manipulations: while the amine-specific dense cores persisted after α-MPT, they could no longer be detected after reserpine or *p*-CPA (Figures 3–5).

That electron dense cores could be observed in non-osmicated tissues from control animals and none in those treated with reserpine is strong evidence that the electron dense material represents a biogenic amine[12]. Moreover, since α-MPT and *p*-CPA have been reported to have somewhat specific synthesis blocking properties for catecholamines[13] and 5-hydroxytryptamine[14,15] respectively, we can conclude that the endogenous monoamine localized is most probably 5-HT. These results therefore confirm and extend our fluorescence histochemical findings[8,9] which revealed the presence, above the ependymal cells, of a yellow fluorescence which took the form of small spots or a thin spotted layer. Although from the present electron microscopic examination of the brain regions investigated it cannot be excluded that in addition some nerve terminals other those storing 5-HT exist, it seems very probable that the majority of the supra-ependymal nerves in these regions are indoleaminergic. There is no clear evidence as yet to indicate whether the indoleamine stored in these nerves is released to act locally on the ependymal cells or whether it is released into the cerebrospinal fluid (CSF) to have its effects elsewhere in the brain.

It appears that supra-ependymal nerve fibres storing 5-HT have a widespread distribution in the ventricular system of the rat[9]. Their occurrence in the ventricles of the human brain has not yet been demonstrated although supra-ependymal nerve fibres have been found in various other mammals[16–21] where it remains to be shown whether they too store 5-HT. This may be of some importance since the role of CSF indoleamines in a number of central nervous functions e.g. affective disorders, has recently been discussed [22]. Moreover, the changes in CSF levels of an acid metabolite of 5-HT, 5-hydroxyindoleacetic acid, reported for patients with psychiatric disorders, although conflicting, may hold some clue as to their function e.g. a role in affecting mood[22].

In summary, a monoamine can be localized in the small and large vesicles of supra-ependymal, varicose nerve fibres upon electron microscopy. The reaction of the electron dense material (amine) in both vesicle types to various pharmacological manipulations strongly suggests the presence of an indolealkylamine, most probably 5-HT.

Résumé. Les terminaisons nerveuses supra-épendymales du rat ont été examinées au microscope électronique à l'aide de techniques cytochimiques et cytopharmacologiques. Il est apparu que les vésicules contenues dans ces terminaisons nerveuses renferment une indolealkylamine, très probablement de la 5-hydroxy-tryptamine.

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Oncogenic Viruses in the Thrombocytopenic Stage of Experimental HIPA - Plasmacytoma

The intraperitoneal inoculation of HIPA agent, a 'C' virus like particle, induces a biphasic thrombocytopenia in BALB/c-mice. The first thrombocytopenia occurs 1 day after HIPA agent inoculation and lasts about 4 days. The second thrombocytopenia occurs approximately 13 days after the inoculation and persists through the development of mesenteric HIPA plasmacytoma around the 21st day until the death of the mice by haemorrhagic ascites at about the 28th day[1,2].

In order to demonstrate a causal relationship between oncogenic viruses and thrombocytopenia in HIPA plasmacytoma, 2-month-old BALB/c-mice of both sexes were studied.

Six groups of 2 mice each were inoculated with 1 AE/mouse ultracentrifugate from HIPA tumor ascites[2]. On the 1st, 3rd, 8th, 10th, 13th and 24th day after inoculation, platelets were counted in group A, B, C, D, E and F, respectively. (see Table). After sacrifying the mice by ether inhalation, platelets were concentrated and prepared for electron microscopy as previously reported[3]. Furthermore the spleen tissues were prefixed in 2% glutaraldehyde, postfixed in osmium tetraoxide, dehydrate in ethanol and embedded in Epon. Platelets and spleen from 2 healthy BALB/c-mice were used as control. The results are summarized in the Table.

On the 1st and 3rd days following inoculation with HIPA ultracentrifugate, mice of group A and B respectively had a thrombocytopenia averaging 6×10^5 platelets. Similar to the controls, the mice of groups C and D, which were tested 8 and 10 days after inoculation, exhibited no thrombocytopenia.

Electron microscopic examination of the platelet concentrates and spleens from these mice did not reveal any virus-like particles.

Mice of group E (13 days after inoculation) and those of group F (24 days) showed a relative thrombocytopenia. At this time the mice of group F had already developed mesenteric tumors with haemorrhagic ascites. Also virus particles were frequently found in spleens of group E and F mice at the same time of the second thrombocytopenia. Virus particles were never found in any of the platelet concentrates.

In the spleen the virus particles were of the enveloped A-type lying free in the intercellular spaces and between channels of megacaryocytes or budding at cytoplasmic

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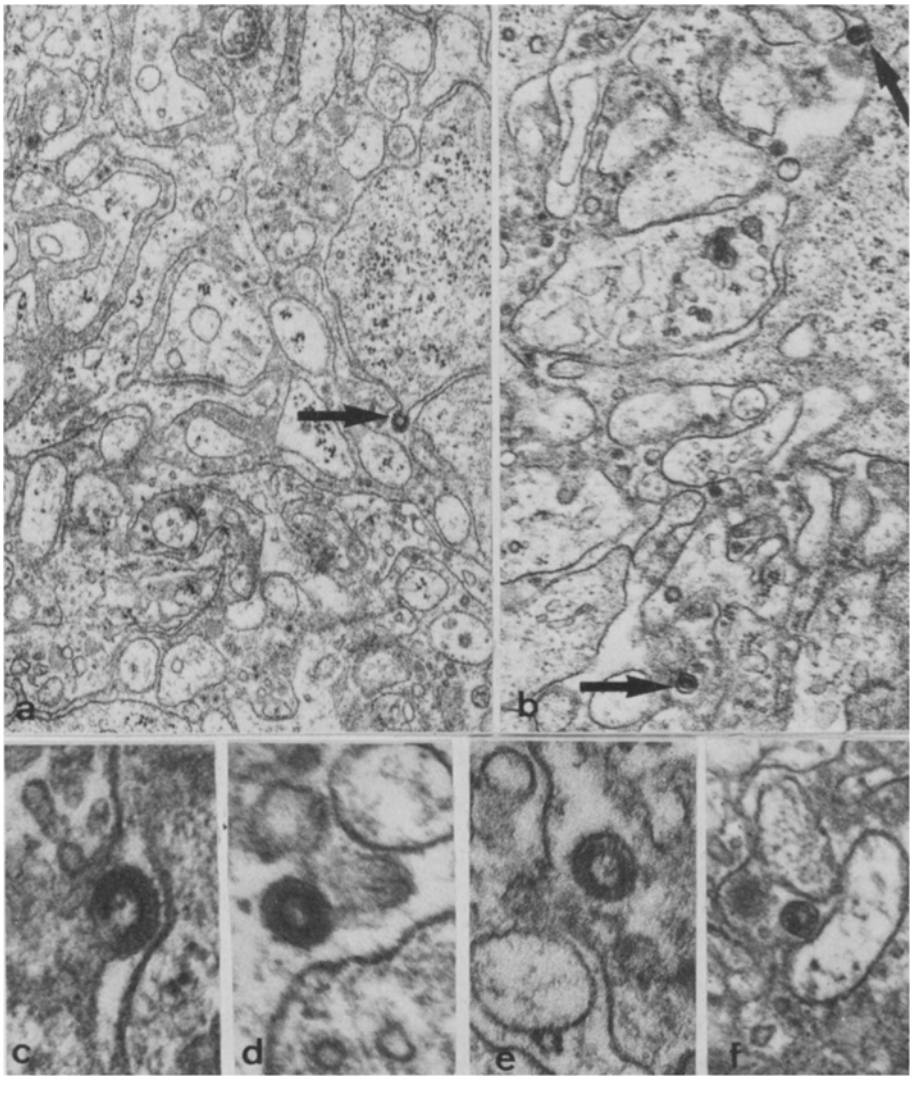
Mice group	Day of examination [a]	Platelets Count + 10^5	Virus-like part. into spleen	Virus like part. in plat. conc.
A	1st	80 95	∅	∅
B	3rd	65 55	∅	∅
C	8th	98 95	∅	∅
D	10th	90 89	∅	∅
E	13th	60 53	++ +	∅
F	24th	50 43	+ ++	∅
Control Mice		98 99	∅	∅

[a] After i.p. HIPA agent inoculation.



The illustrations are of thin sections of spleens of BALB/c-mice. a) Virus budding out from megacaryocyte membrane (arrow) × 30,000. b) Upper arrow points to virus-budding, lower arrow to free C-type virus-particle. × 30,000. c) and d) Two different development stages of virus-budding. ×115,000. e) and f) Enveloped A-type or 'immature' C-type particles. e) ×90,000; f) ×54,000

membranes. (Figures a, b, c and d.) The free particles were roundish, averaging 90–100 nm in diameter, and exhibited an outer and an inner membrane (envelopes A-type or C-type; Figures b, e and f.) The centre of the particles was generally electronlucent.

The results of these experiments thus demonstrate the appearance of virus particles in the spleens of BALB/c-mice concomittantly with the second thrombocytopenic phase in the development of HIPA tumors.

Viruses in the spleen of BALB/c-mice inoculated with HIPA-tumor cell-free extracts might represent non-specific contamination. However, the following facts suggest special relationship between the HIPA virus and the BALB/c-mouse spleen: 1. Virus particles are never found in the spleens of healthy BALB/c-mice. 2. Tumor-free spleen homogenate from an oil-pretreated BALB/c-mouse was able to induce the original HIPA tumor[4] wherever oncogenic viruses could be demonstrated.

Another point concerns the relationship between oncogenic viruses and thrombocytopenia associated with HIPA tumor development. Why virus particles type 'C' were found with the second and not with the first thrombocytopenia is a moot question. Possibly, HIPA viruses induce thrombocytopenia by a mechanism similar to that active in Rauscher leukaemia or in Friend leukaemia, the thrombocytopenia being due to direct destruction of megacaryocytes and platelets[5,6]. However, at present there is not sufficient evidence for the existence of such a mechanism in our case.

Zusammenfassung. Die i.p. Inokulation von HIPA-Tumor Agens führt zu einer byphasischen Thrombozytopenie in BALB/c-Mäusen. Nach regelmässig durchgeführten Abständen von elektronenmikroskopischen Untersuchungen zeigten sich onkogene Virus-Partikel in den Milz-Megakariozyten gleichzeitig mit dem Erscheinen der zweiten Thrombozytopenie (13 Tage). Die Beziehungen zwischen solchen Virus-Partikeln und Thrombozytopenie werden diskutiert.

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Cytogenetic Characterization of C_{1300} Neuroblastoma Cells

C_{1300} murine neuroblastoma is a spontaneous and transplantable tumor of the neural crest. The tumoral cells maintained in culture have retained the ability of neuronal-like differentiation when submitted to various molecular environments. This evolution is characterized by morphological changes (growth of cytoplasmic expansions, decrease of nucleocytoplasmic ratio, Schubert et al.[1]) by tinctorial affinity to silver impregnation, (Schubert et al.[1]), by increase of enzymatic activities involved in the neurotransmission (Augusti-Tocco et al.[2]), by appearance of a neuronal-like sensitivity to neurotropic drugs (Angeletti et al.[3]), by changes in oxydative metabolism (Tholey et al.[4]). In this work, the question was to know if clonal lines from C_{1300} neuroblastoma could be used as a tool for genetic mapping.

Material and methods. The results reported concerned 'adrenergic', 'cholinergic' and 'inactive' clonal lines issued from $N_{115-1-1E}$, S_{21} and N_9 clones (these clones were kindly provided by M. Nirenberg). 'Adrenergic' and 'cholinergic' characteristics are referred to their respective high levels of tyrosine hydroxylase or choline-*O*-acetyltransferase.

The cells were maintained in culture in Eagle-Dulbecco's medium. The cultures were transplanted 24 h before harvesting mitosis. After colchicine treatment (0.4 µg/ml/3 h), osmotic shock was induced by medium dilution with distilled water (1:5/30min/37 °C). Mitotic cells get loose easily. After centrifugation, they were fixed with acetic acid methanol (1:3) mixture for 30 min. Smearing was followed by staining according to Giemsa. The caryotype of control mice was performed using embryonic fibroblasts and bone marrow cultures. Tumoral cells were studied by direct examination.

Results. The normal murine cells possessed 40 telocentric chromosomes. The tumoral cells exhibited from 60 to 63 chromosomes, with 3 to 5 markerchromosomes (1 or 2 mediocentrics of submediocentrics of great size, 1 mediocentric of middle size Figure 1).

55 caryotypes from S_{21} clonal line were analyzed, after 20 passages in our laboratory. The number of chromosomes varies from 17 to 175 (Figures 1 and 2). Their distribution exhibits 2 modal values: 55–65 and 85–95. The marker-chromosomes were morphologically similar, but their number varied, without correlation to the total number of chromosomes. These markers appear to be the same as those observed in the tumor; however, other markers can be observed: great telocentrics, 'dubble-minute' chromosomes, median-sized acrocentrics. But the great mediocentrics and median-sized acrocentrics occur more frequently and constantly. The size of nuclei remains rather homogenous. Breaks and pulverization correlated to viral action are rarely observed.

Then 90 cells issued from $N_{115-1-1E}$ clonal line were analyzed after 18 passages. The number of chromosomes varies from 18 to 89 with 80–85 as modal value. Every cell exhibits marker-chromosomes (Figures 1 and 2). These markers are the same as those observed in S_{21} clonal line. However large sized telocentrics occur more frequently. Moreover a peculiar marker may be observed: it is characterized by a secondary constriction. The nuclear size is rather homogenous. Similar breaks or pulverization, as previously reported, may be observed.

For 66 cells of N_9 clone analyzed after 35 passages, the number of chromosomes varies from 25 to 330. No modal value can be established, because of the high heterogeneity of the distribution. Groups of 10 or less chromosomes are considered a technical lose (micronuclei were never

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