Experimental Model for Induction of Lymph Node Metastases with a Human Heterotransplanted Melanoma in Rat

In previous papers POPP et al. reported a new rat tumour IOB hR 18 derived from a human melanocarcinoma transplanted in WAG rats by intra-embryonic route[1-3]. Tumoural outgrowth became evident in suckling rats. Isologous transplantations were performed in embryos, new-born and adult animals, as well as in adult Wistar rats and were always followed by tumoural take. At present the tumour has a history of 25 generations, being maintained in Wistar rats by s.c. challenge every 3 weeks.

The tumour does not metastasize; it grows within a capsule and may sometimes reach the size of the host, killing it in about 30 days.

Microscopic examination showed an abundantly pigmented melanocarcinoma. After several rat passages, the aspect became that of a massive anaplasic carcinoma, the cells being arranged in rows or in close, well vascularized clones.

Studying the development of intratestis inoculated tumours[4-10], we were able to set up an experimental model of abdominal lymph node metastases of IOB hR 18 tumour.

Material and methods. Sixty adult Wistar-London rats from the Oncological Institute closed colony were used.

A tumoural fragment of about 1 g was minced and then ground in saline. After 5 min sedimentation, 0.2–0.3 ml of the supernatant were picked in a syringe with a No. 16 needle and injected into each testicle.

Results. The intratesticle grafts of IOB hR 18 tumour resulted in 100% takes, while the percentage of abdominal lymph node metastases was of the order of 90%. The bilaterally inoculated animals developed metastases in 15–20 days. The size of metastatic tumours reached that of a dry nut, while the para-aortic and para-renal lymph node metastases ranged between that of a pea and that of a nut. 25% of the inoculated animals developed a tumour cell rich ascites of 0.5–10 ml. The metastases achieved by the above procedure are very similar to the lymph node metastases developing in human melanoma and carcinoma.

This experimental model of intratesticular tumours inoculation is of practical importance because of its neatness and its homogenous and constant results, which permit investigations of free neoplastic cells (malignant monerocytoma[11]) and, in the experimental study, of the pathogeny and therapy of tumoural metastasis.

Résumé. Le travail décrit une technique expérimentale qui permet d'obtenir de grosses métastases ganglionnaires chez le rat après inoculation intratesticulaire de la tumeur IOB hR 18. Ce modèle expérimental peut être utilisé dans diverses études concernant la pathogénie et la thérapie des métastases.

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Binding of 1-3,4-Dihydroxyphenylalanine and Dopamine in Cytoplasmic Granules of Paneth Cells

Several tissues have the ability to take up and bind catecholamines (CA) but it is now generally accepted that this binding occurs predominantly in the adrenergic nerve system. The mammalian intestinum contains 5-hydroxytryptamine (5-HT) storing enterochromaffin cells[1] and in rodents there are also 5-HT-containing mast cells[2]. Certain cells in the glandular part of the rat stomach are capable of storing monoamines in vivo[3] and some human intestinal epithelial-like cells store 1-3,4-dihydroxyphenylalanine (D) in vitro[4]. The intestinal epithelium and the intestinal glandular cells normally contain neither CA nor 5-HT in chemically or histochemically demonstrable amounts. In the present study the binding of i.v. administered D and dopamine (DA) was studied in the duodenum of the mouse with special reference to cytoplasmic particles of Paneth cells.

150 mice, 20–25 g, were studied. The animals received no food for 24 h before experiments. D and DA (15–150 mg/kg) were injected slowly into the tail vein of mice and the animals were killed 10 and 30 min, 1, 2.5, 4 and 8 h later by decapitation. The freeze-drying procedure followed the principles given by ERÄNKÖ[5]. Duodenal pieces, 5 mm in length, including the entire transverse section of an opened intestinal wall, were frozen in isopentane precooled in liquid nitrogen and dried in vacuo at $-40\,^{\circ}C$ for 2 days. Specimens were warmed up to room temperature in vacuo for 5 h and after treatment in formaldehyde vapour derived from paraformaldehyde for 1 h at 80 °C. 5-μ sections were cut and the paraffin wax was removed by xylene before fluorescence microscopy. Histochemically monoamine oxidase (MAO) activity was demonstrated by the method of GLENNER et al.[6]. In inhibition experiments of MAO mice were pre-treated with a 500-mg/kg dosis of Niamid® 4 h before amine administration.

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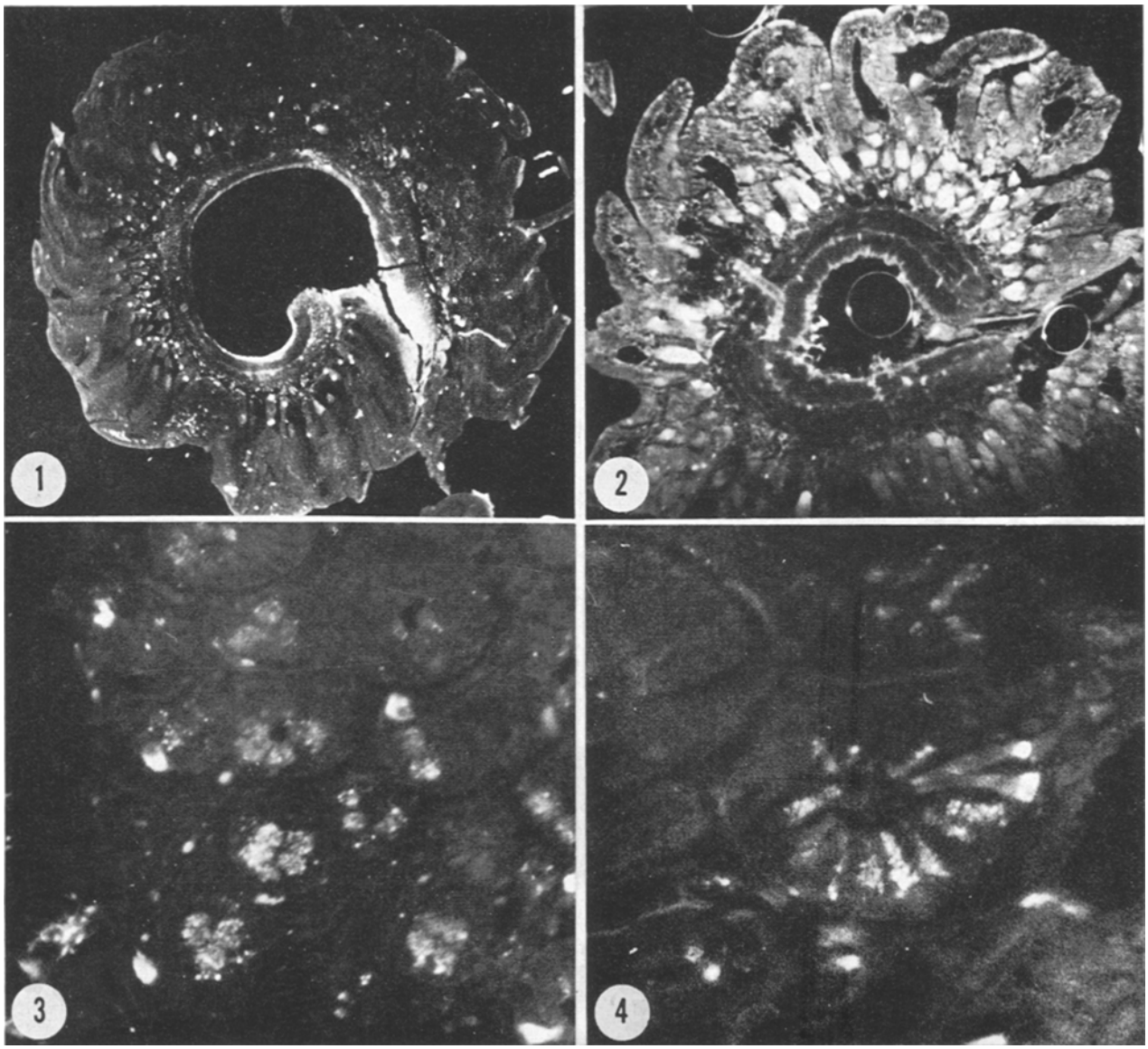
In non-treated animals yellow-fluorescent enterochromaffin and mast cells as well as greenish-fluorescent nerve fibres were found in the intestinal wall but the epithelium and the glandular cells at the crypts of Lieberkühn were devoid of monoamine fluorescence induced by formaldehyde (Figure 1). 10 min after D or DA administration the whole mucosa of the duodenum turned greenish-fluorescent, as shown in Figure 2. The most intense fluorescence was located at the basal area of intestinal glandules and in the epithelial sheet of the villi. In specimens taken 30 min after D or DA administration the fluorescence in the mucosa was in general weak, but the cytoplasm of Paneth cells at the crypts of Lieberkühn exhibited a strong fluorescence with a condensed greenish colour in coarse particles of the apical cytoplasm (Figures 3 and 4). D and DA accumulated specifically in Paneth cell granules and occasionally also in the cytoplasm of enterochromaffin cells. Normally the yellow 5-HT fluorescence in the basal area of enterochromaffin cells was so strong that it obviously quenched the CA fluorescence in cytoplasmic organelles of the cell basis.

The fluorescence intensity decreased in mucosa structures with lowering of the D or DA doses and with increasing of the post-injection observation time. In specimens taken 1 h after injections the mucous membrane was otherwise of normal appearance, but strongly or moderately fluorescent Paneth cell granules were found and a greenish fluorescence in adrenergic nerve fibres was more prominent than normally. This was probably also a consequence of an uptake of D and DA. 2.5–8 h after



Figs. 1–4. Formaldehyde-induced fluorescence in the duodenum of the mouse. (1) Duodenal wall of an untreated animal. Fluorescent nerve fibres and some strongly fluorescent cells are seen in the non-fluorescent background. – 50. (2) Duodenal wall 10 min after i.v. administration of 150 mg/kg of dopamine. Note a strong fluorescence all over the mucosa but especially in the basal area of crypts of Lieberkühn. – 60. (3) Duodenal mucosa 30 min after i.v. administration of 150 mg/kg of dopamine. Some crypts of Lieberkühn are seen. Note the binding of dopamine in apical cytoplasmic organelles of Paneth cells. Some strongly fluorescent enterochromaffin cells are also seen. – 350. (4) Experimental conditions as in Figure 3. Note a strong fluorescence in Paneth cell granules. In contrast to enterochromaffin cells CA fluorescence is located in coarse cytoplasmic granules of Paneth cells. – 800.

D and DA injections duodenal CA fluorescence was as in controls.

Reserpine (5 mg/kg) administered 24 h before D and DA injections was not able to inhibit the binding of the above-mentioned biogenic substances in Paneth cells and had no effect on the fading of their fluorescence. The pretreatment of mice with Niamid® prolonged the disappearance of administered drugs in granules of Paneth cells as well as in other intestinal structures.

The ability of Paneth cell granules to bind CA is surprising, since, at least in the rat, these cytoplasmic organelles fulfil the criteria of lysosomes[7] defined by DE DUVE[8]. However, Paneth cell granules have many specific staining and enzymatic characteristics and therefore they cannot be regarded as typical lysosomes found in other types of cells. In the rat[7], as well as in the present study on the mouse, Paneth cell granules exhibit histochemically a MAO activity. Obviously the detoxification mechanism of monoamines by MAO operates in the mouse, since the fading of administered biogenic substances was so rapid and prolonged by MAO inhibitors in the present study.

The specificity of the histochemical fluorescence reaction used has been well established chemically[9–11]. The similar accumulation of D and DA by Paneth cells supports a similar binding mechanism for these biogenic substances but the possibility that D is first converted in vivo to DA and that this amine accumulates in Paneth cell granules is not excluded. Reserpine is known to block the CA storage mechanism in many types of organelles of nervous origin[12,13] but, in the Paneth cells of the present study as in certain other cells which normally do not contain CA[14], reserpine has no effect on the amine binding or fading. On the basis of the present study, the binding type between histochemically identified protein-polysaccharide matrix of granules[7] and administered substances remains obscure.

The physiological function of Paneth cells is largely obscure but they participate in the production of digestive enzymes[15]. The present results indicate that granules may also participate in the binding of biologically active amines and in their elimination by oxidative detoxification mechanism[16].

Zusammenfassung. Nach spezifischer i.v. Injektion wird gefunden, dass Dopamin und 3,4-Dioxyphenylalanin-aminosäure im Zytoplasma der Panethschen Zellen des Mäuseduodenums angehäuft sind. Während Niamid® das meist rasche Verschwinden der Formaldehyd-verursachten Aminfluoreszenz in den Granula von Panethschen Zellen verzögerte, blieb Reserpin wirkungslos.

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Response of Neuronal Lysosomes to Anoxia in Tissue Culture of Mammalian Cerebellum

Cytopathological changes in the brain due to oxygen deficiency have been extensively studied although the precise mechanism is still not understood[1]. More recent experiments have demonstrated enzyme loss in the brain of experimental animals following periods of anoxia[2–4].

It has also been reported that neuronal lysosomes showed cytopathological changes following exposure to anoxia[4,5].

The present communication deals with similar changes demonstrated in mammalian cerebellar neurons grown in tissue culture as exhibited by histochemical acid phosphatase reaction.

Material and methods. Tissue culture explants were made of new-born kitten cerebellar cortex using the flying coverslip-roller tube method as previously described[6–8]. The cultures were fed once a week with nutrient fluid consisted of 50% Gey's solution, 45% human cancerous ascitic fluid and 5% embryonic extract of 8-day-old chick embryo.

After 2–4 weeks in vitro, cultures were divided into 2 groups (control and experimental). The experimental cultures were then exposed to anaerobic conditions for times ranging from 30 min to 24 h, while the control cultures continued to grow under normal condition.

To produce an anaerobic condition, the alkaline pyrogallic acid method was used[9,10]. A cotton plug was pushed into the test tube so that the upper portion of the plug was 1.5 inches below the lip of the tube. 1 g of pyrogallic acid and 1 g of sodium carbonate were mixed and inserted on top of each cotton plug. A rubber stopper was placed tightly in the tube and the boundary of the tube and stopper was sealed with paraffin.

The basis of the alkaline pyrogallic acid method lies in the fact that pyrogallic acid, when placed in alkaline pH, will absorb a large quantity of oxygen. At varying intervals, cultures of both the experimental and control groups were fixed in cold formol-calcium for 5 min at 4 °C and then placed in Gomori's glycerophosphate-lead medium for 30 min at 37 °C. After incubation they were

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