varicose-like structures could be seen. Both bipolar and multipolar cells were found throughout the period of development studied (6–24 days). Morphological changes in the CAJAL cells of the rabbit cerebral cortex are therefore minute from the 6th day onwards.

If CAJAL cells serve a distinct function, it is possible that they undergo biochemical changes with age. Such changes could make the staining of the adult cells rather difficult, using techniques which visualize them so readily in the brain of embryonique and inmature animals. Taking proper precautions in the preparation, we have, however, been able to see CAJAL cells by the Golgi staining in the cerebral cortex of adult animals, thus confirming data from investigations on the canine brain by FOX and INMAN[11].

Further work should concentrate an electrophysiological investigation of CAJAL cells, since, lacking an axon, these cells might exhibit a possible function through slow, non-conducted membrane potential variations[12].

Resumen. Las células de CAJAL en los animales estudiados, no poseen axon. Morfológicamente son similares a las grandes amacrinas de la plexiforme interna de la retina.

M. BARÓN and A. GALLEGO

Department of Physiology and Biochemistry, Madrid Medical School, Madrid (Spain),
7 September 1970.

[11] M. W. FOX and O. INMAN, Brain Res. *3*, 192 (1966).
[12] Supported by a grant from The Seguridad Social, Instituto Nacional de Previsión.

The Localization of Tellurium in Tellurium-Induced Hydrocephalus[1]

If tellurium is experimentally fed or injected into adult animals or birds, it can result in the deposition of dark bodies in cerebral neurons, the so-called 'black brain'[2–4]. Electron-microscope studies of the brains of rabbits and Syrian hamsters given tellurium have suggested that the tellurium particles are localized in the lysosomes of neurons and glial cells[5–7]. It has been suggested that the tellurium is present in cerebral tissues as tellurous acid[7–9].

Small amounts of elemental tellurium included in the normal diet of pregnant Wistar and Long-Evans rats can result in hydrocephalus of the fetuses and newborns[9–11]. Radioactive tellurium 127m, as tellurous acid, included with this diet will localize in the fetal brain[9,12], and can be visualized with the light-microscope using autoradiographic techniques[12]. Radioactive tellurium 127m, being a strong γ-emitter (99.2%) and a very weak β-emitter (0.8%), is adequate for light microscopy autoradiography but not for electron-microscopy autoradiography[12,13]. This communication reports observations of small dark particles, presumably telluric, in lysosomes of neurons and glial cells in the tellurium-induced hydrocephalic brains of newborn rats.

[1] This work was supported by USPHS research grant No. NSO-8891.
[2] F. JAHNEL, I. H. PAGE and E. MULLER, Z. ges. Neurol. Psychiat. *142*, 214 (1932).
[3] A. PENTSCHEW, Arch. Psychiat. Nervenkr. *102*, 749 (1934).
[4] W. W. CARLTON and W. A. KELLY, Toxic. appl. Pharmac. *11*, 203 (1967).
[5] H. HAGER, Arch. Psychiat. Nervenkr. *201*, 53 (1960).
[6] K. BLINZINGER and H. HAGER, Verh. dt. Ges. Path. *49*, 357 (1965).
[7] R. NIZUMO, Yokohama med. J. *20*, 101 (1969).
[8] R. H. DEMEIO, J. Ind. Hyg. Toxic. *28*, 229 (1946).
[9] W. F. AGNEW, F. M. FAUVRE and P. H. PUDENZ, Expl Neurol. *21*, 120 (1968).
[10] F. GARRO and A. PENTSCHEW, Arch. Psychiat. Nervenkr. *206*, 272 (1964).
[11] S. DUCKETT, in Report of the VIth International Congress of Neuropathology, Paris (1970).
[12] S. DUCKETT and K. A. O. ELLEM, in preparation.
[13] W. F. AGNEW, private communication.

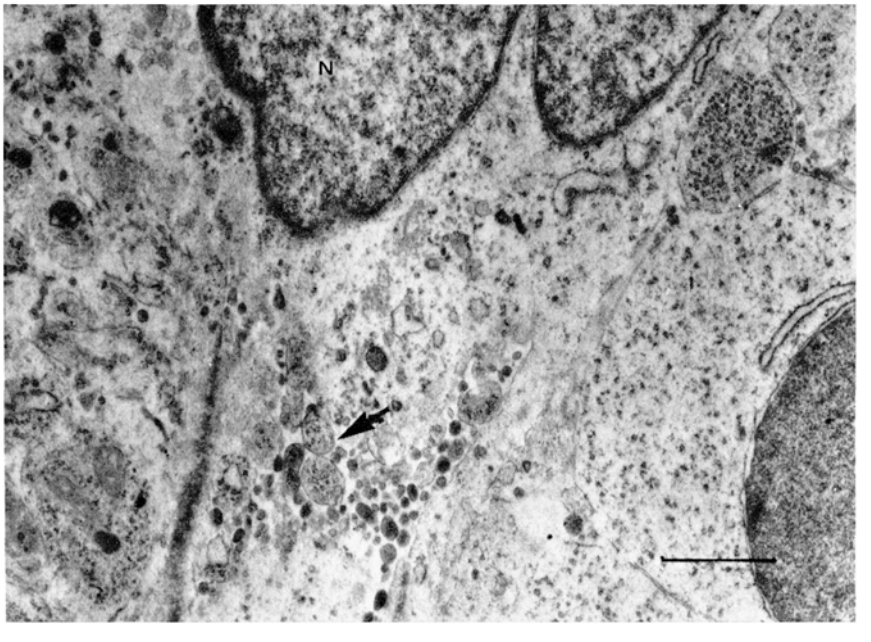

Fig. 1. A Neuroblast from the telencephalon of a rat fetus, whose mother was fed tellurium, showing black particles within membrane-bound bodies in the cytoplasm. The arrow points to the bodies shown in Figure 2. N = nucleus.

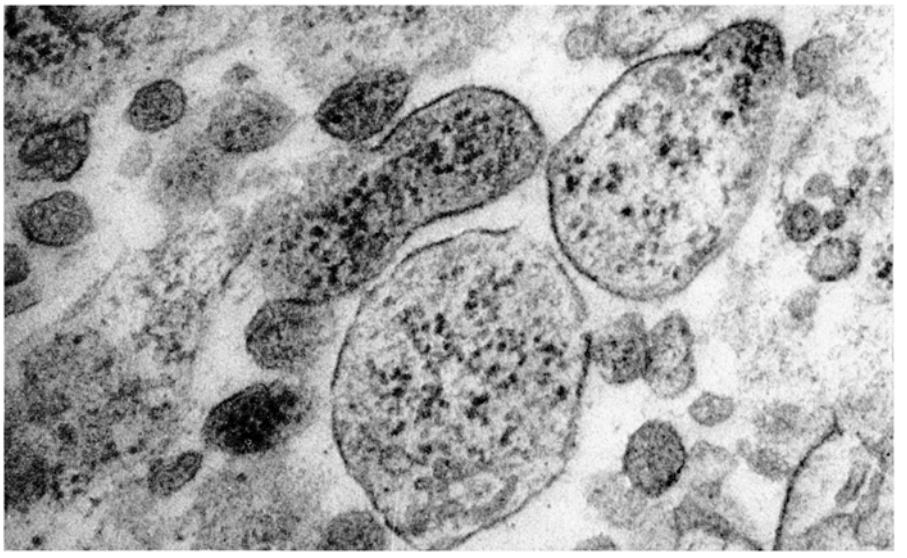

Fig. 2. Magnification of the area designated by the arrow in Figure 1. ×57,000.

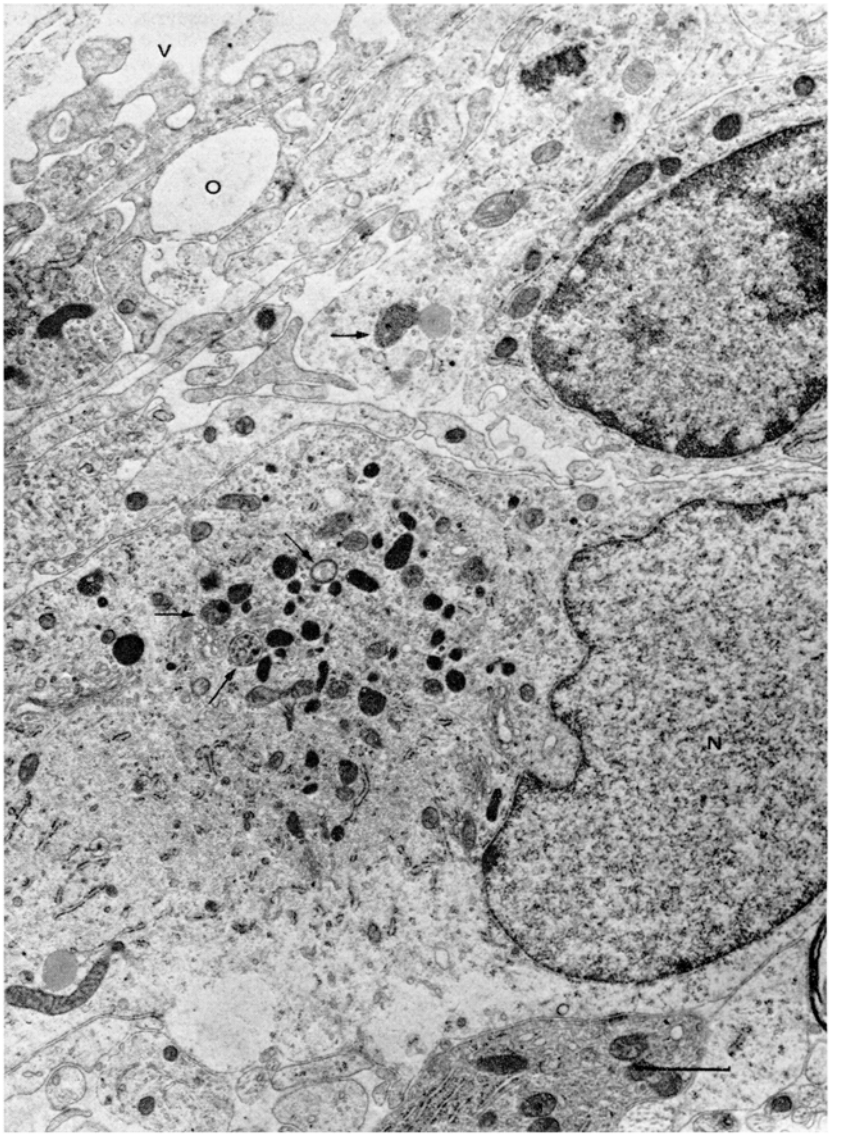

Fig. 3. A neuron containing in its cytoplasm membrane-bound bodies with black bodies (see arrows) in the brain of an 8-day-old rat with tellurium-induced hydrocephalus, situated near the ventricle. V = ventricle; O = edema. ×12,500.

Materials and methods. A diet of normal rat food (Purina Chow) containing 3000 ppm of elemental tellurium (K. and K. Labs., Plainview, N.Y.) was fed to 24 Wistar rats during gestation. These animals ate approximately 15 g of this diet per day. 20 rats gave birth to litters of hydrocephalic animals. 9 hydrocephalic new-born rats were anesthetized with ether and sacrificed. Brain tissues were fixed in 2.5% cold gluteraldehyde buffered with 0.1 *M* sodium cacodylate, pH 7.2, for 1 h, washed for 1 h in the buffer alone, postfixed with 1% osmic acid buffered with 0.1 *M* veronal acetate, pH 7.2, for 30 min, washed in the buffer alone for 1 h, dehydrated in graded alcohols and embedded in Epon 812. Tissues from 3 of these brains were not postfixed in osmic acid. Thin sections were cut and subsequently stained with 1% uranyl acetate followed by 1% lead citrate (Reynolds), carbon-coated and examined with an RCA EMU4 electron-microscope. The tissues for examination by light microscopy were stained with hematoxylin-eosin or cresyl violet (Nissl).

Observations. Paraffin and celloidin sections of the cerebral tissues examined with the light microscope did not reveal intracellular deposits. Examination with the electron-microscope of cells in postfixed and nonpostfixed tissues in the cerebral cortex and ependyma showed small dark particles within lysosomes in the cytoplasm of some neurons and glial cells. Such particles were not free in the cytoplasm. As these electron-dense particles were present in stained and unstained sections of postfixed and nonpostfixed tissues, it was presumed that they were neither lipofucsin nor glycogen.

Discussion. The purpose of this communication is to report the presence of anomalous electron-dense particles in the lysosomes of neurons and glial cells in the brains of new-born rats with tellurium-induced hydrocephalus. Similar electron-dense bodies have been identified in the neurons and glial cells of adult animals intoxicated with tellurium[7]. It is therefore suggested that the dark bodies reported here are also telluric in nature.

The importance of identifying the presence of tellurium in the hydrocephalic brain is that this would demonstrate the presence of the teratogenic agent directly in the tissues. GARRO and PENTSCHEW[10] noted similarities of the hydrocephalus caused by tellurium and that caused by vitamin B_{12} deficiency and referred to vitamin-imbalance and metabolic-disorder in attempting to explain the cause of tellurium-induced hydrocephalus. The work done with autoradiography[9,12] and the present observations show that the tellurium is present in the cerebral tissues.

The reason for the macroscopic appearance of the tellurium-induced non-hydrocephalic 'black brain' of adult animals was the large doses of tellurium given[2-4]. The absence of macroscopically visible dark deposits in the hydrocephalic brains of the developing rats is presumably because of the very small doses of tellurium given.

Résumé. L'étude présente concerne la localisation du tellure dans le cytoplasme de cellules nerveuses fœtales et post-natales chez des animaux dont la mère a ingéré du tellure durant la grossesse.

S. DUCKETT and T. SCOTT

Department of Neurology, Jefferson Medical College, Philadelphia (Pennsylvania 19107, USA),
26 October 1970.

[14] Acknowledgments. We thank Miss THERESA POWERS and Mr. EARL SPANGENBERG of the Department of Anatomy for the photography.

Oncogenicity of a Cell Line Derived from Adenocarcinoma of the Salivary Gland of C3H/He Mouse

The data concerning the occurrence of viral particles in cultured neoplastic cells and their modifications are somewhat discrepant. Especially as far as Bittner virus is concerned, it has been found that the cells isolated from the mammary tumor show a different morphological pattern in relation to the presence of viral particles[1,2].

In the course of a systemic study on the properties of mouse salivary gland adenocarcinoma[3], induced by a Bittner-like virus[4], investigations have been made on the behaviour of cultured cell lines correlated to the modifications occurring during the maturation of the viral particles.

The tumor was removed on 18th day, freed from the necrotic areas, minced and trypsinized at +4 °C overnight. The cells were washed 3 times with the growth medium and suspended at concentration of 100,000 cell/ml in Eagle basal medium supplemented with addition of 10% of inactivated calf serum. The pH was adjusted to 7.2 by CO_2 flowing. Subcultures were prepared by the standard method and the morphological features were studied by conventional methods. Occasionally the cell function was checked by measurements of O_2 consumption.

The cultured cells grew rapidly and gave an uniform and complete monolayer. In the primary culture the cells sheet was obtained within 72 h, while for the subsequent passages the time was longer (5–6 days). At the 4th subculture symptoms of growth modifications were observed. Essentially the cultures lost their monolayered aspect and the cells clumped as isolated colonies, being epithelial, nevertheless. When analyzed at light microscopy, they maintained their morphology.

The non-subcultured cells suffered regular cycles of an almost complete destruction and a partial reconstitution of the cellular layer originating from the surviving cells. After this time the cultures appeared to be completely destroyed; nevertheless the bottles were kept at 37 °C and the growth medium changed weekly. 97 days after the first passage, a clonal type growth was observed and on the 120th day the cells were completely monolayered. These cells were tested for their oncogenicity in C3H/He Ar/IRE mice by injecting s.c. different quantities (Table).

[1] Y. TSUBURA, K. TOKYOSHIMA, S. SANO and T. WATANABE, *Cancer Cells in Culture* (1968), p. 216.
[2] J. A. SYKES, J. WITHESCANVER and L. BRUGGS, J. natn. Cancer Inst., USA *41*, 1315 (1968).
[3] A. CAPUTO and L. ORCI, Z. Krebsforsch. *73*, 46 (1969).
[4] L. CASTELLI and A. CAPUTO, Experientia *26*, 780 (1970).