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.

1 With the technical assistance of Mr P.-A. Milliquet.

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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

Binding of [^{3}H]GABA and [^{3}H]muscimol to a particulate fraction of a neurone-enriched culture of embryonic mouse brain

Radioactive ligand and concentration	Distribution ratio Control	In presence of 10^{-3} M unlabelled GABA	Amount bound (fmole/mg protein) Control (A)	In presence of 10^{-3} M unlabelled GABA (B)	'Specific' binding (A minus B)
[^{3}H]GABA, 15.4 nM	1.39 ± 0.04	1.13 ± 0.05**	327 ± 6	262 ± 19**	65
[^{3}H]GABA, 6.2 nM	1.57 ± 0.06	1.15 ± 0.05**	139 ± 11	104 ± 6*	35
[^{3}H]Muscimol, 15.4 nM	2.74 ± 0.13	1.08 ± 0.22**	675 ± 8	286 ± 52**	389
[^{3}H]Muscimol, 6.2 nM	3.51 ± 0.31	1.33 ± 0.26**	344 ± 22	128 ± 20**	216

Means ± SD for 3 separate samples in all cases, except for 'specific' binding which was calculated; * and ** indicate $p < 0.01$ and $p < 0.001$ with respect to corresponding control values (Student's t-test; 2-tailed). Distribution ratio = dis per min ^{3}H/g pellet/dis per min ^{3}H/g supernatant.

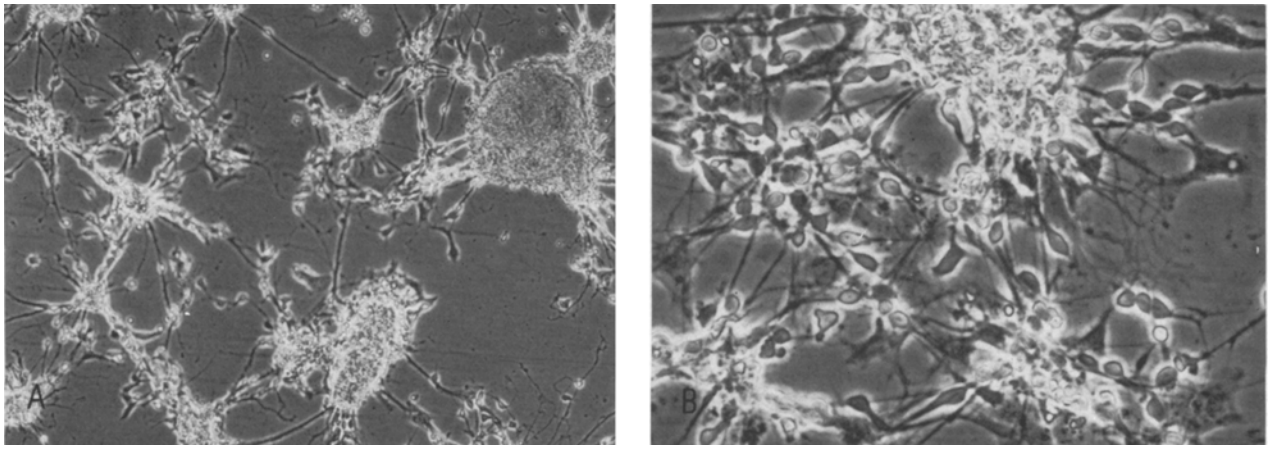
trapped in the pellets. Both [^{3}H]GABA and [^{3}H]muscimol were maximally bound under the conditions employed, and both ligands were maximally displaced by 10^{-3} M unlabelled GABA. γ-[2,3-^{3}H(N)]Aminobutyric acid (36.12 Ci/mmole), [methylene-^{3}H(N)]muscimol (13.68 Ci/mmole), and [U-^{14}C]sucrose (673 mCi/mmole) were purchased form New England Nuclear.

Results and discussion. Representative phase-contrast photomicrographs of 5-day neurone-enriched cultures are shown (figure). Note the clumps of cells and the developing fibre network.

For binding experiments, final pellets weighed 5.1 ± 0.4 mg (24) and contained 66 ± 5 mg protein/g (24); means ± SD, numbers of samples in parentheses. Pellet/supernatant distribution ratios for [^{14}C]sucrose remained constant at 0.85 ± 0.06 (24) under all experimental conditions and were always significantly lower than corresponding distribution ratios for [^{3}H]GABA or [^{3}H]muscimol (table). Addition of excess unlabelled GABA (10^{-3}M) significantly decreased the distribution ratios of both [^{3}H]GABA and [^{3}H]muscimol at both ligand concentrations used, and [^{3}H]muscimol distribution ratios were always greater than corresponding values for [^{3}H]GABA (table). In the absence of excess unlabelled GABA about 2–2.5 times more [^{3}H]muscimol than [^{3}H]GABA was found in the pellets. Calculation of 'specific' binding (i.e., the difference between control values and those obtained in the presence of 10^{-3} M unlabelled GABA) revealed that about 6 times more [^{3}H]muscimol than [^{3}H]GABA binding sites existed in the particles at the ligand concentrations used.

In accord with previous results[7–9], these data have revealed that 'specific' binding of [^{3}H]GABA and [^{3}H]muscimol, indicative of GABA-receptors, can be detected in neurone-enriched cultures of embryonic rodent brain using ligand-binding techniques. Furthermore, these receptors exist during a period of development characterized by very few (if any) synapses[12]. Thus, the development of GABA-receptors, like that of cholinergic receptors (e.g., Vogel et al.[13]) appears to precede the development of synaptic contacts. Other recent data which have revealed that the dissociation constant and maximal binding capacity of this binding of [^{3}H]muscimol, at values of about 9×10^{-9} M and 270 fmoles/mg protein, respectively[8], are of the order expected for GABA-receptor interaction, and the finding that the substrate-specificity of [^{3}H]muscimol binding in particles of neuronal cultures is similar to that in cerebral membrane preparations[9] further support the contention that GABA-receptors are involved. The finding that greater amounts of [^{3}H]muscimol than of [^{3}H]GABA could be displaced by excess unlabelled GABA was expected, since similar results have been obtained with preparations of rat whole brain homogenates[14,15] and neurone-enriched cultures of embryonic rat brain[7]. As with neurone-enriched cultures of rat brain[7], the present study revealed about six times more 'specific' [^{3}H]muscimol than [^{3}H]GABA sites. Since some of the binding sites that are labelled by [^{3}H]muscimol are not labelled by [^{3}H]GABA, further evidence is provided for the existence of different populations of 'GABA-receptors'.

These results have provided evidence that the so-called 'specific' binding of [^{3}H]GABA (or [^{3}H]muscimol) is not necessarily associated with synaptic GABA-receptors. However, since no 'specific' [^{3}H]GABA or [^{3}H] muscimol binding occurred to particles prepared from cultured glioblasts[7,10], it seems that both labelled ligands can serve as useful 'neuronal markers'.



Phase-contrast photomicrographs of a neurone-enriched culture of the cerebra of 14-day-old mouse embryos that was grown for 5 days on a polylysine-coated surface. Note the extensive neurite formation: *A* × 165; *B* × 265.

1 This study was supported by C.N.R.S., ATP N° 3356 and by Commissariat à l'Energie Atomique, Departement of Biologie. Thanks are due to Mlle Marie-Anne Frenkel for her excellent technical assistance.
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Isolation of biologically active alkaloids from Korean mistletoe *Viscum album, coloratum*[1]

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Summary. Anticancer activity of certain highly cytotoxic alkaloids present in Korean mistletoe has been demonstrated in experimental animals. Unlike European mistletoe, no cytotoxic proteins were found in the Korean mistletoe.

Mistletoe plant belongs to genus *Viscum* which contains a variety of plants found all over the world. Mistletoe plant is a parasite and is found to grow on a variety of deciduous trees including apple, ash hawthorn, lime and acorn. The use of mistletoe for the treatment of human cancer had been suggested by Steiner[3]. Subsequent work using injectable preparations of European mistletoe 'Iscador' reported favorable effects against human malignancies[4-9].

The toxic properties and the toxic components of European mistletoe, *Viscum album* L., have been analyzed and described by Vester and reviewed by Evans and Preece[10]. Vester et al.[11-16] used extract of European mistletoe to demonstrate its anticancer activity against cultures of sarcoma 180 and HeLa cells. The antitumor activity of mistletoe extracts against sarcoma 180 in Swiss mice was also demonstrated. The most active component was present in a protein fraction whereas some activity was also found in protein free fractions. In these studies toxicity in vivo or cytotoxicity in vitro were not correlated with the carcinostatic (in vivo) effects of mistletoe. Samuelsson[17] has studied and isolated the toxic component of mistletoe *Phoradendron serotinum* growing on *Juglans hinsii* Jepson (collected in Martinez, California) and identified it as a protein (Phoratoxin) with mol wt of 13,000. Phoratoxin produced pharmacological effects which were similar to viscotoxin obtained from *Viscum album* L., but on weight basis Phoratoxin was 10 times less effective than viscotoxin. These proteins unlike normal chemotherapeutic agents were not immunosuppressive. There are literature[18] suggestions that some protein fractions may activate the immune competence of the host. Samuelsson has also described the isolation of Phoratoxin from mistletoe. *Phoradendron tomentosum* (DC) Engelin subsp. *macrophyllum* (Cockerell) Wiens[19]. The purified toxic protein was shown to have a mol. wt of 5000 and LD_{50} (lethal dose 50) in mice 0.57 mg/kg. The anticancer activities of the purified proteins have not been described. There is no literature describing toxic effects of alkaloidal components of European or Californian mistletoe. Here we wish to report the isolation of alkaloids from *Viscum album, coloratum* (a Korean mistletoe) and describe their anticancer activities.

Isolation of mistletoe alkaloids. Twigs and leaves from *Viscum album, coloratum* were ground and chopped in a blender and then extracted with aqueous acetic acid (2%) by continuous agitation of the suspension for 24, 48 and 72 h. At this time the suspension was filtered and the filtrate carefully lyophilized. Under these conditions a brown pow-

Table 1. Activity of mistletoe extract and mistletoe alkaloid fractions against in vitro cultures of leukemia L1210*

Concentration (μg/ml)	Growth inhibition (%)											
	Crude mistletoe extract	Alkaloid extract	Alkaloid fraction No.**									Iscador
			I	II	III	IV	V	VI	VII	VIII	IX	X	
810.00	100	100	–	–	–	–	–	–	–	–	–	–	92
230.00	100	100	–	–	–	–	–	–	–	–	–	–	44
23.00	23	100	100	100	100	100	41	98	44	40	45	41	6
2.30	20	21	17	100	78	86	–	26	–	–	–	–	–
0.46	6	–	7	95	18	51	–	12	–	–	–	–	–
0.23	–	–	14	63	19	33	–	26	–	–	–	–	–
0.09	–	–	7	10	9	11	–	7	–	–	–	–	–

* Suspension cultures of L1210 cells (6×10^4 cells/ml) were incubated at 37 °C in a CO_2 incubator for 48 h with indicated concentrations of various materials obtained from mistletoe. The results are expressed as number of cells counted in the drug treated dishes as compared to the untreated controls at the end of the incubation period.

** Fractions were obtained as chromatographically homogeneous components by preparative tlc of the mistletoe alkaloid extract.