

Intranukleäre Einschlusskörper in Neuronen alternder Ratten

Intranukleäre Einschlusskörper verschiedener Strukturen sind von einer Anzahl von Autoren sowohl im normalen^{1,2} als auch im pathologischen³⁻⁵ Nervengewebe als elektronenmikroskopische Befunde beschrieben worden. Bei letzterem handelte es sich um Geschwülste, Infektionen und chemische Induktion. Man glaubte, diese Befunde als Ausdruck einer zellulären Hyperaktivität bewerten zu können³.

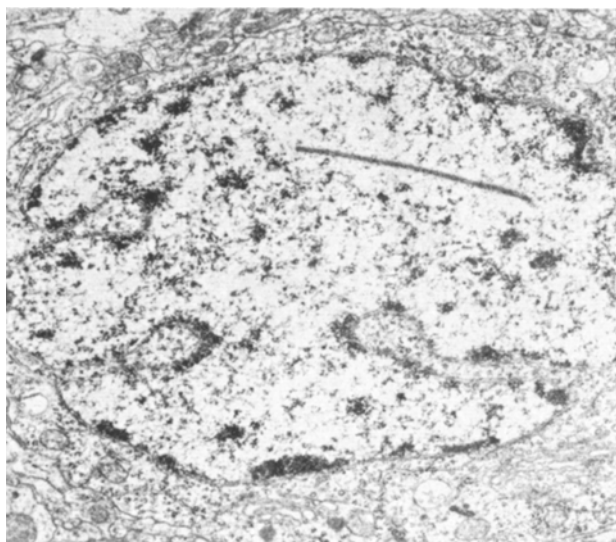


Fig. 1. Stabförmiger Kerneinschlusskörper im kortikalen Neuron einer 25 Monate alten Ratte. $\times 5000$.

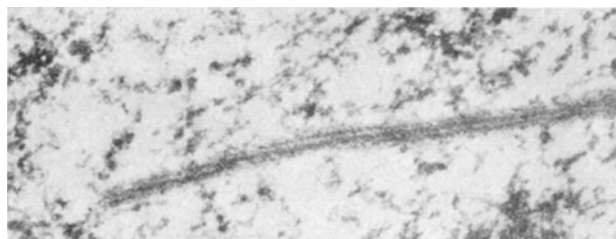


Fig. 2. Im Ausschnitt ist die lamellierte Struktur mit leichter spiralförmiger Verdrehung sichtbar. $\times 20000$.

Eine besondere Form dieser intranukleären Einschlusskörper wurde von RAIN und FIELD⁶ im menschlichen Biopsiematerial von Multiple-Sklerose-Kranken gefunden. Es handelte sich dabei um einen stabförmigen Körper mit lamellierter Struktur.

Bei der Untersuchung des sensomotorischen Kortex in alternden Ratten fand sich jedoch mit grosser Regelmässigkeit ein strukturell gleicher Befund. An osmiumfixiertem Material zeigen diese Ultrastrukturen ebenfalls die typische längsverlaufende Lamellierung. Teilweise war diese in der Längsachse um wenig spiralig verdreht. Neben den Stäbchen fanden sich, allerdings weniger häufig, Fibrillenbündel, wie sie im Karyoplasma von Neuronen jüngerer Ratten bereits beschrieben sind¹.

Diese Beobachtung lässt schliessen, dass nicht alle intranukleären Einschlusskörper im Sinne einer zellulären Hyperaktivität zu deuten sind. Möglicherweise sind einige dieser Kerneinschlüsse – und wir möchten das für die hier beschriebenen annehmen – als Zeichen eines unspezifischen involutiven oder degenerativen Prozesses aufzufassen. Eine direkte Assoziation zur MS erscheint fraglich^{7,8}.

Summary. Intranuclear rodlet shaped structures similar to those recently observed in human multiple sclerosis biopsy material were found in cortical neurons of aging rats. The morphology of these structures is described and their possible significance discussed.

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⁷ Herrn Dr. WILK von der Firma Dr. Karl Thomae sei für die Überlassung der untersuchten «Specific-Germ-Free»-Sprague-Dawley-Ratten gedankt.

⁸ Mit Unterstützung des Bundesministeriums für Gesundheitswesen (No. I A 3-92-75/68).

Uptake of Labelled Vitamin B₁₂ and 4-Iodophenylalanine in some Tumours of Mice

During the last years there has been a rapid improvement of the equipment for external scintillation counting, a method of increasing importance in tumour diagnosis¹. There is, however, a demand for gamma-emitting substances with selective affinity for malignant tumours. Using whole body autoradiography² we have obtained some results which seem to be of interest in this respect. Whole body autoradiography has the advantage, in screening substances for tumour diagnosis, that the distribution within the tumour can be studied and that the tumour uptake can be compared with the uptake in all normal tissues.

Material and methods. Labelled vitamin B₁₂ (⁵⁷Co, ⁶⁰Co) was administered i.v. to 4 groups of mice with different kinds of tumours, which had been transplanted s.c. into the neck region and grew as solid masses: ⁹⁰Sr induced soft fibroblastic osteosarcoma, spontaneous mammary carcinoma, Moloney-virus induced tumour, and Ehrlich

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ascites cell tumour. The animals were killed by ether anaesthesia at intervals from 15 min to 4 days after injection, and then immersed in a mixture of hexane and solid carbon dioxide (-70°C). Sagittal $20\ \mu$ sections through the frozen animals were cut and dried at -10°C . Autoradiographic exposure was obtained by apposition against Gevaert Structurix X-ray film.

^{125}I -labelled DL-4-iodophenylalanine was given i.v. to 3 mice with the following transplanted tumours: ^{90}Sr induced soft fibroblastic osteosarcoma, Ehrlich ascites cell tumour, and lymphatic leukemia. The animals were killed 15 min after injection and subjected to the same procedure as cited above.

The whole body autoradiography technique has been described in detail previously².

Results and discussion. Vitamin B_{12} . The ^{90}Sr induced soft fibroblastic osteosarcoma, which was the most rapidly growing tumour, showed the greatest uptake of radio- B_{12} , the concentration being higher than in any normal tissue (Figure 1). The other tumours also accumulated the substance although less extensively. In all tumours the radioactivity was localized to the peripheral actively growing parts.

The results suggest that B_{12} is needed for rapid cell multiplication. This may be related to the findings of several authors claiming that B_{12} participates in the synthesis of nucleic acids (e.g.³).

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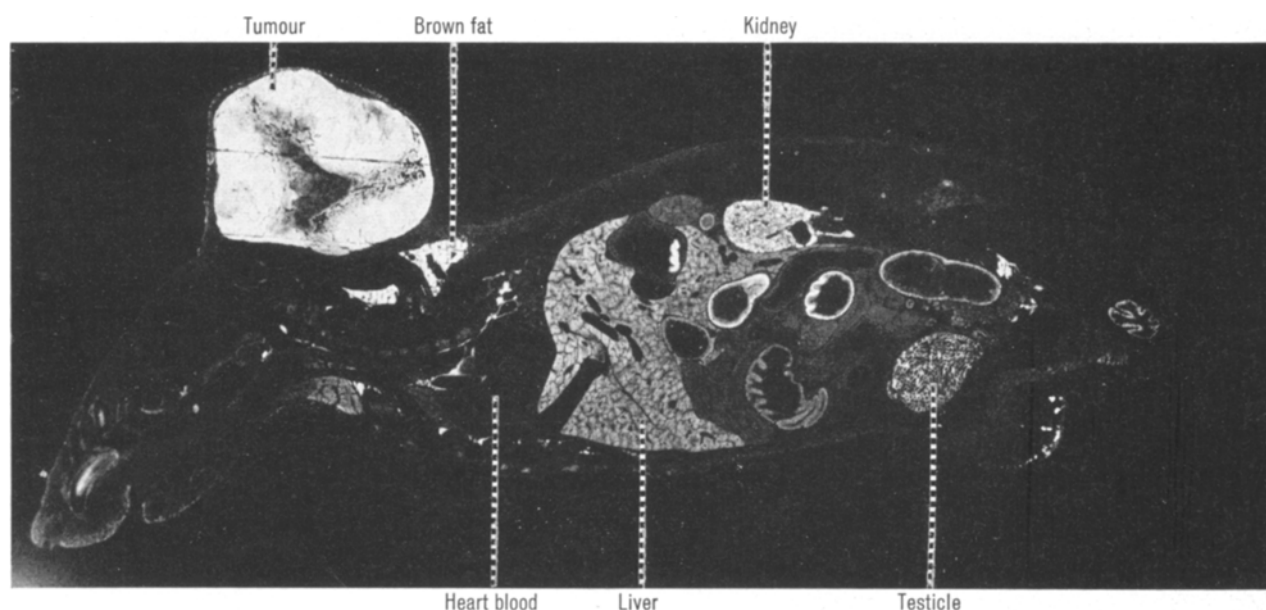


Fig. 1. Autoradiogram of mouse carrying a transplanted ^{90}Sr -induced soft fibroblastic osteosarcoma in the neck region. The animal was sacrificed 4 days after i.v. injection of ^{60}Co -vitamin B_{12} . Note high accumulation of radioactivity in the actively growing peripheral parts of the tumour. Accumulation is also seen in the gastric and intestinal mucosa, renal cortex, testis, liver and brown fat.

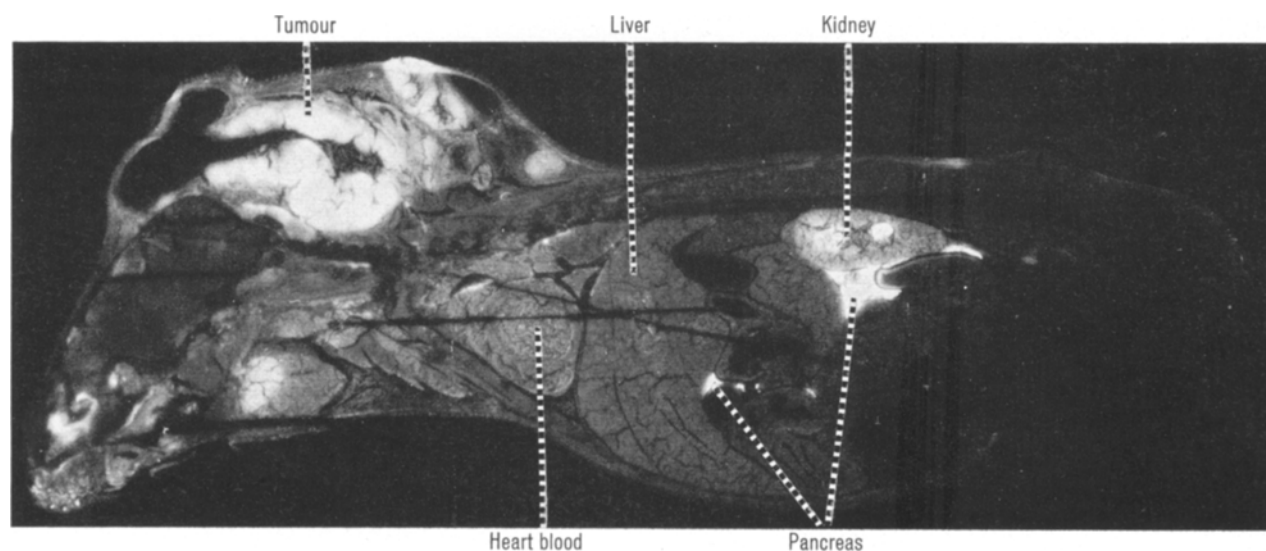


Fig. 2. Autoradiogram of mouse with a transplanted Ehrlich ascites cell tumour 15 min after i.v. injection of ^{125}I -4-iodo-phenylalanine. Note high uptake in the growing parts of the tumour. In addition to the tumour, the substance is mainly concentrated in the pancreas and kidney.

It has been claimed that patients with neoplasms have an increased requirement for vitamin B₁₂. Carcinoma cells, like normal cells, need B₁₂ and the infiltrative growth of the carcinomas could possibly increase the demand for B₁₂⁴. Following injection of vitamin B₁₂ increased growth has been reported in some sarcomas⁵.

4-Iodophenylalanine. This amino acid has earlier been found to localize almost selectively in the exocrine pancreas of normal mice⁶. The substance appeared to be transported across the pancreatic cell membrane similarly to an 'ordinary' amino acid but not to be accepted in the protein synthesis and therefore rapidly returned to the blood. In the rest of the body the distribution was very even, almost all tissues having lower concentration than the blood.

In the present investigation, a concentration of 4-iodophenylalanine estimated to approximately 3 times that of blood was seen in the actively growing parts of the Ehrlich ascites cell tumour (Figure 2). The lymphatic leukemia showed somewhat higher concentration than the blood, while the soft fibroblastic osteosarcoma did not take up the drug.

Unlike vitamin B₁₂ 4-iodophenylalanine did not show an uptake which correlated with the growth rate of the tumour, since the soft fibroblastic osteosarcoma grew faster than the Ehrlich ascites cell tumour but did not concentrate the drug. Rather, it would seem that the Ehrlich ascites cells and to a less extent the lymphatic leukemic cells have a membrane transport system which can accept 4-iodophenylalanine, which is not the case for the cells of the soft fibroblastic osteosarcoma or the cells of most normal tissues. This may be of interest with regard to the possibility of slightly modifying naturally occurring compounds, such as amino acids, sugars, nucleosides, vitamins etc., to obtain selective localization in tumours. Another non natural amino acid which has

been found to accumulate in certain tumours without being incorporated into proteins is 1-aminocyclopentane carboxylic acid⁷.

One practical consequence from our findings might be the use of gamma-emitting vitamin B₁₂ or 4-iodophenylalanine for clinical localization of tumours. However, these compounds do not accumulate equally in all tumours and a comprehensive mapping of their uptake in different neoplasms in various species must be done before their possible use as tumour diagnostic agents can be appraised⁸.

Zusammenfassung. Die Verteilung von radioaktivem Vitamin B₁₂ und 4-Jodphenylalanin in tumortragenden Mäusen wurde ganztierautoradiographisch untersucht. Die grösste Aufnahme von B₁₂ wurde im weichen fibroblastischen Osteosarkom gefunden, während 4-Jodphenylalanin in einem Ehrlich-Ascites-Tumor aufgenommen wurde.

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⁸ Acknowledgments. This work has been supported by research grants from the Swedish Cancer Society (No. 68:37) and from Söderbergs Stiftelse.

Hepatic Carcinogenesis Threshold and Biphasic Mitochondrial Swelling Response in the Guinea-Pig During Diethylnitrosamine Administration¹

The refractoriness of the guinea-pig to the carcinogenic action of amino azo dyes and aromatic amines, and the resistance of this species to carcinogenic agents in general, is well known. It was reported previously² that feeding of the potent hepatocarcinogen, 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB), to guinea-pigs, even at the high level of 0.12%, does not affect the pattern of swelling of isolated liver mitochondria. Although this is in striking contrast with the observations on rats³⁻⁵, it is not actually unexpected in view of the refractoriness of the guinea-pig to azo dye carcinogenesis and the well-established correlation between the onset of carcinogenesis and alterations of mitochondrial swelling^{2,3,5} in the rat. The report by ARGUS and HOCH-LIGETI⁶ on the potent hepatocarcinogenic activity of diethylnitrosamine (DEN) in the guinea-pig eliminated the notion that this species is endowed with specific cancer resistance. Hence, this finding prompted an investigation of the possible effect of DEN administration on the swelling response of hepatic mitochondria of the guinea-pig and the correlation, if any, between the swelling response and the threshold of DEN-induced hepatic tumorigenesis.

Materials and methods. Random-bred male guinea-pigs (smooth-haired variety), housed singly in a cage, were

used. The weight range at the beginning of the experiments was 170–260 g. The animals were maintained on Purina rabbit chow ad libitum, and vitamin C was supplied during DEN administration by depositing on the back of the tongue, 3 times weekly, 100 mg ascorbic acid in solution in 1 ml water; in the dose-response study, after the respective periods of DEN administration the vitamin C dosing was substituted by an abundant daily

¹ Supported by Research Grants Nos. CA-05431 and CA-05793 from the National Cancer Institute, United States Public Health Service. The expert technical assistance of Mrs. SHIRLEY B. BEMIS with the mitotic indexes is gratefully acknowledged.

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