23 L.S. Johnson and F.M. Sinex, J. Neurochem. *22*, 321 (1974).
24 J.V. Small and A. Sobieszek, J. Cell Sci. *23*, 243 (1977).
25 E. Lazarides and B.D. Hubbard, Proc. natl Acad. Sci. USA *73*, 4344 (1976).
26 W.W. Franke, E. Schmid, M. Osborn and K. Weber, Proc. natl Acad. Sci. USA *75*, 5034 (1978).
27 J.M. Starger, W.E. Brown, A.E. Goldman and R.D. Goldman, J. Cell Biol. *78*, 93 (1978).
28 E. Lazarides and D.R. Balzer, Jr, Cell *14*, 429 (1978).
29 G.S. Bennett, S.A. Fellini and H. Holtzer, Differentiation *12*, 71 (1978).
30 S.A. Fellini, G.S. Bennett, Y. Toyama and H. Holtzer, Differentiation *12*, 59 (1978).
31 H. Shibayama and J. Kitoh, Acta Neuropath. *41*, 229 (1978).
32 H.M. Wisniewski, H.K. Narang and R.D. Terry, J. Neurol. Sci. *27*, 173 (1976).

Secretory phenomenon of the adenohypophyseal cells viewed with the scanning electron microscope

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Summary. Exocytosis from rat adenohypophyseal cells, probably somatotrophic, was studied in the scanning electron microscope after freeze fracturation of the glands. This technique permitted the distinguishing of a clearly delimited exoplasm interrupted by the passage of the secretory granules. It was postulated that the exoplasm could be involved in the control of the release of secretory granules in the endocrine cells studied. At the basal cell surface a simultaneous discharge of several secretory granules has been detected.

Exocytosis from different endocrine cells has been studied in the transmission electron microscope (TEM) on sections and on freeze-etched replicas[2-8], so that this phenomenon is now well understood. However, the scanning electron microscope (SEM) offers some new details concerning the discharge of hormonal granules which may further our knowledge about this phenomenon.

Materials and methods. Following perfusion through the abdominal aorta of adult male Wistar rats with Ringer's solution (pH 7.3; 330 mosmoles; +4 °C; 13.6 kPa), the hypophyses were prefixed with a mixture composed of 2% glutaraldehyde and 1% formaldehyde in 0.15 M cacodylate buffer (pH 7.2; 710 mosmoles; 13.6 kPa) for 2 min. The glands were then prepared and stored in the same mixture for 45 min. After postfixation in 1% osmium tetroxide, the hypophyses were washed in a fresh buffer solution, dehydrated in increasing concentrations of alcohol, transferred to liquid nitrogen, carefully fractured according to Tokunaga et al.[9] and critical-point dried with Freon 13. After gold metallization, the specimens were observed in a JEOL JSM-35 scanning electron microscope.

Observations. In freeze-fractured cells, probably somatotrophic cells, the secretory granules are clearly visible and measure about 0.3–0.5 µm (figures 1 and 2, G). They are concentrated in the cell pole facing the blood capillary (figure 1), the endothelial cells of which are very flattened (figures 1 and 2, C). The spherical secretory granules (figure 1, arrow) are seen perforating the thin cytoplasmic rim, the exoplasm (figure 1, E) and partially penetrating into the narrow pericapillary space (figure 1, S). Other granules lie in a cytoplasmic protrusion (figure 1, P) that is in contact with the outer aspect of the capillary endothelium. In some areas, the exoplasm shows delimited interruptions (arrowheads), the borders of which may fuse with those of the secretory granules (asterisk). Some irregularly shaped and partially dissolved granules are seen within the pericapillary space (white arrowhead).

On the basal cell surface (figure 2, BS) the secreting adenohypophyseal cells show smaller and larger apertures in which the expulsion of a single granule (figure 2, arrowhead) or more (figure 2, encircled area) is seen.

Discussion. The secretory phenomenon can be studied under the scanning electron microscope on freeze-fractured specimens only, since sectioning of the organs causes serious artifacts.

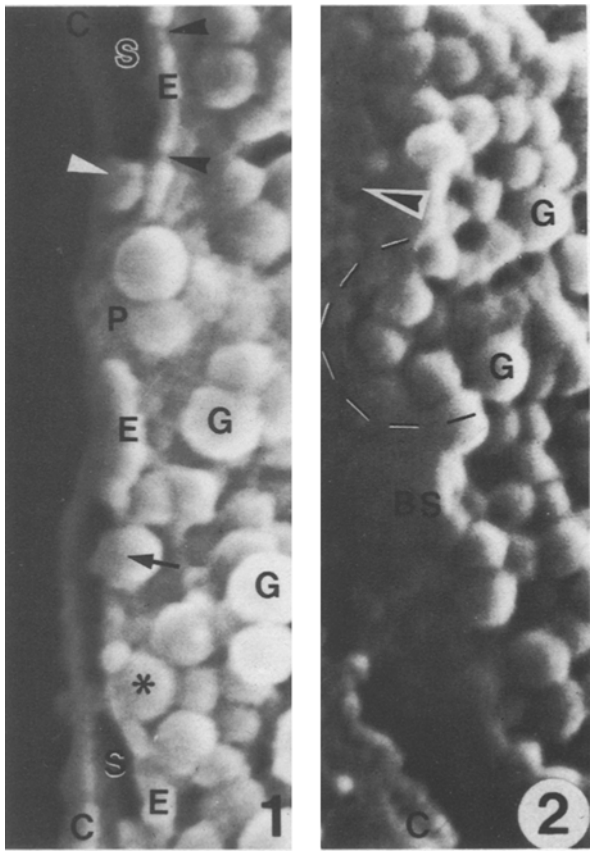

Fig. 1. Profile image of the secretory phenomenon. From the cell body (right) the secretory granules (G) pass a thin exoplasm (E) which shows some interruptions (arrowheads). The arrow points to a hormone granule protruding into the pericapillary space; the white arrowhead shows the remnants of such a granule. An asterisk indicates a hormone granule, the borders of which fuse with the exoplasm. × 22,500.

Fig. 2. Overview of the fractured basal cell surface (BS). An arrowhead shows the protrusion of a single secretory granule. At least 3 secretory granules in the course of exocytosis leave the cell through a large opening (encircled area). × 22,500.

The 3-dimensional image of exocytosis in the adenohypophyseal cells corresponds to that obtained in the TEM on the sections as profile images[2] and on the freeze-etched replicas as overviews[3]. The SEM gives both projections at the same time, but with some loss of details due to its weaker resolving power. Therefore we could not observe the accumulation of granules in the deep intracellular invaginations[2] nor the fusion of the granule membrane with the plasmalemma[2-7]. Nevertheless, the release of the hormone granules in the pericapillary space is very clear, as is the presence of their remnants in this space. The interruptions of the cytoplasmic rim (figure 1, arrowheads) may correspond to the exocytotic stomata left after the extrusion of the granules from the cell[7].

The exocytosis of single hormone granules viewed on the basal cell surface agrees with observations made on the freeze-etched replicas. The phenomenon of the grouped extrusion of secretory granules through a large opening on the basal cell surface is a new observation made with the SEM. This fact speaks in favour of the ability of the adenohypophyseal cells to empty very rapidly during moments of increased functional demand.

Another new observation made with the SEM is the distinct delimitation of the 0.12–0.25 µm large exoplasm. This finding is related to the presence of microfilaments in this zone[5,10,11] which allows a greater density to the exoplasm than to the underlying cytoplasm. This fact proves that the exocytosis from the adenohypophyseal cells (and probably from other endocrine cells) is an active phenomenon depending upon the contraction or relaxation of the exoplasmic cytoskeletal web.

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2 H. Ueberberg and Ch. Hohbach, Z. Zellforsch. *132,* 287 (1972).
3 R. Stoeckart, H.G. Jansen and A.J. Kreike, Z. Zellforsch. *131,* 99 (1972).
4 B. Krisch, K. Becker and W. Bargmann, Z. Zellforsch. *123,* 47 (1972).
5 S. Daikoku, T. Takahashi, H. Kojimoto and Y.C. Watanabe, Z. Zellforsch. *136,* 207 (1973).
6 L. Orci, in: Proceedings of the 5th International Congress of Endocrinology, vol.2, p.7. Ed. V.H.T. James. Excerpta Medica, Amsterdam 1976.
7 L. Orci, A. Perrelet and D.S. Friend, J. Cell Biol. *75,* 23 (1977).
8 D.T. Theodosis, J.J. Dreifuss and L. Orci, J. Cell Biol. *78,* 542 (1978).
9 J. Tokunaga, M. Edanaga, T. Fujita and K. Adachi, Archs histol. jap. *37,* 165 (1974).
10 R.W. Hull, J. Protozool. *8,* 351 (1961).
11 B.S. Spooner, K.M. Yamada and N.K. Wessells, J. Cell Biol. *49,* 595 (1971).

Binding of [^{3}H]GABA and [^{3}H]muscimol to subcellular particles of a neurone-enriched culture of mouse brain[1]

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Summary. Binding of [^{3}H]GABA and [^{3}H]muscimol, indicative of GABA-receptors, has been demonstrated in a neurone-enriched culture of embryonic mouse brain using a ligand-binding technique. Evidence is provided for the existence of different populations of GABA-receptors.

Physiological, pharmacological, and biochemical studies have revealed that γ-aminobutyric acid (GABA) appears to be a major inhibitory neurotransmitter in the vertebrate CNS[2-4]. High-affinity binding of GABA and of the potent GABA-agonist muscimol, in the absence of added Na^+, has provided further evidence for the existence of synaptic GABA-receptors[4-6]. Recent studies from our laboratory have revealed further that such binding processes occur in particles prepared from neurone-enriched cultures of embryonic rat brain, but not in those prepared from cultured astroblasts of neonatal rat brain[7-10]. Herein, we report that 'specific' binding of both [^{3}H]GABA and [^{3}H]muscimol occurs to particles prepared from a neurone-enriched culture of embryonic mouse brain.

Materials and methods. The method of Sarlière et al.[11] was used to prepare neurone-enriched primary cultures of mouse brain. The cerebra of 14–15-day-old mouse embryos (ICR strain) were excized and placed in Eagle's medium (as modified by Dulbecco; GIBCO, Grand Island, N.Y.) supplemented with glucose (600 mg%), Na^+-bicarbonate (0.20%), penicillin (100 units/ml), streptomycin-SO_4 (100 µg/ml) and fungizone (GIBCO, 250 ng/ml). Cerebra were then freed of meninges and blood vessels and passed through a sterile nylon sieve (82-µm pore size). Dissociated cells were collected in 6.3 ml of the above-mentioned medium containing 20% heat-inactivated fetal calf serum. Aliquots (1.5 ml) of cell suspension, representing 3 cerebra, were added to each polylysine-coated[12] plastic flask (Nunclon; 250 cm^3) which contained 8.5 ml of medium, and flasks were incubated at 37 °C in a humid atmosphere of 95% air/5% CO_2. For the coating procedure, 200 µg of poly-L-lysine (Sigma Chemical Corp.; mol. wt 130,000) in 8 ml of water were left in contact with the plastic surface for at least 1 h. Development of the cultures was viewed by phase-contrast microscopy.

After 4 days in culture, cells from 18 flasks were pooled and collected by centrifugation at 1000 × g, 10 min, and frozen at −25 °C for 2–14 days. Frozen samples were resuspended in 20 ml of deionized water, homogenized, allowed to stand at 23 °C for 20 min, and then centrifuged at 50,000 × g, 20 min. This cycle of washing and centrifuging was repeated twice more, and then the pellet (weighing about 280 mg) was resuspended in 3.0 ml of Na^+-free, Tris-citrate medium (50 mM; pH 7.1). All further operations were conducted at 0–4 °C using Tris-citrate medium. Aliquots (0.1 ml) of tissue suspension (about 0.4 mg protein) plus 0.1 ml of medium, either free of added substance or containing 10^{-3} M unlabelled GABA (final concentration) were mixed and allowed to stand for 10 min. Then, 0.25 ml of medium was added, containing (as final concentrations) [^{3}H]GABA or [^{3}H]muscimol at 6.2 or 15.4 nM plus [^{14}C]sucrose at 0.12 or 0.31 µM; samples were mixed, allowed to stand for 20 min, and then centrifuged at 57,000 × g, 5 min. The high concentration of unlabelled GABA was used to estimate 'specific' binding of the labelled ligands; [^{14}C]sucrose was used to estimate the amounts of supernatant fluid