fall is noticed above all in the radioactivity of the former with a less pronounced percentual reduction in the radioactivity of the latter.

Apart from these variations in the percentage values of the radioactivity distribution of the labelled glycine between the proteins and polypeptides of the liver and the serum respectively, the fundamental datum that remains as the result of these present enquiries is that of a clear slowing down of the intake of the glycine itself in the proteins and polypeptides as a consequence of the massive irradiation of the rat.

These data agree with the alterations of the involutive type that the irradiated animals present, including the loss of weight, while contrasting with what we discovered in relation to the utilization in vitro of the labelled cystine, which induced us to think that the present data obtained by us with glycine correspond more nearly to the direction that the nitrogen balance assumes in the irradiated animal, a direction essentially of the deficiency type. It is moreover possible that the modifications described above for the cystine may be ascribed to an increased request by the tissues of the irradiated animal for sulphur compounds. Further researches conducted with the latter substance in vivo instead of in vitro may confirm or not such a supposition, in favour of which are to be placed the positive results of the therapeutic use of the same either alone or associated with cysteamine in the treatment of radiation disease.

Riassunto. Gli autori hanno esaminato il comportamento dell'utilizzazione della glicina-1-^{14}C da parte del fegato di ratti irradiati e la sua incorporazione nelle proteine e nei polipeptidi del fegato e del siero. I risultati ottenuti hanno messo in evidenza un abbassamento dell'utilizzazione della sostanza, con riduzione, rispetto agli animali di controllo non irradiati, della radioattività delle proteine e rispettivamente dei polipeptidi sia epatici che plasmatici.

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DOPA Decarboxylase Activity in a Transplantable Hamster Melanoma

The urine of patients with generalized melanoma contains large amounts of homovanillic acid[1], a natural metabolite of 3,4-dihydroxyphenylalanine (DOPA)[2], which is actively produced in melanotic tumours[3] and which is believed to be the precursor of melanin[3]. Homovanillic acid may be produced from DOPA via dopamine, and accordingly it was assumed that the increased urinary excretion of homovanillic acid in melanoma patients was due to an increased production of dopamine. This hypothesis prompted an investigation into the dopamine content and the DOPA decarboxylase activity of a melanotic tumour.

For this purpose a transplantable malignant hamster melanoma (type M. Mel. 1 of Fortner, described by ŠALAMON and STORCK[4]) was used[5]. Histologically the tumour showed signs of active melanin formation during the first passages but, coincident with more vigorous growth, the melanin formation decreased and only small amounts of melanotic pigment were revealed by staining according to MASSON and LILLIE[6].

Catechol amines were extracted and isolated by means of an ion exchange procedure[7] and determined fluorimetrically[8]; DOPA decarboxylase was studied by incubating the tumour tissue with C^{14}-labelled DOPA. If not otherwise stated, 10 mg samples of carefully minced tumour tissue were incubated with 3 μg C^{14}-DL-DOPA in 1 ml 0,1 *M* phosphate buffer, pH 7.0, in an atmosphere of nitrogen at 37°C. The DOPA decarboxylase activity was determined by measuring the amount of C^{14}-dopamine produced in 1 h[9,10]. The values are given in mμg dopamine (free base) produced. The activity of monoamine oxidase was determined by means of a method using C^{14}-labelled serotonin as substrate[10,11].

After the fifth passage the dopamine content of the tumour was studied in the following four passages. In the first of these passages significant amounts of dopamine (3.9, 6.7, 7.2 and 7.9 μg/g wet weight) were found in the tumours of the four animals studied. At this stage the tumour was stored for about two months in the deep freeze before renewed transplantation. In all tumour transplants following this treatment dopamine was absent. No explanation of this change of characteristics of the tumour can be offered at present. Neither noradrenaline nor adrenaline could be demonstrated in any experiment before or after deep-freezing the tumour. After administration of DL-DOPA (200 mg/kg i.p.) to tumour-bearing animals, dopamine (mean value 0.7 μg/g wet weight, range 0.2–1.6 μg) could be demonstrated in the tumour tissue 2 h after injection. Similar treatment with L-tyrosine (100 mg/kg) failed to produce any increase in the dopamine content.

The DOPA decarboxylase activity of the tumour tissue was found to be high throughout the experimental period and was unaffected by the deep-freezing. In a consecutive series of determinations on tumours of 16 animals, the

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[5] The tumour has been successfully maintained by serial transplantation since September 1963 at the Department of Pathology in Lund. For transplantation, carefully minced tumour tissue suspended in saline was injected intracutaneously. As a rule the transplantation interval was 2–3 weeks.
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mean value of DOPA decarboxylase activity, expressed as mμg dopamine produced per 10 mg tissue, was 130 (range 50–360). Individual variation of the results may be partly explained by varying vitality of the tissue samples and, further, the animals were taken from different transplantations. In one animal with liver metastases, the DOPA decarboxylase activity of the metastases was somewhat lower than in the inoculated tumour; the values were 140 and 190 mμg respectively, which is about 5 times lower than in normal hamster liver tissue. For comparison, the DOPA decarboxylase activity of the skin surrounding the tumour was determined: mean value 4 mμg dopamine produced per 10 mg tissue (range 2–6 mμg). The values were largely consistent with the activity of normal skin from other species[12]. Addition of 1 μg pyridoxal-5-phosphate to the incubation medium more than doubled the DOPA decarboxylase activity of homogenized samples of tumour tissue[13]; the effect was less marked on minced samples.

It remains to be established whether this observation of a high DOPA decarboxylase activity in a hamster melanoma is characteristic of melanotic tumours in general. The present findings indicate that at least some of the urinary phenolic acids of melanoma patients may be derived from dopamine produced in the tumour tissue. There was no evidence, however, that dopamine was deaminated in the tumour, since the monoamine oxidase activity of the tissue was very low.

The significance of the high DOPA decarboxylase activity in hamster melanoma is unknown. The observation made by the present authors that some human naevi had a raised DOPA decarboxylase activity compared with normal skin may be of some interest in view of the histogenetic connection between naevi and melanotic tumours. The possibility of a connection between dopamine formation in the melanotic tumour and the growth rate[14] was tested in a series of experiments in which the animals received α-methyl DOPA (500 mg/kg i.p.) once daily for ten days. After this treatment DOPA decarboxylase activity was more than 95% inhibited as compared with controls; the growth of the tumours was not influenced.

The high DOPA decarboxylase activity of the hamster melanoma studied does not seem to be characteristic of tumours in general; e.g. transplants from a virus-induced tumour (Rous-Ruppin variant) had low enzyme activity, in all cases at least 10 times less than the melanotic tissue.

The present results seem to indicate the possibility of two different metabolic pathways for DOPA in melanotic tumours; apart from oxidative production of melanin precursors[3], DOPA may also be metabolized anaerobically by decarboxylation[15].

Résumé. Une activité considérable de la 3,4-dihydroxyphénylealanine (DOPA) décarboxylase a été démontrée chez un transplantable mélanome malin du hamster. Après l'injection intrapéritonéale du précurseur de la dopamine, l'amine nouveau-formée était trouvée dans toutes les tumeurs. L'inhibition de la DOPA décarboxylase par traitement des hamsters avec α-méthyle-DOPA n'a pas influé sur le progrès des tumeurs.

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Cholinesterase in the Cat Cerebellar Cortex, Deep Nuclei and Peduncles

The distribution of cholinesterase in the cat's cerebellar cortex, as determined by histochemical methods, was described in an earlier report[1]. Acetylcholinesterase (AChE) is concentrated in the granular layer, which stains more intensely in the depths of sulci than at the tips of folia. The other layers of the cortex are less densely stained. Results described in this report provide evidence for the postulates that afferents to the cerebellar cortex and efferents from the cerebellar deep nuclei are cholinergic[2,3].

Technique. These further experiments were carried out on the localization of AChE in the cerebellar cortex, deep nuclei and peduncles. As well as from normal cats, material was derived from cats in which the cerebellar peduncles had been cut bilaterally, using an approach through the foramen magnum and fourth ventricle (four cats). The vermal lobe of the cerebellar cortex was extensively undercut in a second group of four cats. These experiments were terminated after periods of four to eighteen days and after perfusion with saline and 5% formol-saline the cerebelli and peduncles were stained for AChE.

AChE distribution has been determined with the acetylthiocholine technique described by GEREBZTOFF[4] in which the incubated sections are placed in a dilute solution of ammonium sulphide to produce copper sulphide as the end product at sites of enzymic activity. Other sections were incubated in a 'direct-colouring' acetylthiocholine medium containing potassium ferricyanide[5]. A

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