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.

G. Pedio, J. R. Rüttner, B. Odermatt and Dorothea Gut

Institut für Pathologische Anatomie der Universität Kantonsspital Zürich, Schmelzbergstrasse 12, CH–8006 Zürich (Switzerland), 27 September 1973.

[4] G. Pedio, E. Grieshaber and J. R. Rüttner, Path. Microbiol. *31*, 278 (1968).
[5] L. H. Dennis and I. Brodsky, Proc. Soc. exp. Biol. Med. *120*, 683 (1965).
[6] I. Brodsky, E. Ross, S. B. Kahn and G. Petkov, Cancer Res. *28*, 2406 (1968).

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

[1] D. Schubert, S. Humphreys, C. Baroni and M. Cohn, Proc. natn. Acad. Sci. USA *64*, 316 (1969).
[2] Augusti-Tocco and G. Sato, Proc. natn. Acad. Sci. USA *64*, 311 (1969).
[3] R. U. Angeletti and R. Levi-Montalcini, Archs ital. Biol. *108*, 213 (1970).
[4] G. Tholey, J. Ciesilski-Treska, B. Wurtz and P. Mandel, C. r. Acad. Sci. Paris *275*, 1715 (1972).

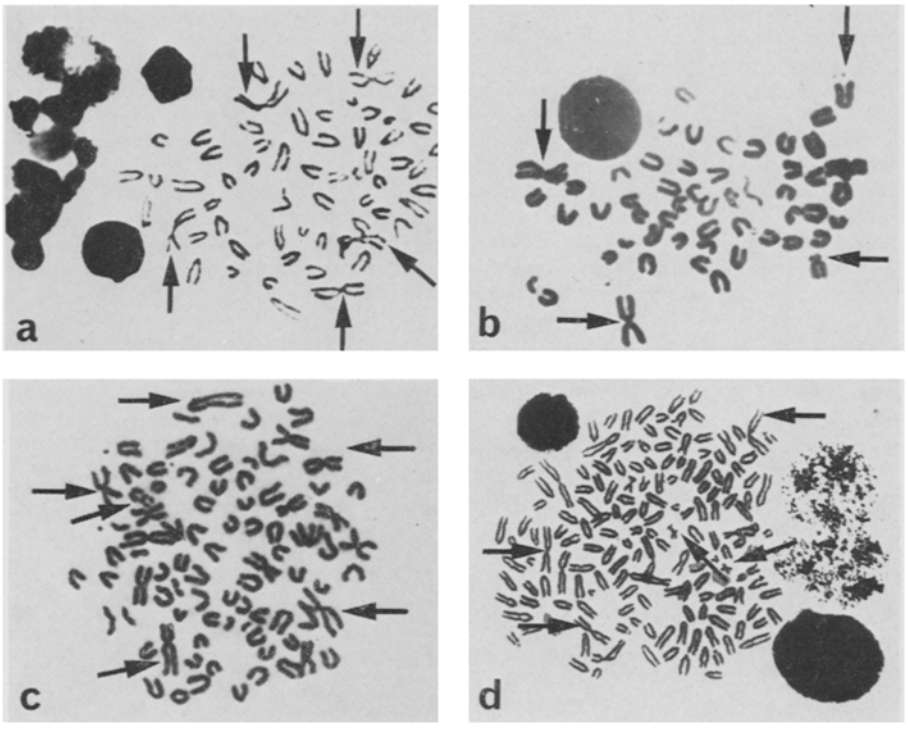

Fig. 1. Metaphase plates of tumoral (a), S_{21} (b), N_{115} (c) and N_9 (d) cells.

observed). All cells possess marker-chromosomes, which are morphologically similar (Figures 1 and 2). But their number varies. Moreover small metacentrics may be observed. The nuclear size is quite heterogenous. Nuclear fusion and viral action occur as precedently.

Discussion. All cells possess marker-chromosomes. Their numbers are independent of the total number of chromosomes. Some markers are present in every cell of every clone and in the tumoral cells. Other markers appear more specific for a peculiar clone: small-sized mediocentric for N_9 (23% of the cells), median sized acrocentric (40% of the cells) for S_{21}, chromosome with secondary constriction (38% of the cells) and large sized telocentric (70% of the cells) for N_{115}. Any peculiar association between markers was never observed. Aneuploidy and polyploidy could indicate an adaptation to the culture conditions. However, the number of chromosomes and their modal distribution from one clone to the other differ netly. Heterogeneity of N_9 caryotypes seems to be increased by the number of passages. Moreover, breaks and pulverization, probably correlated to viral action (STICH et al.[5]), are present everywhere; some viruses possess the ability to induce chromosomal instability, characterized by polyploidy aneuploidy and marker formation.

It is difficult to establish correlations between virus, transplantation, presence of marker-chromosomes and specific clonal characteristics. The clonal lines studied exhibited different responses when they were submitted to various molecular environments, and a shift between morphological appearance and enzymatic equipment. No correlation can as yet be established between chromosomal and cytochemical results. The heterogeneity of these clones requires further studies.

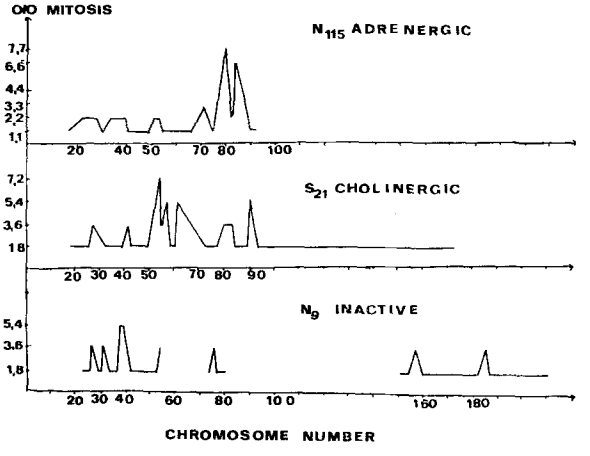

Fig. 2. Variation of chromosome number of N_{115}, S_{21}, and N_9 clonal lines.

Résumé. Le neuroblastome C_{1300} est une tumeur murine de la crête neurale, spontanée et transplantable. Ses cellules, dans certaines conditions in vitro, peuvent se «différencier» dans le sens neuronal. L'étude cytogénétique de 3 clones, de propriétés différentes, issus de cette tumeur est entreprise. La distribution du nombre de chromosomes varie d'un clone à l'autre; des chromosomes marqueurs, dont certains assez spécifiques, sont observés. Les relations existant entre les caractéristiques cytogénétiques et les propriétés cytochimiques doivent encore être précisées.

S. WARTER, J.C. HERMETET and J. CIESELSKI-TRESKA

Institut d'Embryologie, Faculté de Médecine, 11, rue Humann, F-67 Strasbourg (France); and Institut de Chimie Biologique et Centre de Neurochimie, Faculté de Médecine, F-67 Strasbourg (France),
11 July 1973.

[5] H. F. STICH, and D. S. YOHN, Progr. med. Virol. *12*, 78 (1970).