have been used in a similar manner to isolate microsomes from cellular homogenates[9]). The pellet had 80–150 % of the biosynthetic activity of an equivalent volume of 40,000 *g* skim milk.

Examination of the pellet by electron microscopy showed that it consisted mainly of membrane-bounded pieces of cytoplasmic material each with a lipid droplet but no nucleus (Figure 1). Rough endoplasmic reticulum in sheets, vesicles or swollen cisternae was usually present in the pieces with an occasional mitochondrion (Figures 2 and 3). More rarely parts of the golgi apparatus could be identified, together with granule-containing vesicles which were identical to the golgi vesicles containing casein that are typical of the lactating mammary secretory epithelial cell. On any one section the cytoplasmic pieces varied considerably in the degree of structural preservation of the organelles and could be arranged in a hypothetical sequence of degeneration (Figure 1).

No sign of any of the vesicular structures characteristic of the skim milk lipoprotein[6, 7] was found in the precipitate from calcium-treated skim milk. Autoradiography of sections of the pellet of the precipitated material, which

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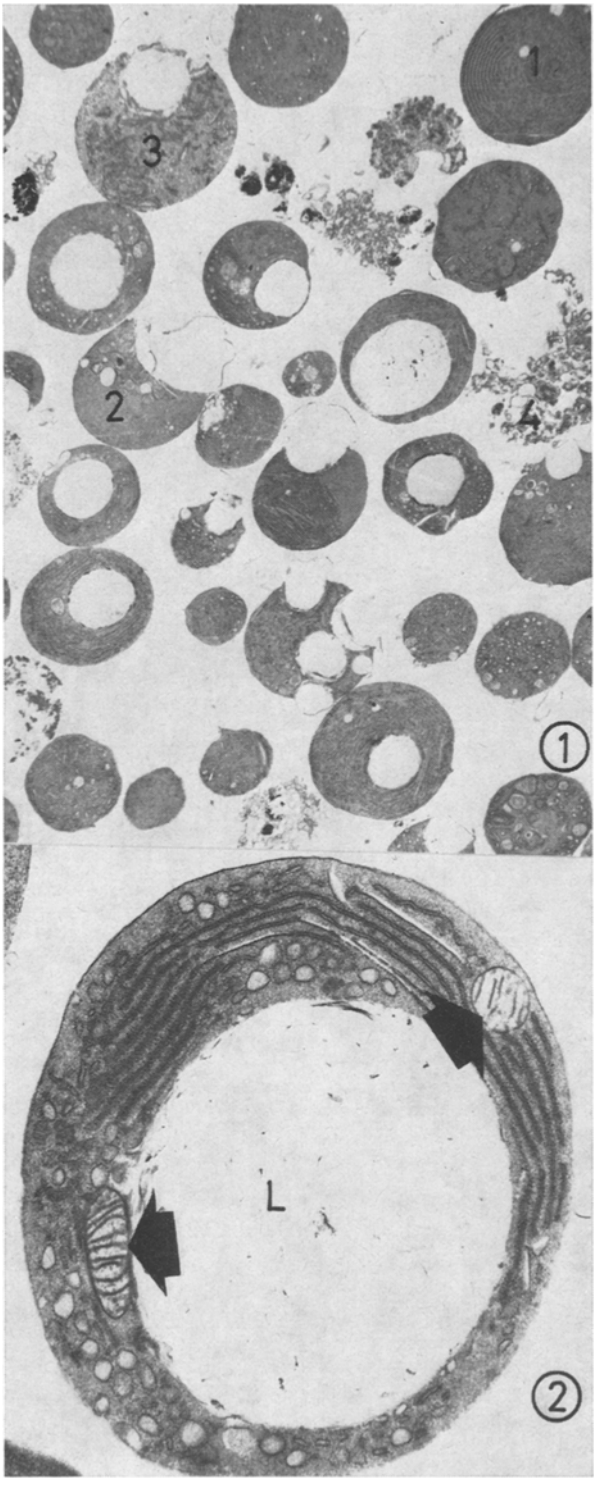

Fig. 1. Section through a pellet produced by adding 0.08 *M* $CaCl_2$ to goat skim milk and centrifuging the resulting precipitate at 400 *g* for 5 min. The skim milk is the supernatant produced by centrifuging (40,000 *g* 15 min) goat milk which is collected at the 2nd of 2-hourly milkings after normal milking. The techniques of processing the pellet for electron microscopy are described fully elsewhere[6]. The pellet consists of membrane-bounded pieces of cytoplasmic material, each containing a large lipid droplet which is not necessarily included in this plane of section. The cytoplasm contains endoplasmic reticulum in variable amount and form. Numbers 1–4 make up a possible sequence in breakdown of the cytoplasmic pieces. × 3,200.

Fig. 2. Detail of a cytoplasmic droplet. Note the numerous lamellae and vesicles of rough endoplasmic reticulum, 2 mitochondria (arrows) and a large central lipid droplet (L). × 15,000.

had been incubated with a radioactive fatty acid before or after precipitation, showed significant incorporation into the lipid droplets contained within each cytoplasmic fragment (Figures 3 and 4), thus confirming their role in the synthesis of triglycerides. In this instance also, virtually all the esterified radioactive fatty acid (largely triglyceride) was associated with the precipitate and very little (less than 5%) with the supernatant layer. Chloroform-methanol extraction of the pellet of precipitated material abolished the autoradiographic reaction. Further evidence that the enzymes were particulate came from an experiment in which the 40,000 *g* skim milk was passed through a 3 μm millipore filter; only 15% of the biosynthetic activity was in the filtrate while the remainder was on the filter.

The reason that such large (up to 5 μm) pieces of cytoplasmic material remained in the skim milk even after a 15 min centrifugation at 40,000 *g* was probably that the lipid droplet acted as a buoyancy chamber, balancing the weight of the cytoplasm. The particles could be sedimented by prolonged centrifugation at 40,000 *g* or higher when the pieces appeared in the fluffy layer on top of the casein pellet. The origin of the cytoplasmic droplets can be accounted for by sequential

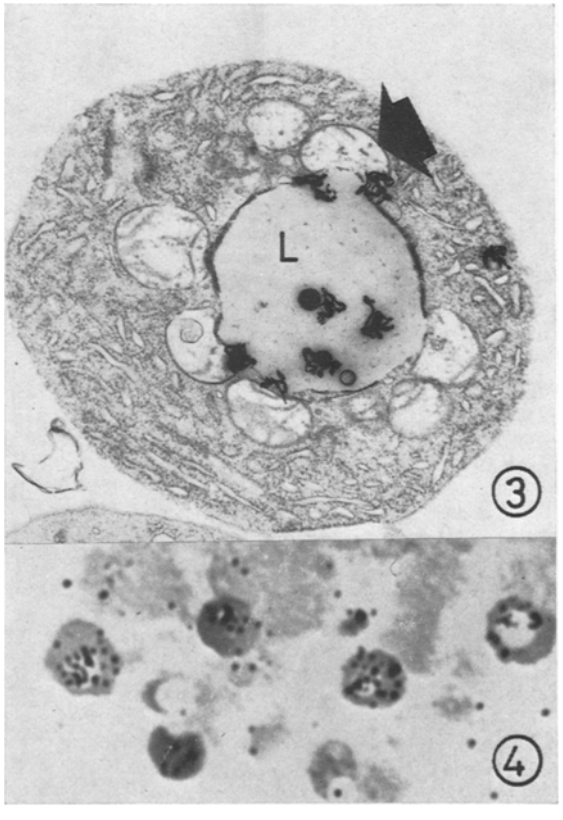

Fig. 3. Electron microscope autoradiograph (for method see[15]) of a cytoplasmic droplet in the calcium-induced precipitate from skim milk incubated with (^{14}C)-linolenic acid. There are 5 silver grains localized over the central lipid droplet. Note the mitochondria (arrow) in the adjacent cytoplasmic piece. × 62,000.

Fig. 4. Light microscope autoradiograph (for method see[15]) of a thick plastic section from thin layers of calcium-induced precipitate from skim milk. The skim milk was incubated with (^{3}H)-palmitic acid prior to calcium treatment. Localization of the silver grains is predominantly to the cytoplasmic droplets. × 1,200.

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.

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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

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