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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[12] R. HÅKANSON and H. MÖLLER, Acta dermat.-venereol. *43*, 485 (1963).

[13] Carefully minced tumour tissue was homogenized by the method of Potter-Elvehjem in three volumes of ice-cold 0.1 *M* phosphate buffer, pH 7.6, and centrifuged at 20,000 *g* for 15 min in a refrigerated centrifuge. The supernatant was used as enzyme source.

[14] R. HÅKANSON, Exper. *17*, 402 (1961).

[15] This study was supported by grants from Riksföreningen mot cancer, Stockholm, and from the Léonie Deshayes' Foundation, Lund. Melanoma-bearing Syrian hamsters were generously supplied by Prof. H. STORCK, Zürich (Switzerland). The supply of α-methyl DOPA from Merck, Sharp and Dohme (USA) is gratefully acknowledged.

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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[2] C. O. HEBB, Nature *192*, 527 (1961).

[3] W. FELDBERG and M. VOGT, J. Physiol. *107*, 372 (1948).

[4] M. A. GEREBZTOFF, *Cholinesterases, a Histochemical Contribution to the Solution of some Functional Problems* (Pergamon Press, London 1959).

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reddish-brown deposit (believed to be copper ferrocyanide) rapidly forms at sites of AChE activity and the method is of particular value for staining the damaged tissues of operated cats; manipulation of the sections is thereby considerably reduced. AChE associated with nerve fibres is more clearly defined by this latter technique. Counter-staining with cresyl-fast-violet (GEREBZTOFF method) and methyl green (direct colouring medium) has been extensively employed. Details of the method of preparation of tissues for histology, and controls with cholinesterase inhibitors, have been described previously[1].

Results. In the granular layer AChE is present in both extra and intracellular locations. Synaptic areas in the cerebellar islands stain throughout the layer with varying degrees of intensity, but whereas AChE is associated with the surface membrane of stained cells in the granular layer near the tips of folia, many neurones in the depths of sulci contain a high concentration of intracellular cholinesterase.

Cell density is also greater in these deep areas, the two factors being jointly responsible for the increased intensity of the histochemical reaction in granular layers at the depths of sulci. Granular layers in the pyramis, uvula and nodular lobes of the posterior vermal cortex tend to stain more densely than those in other areas of the cerebellar cortex. The intracellularly located AChE in granule-layer cells often failed to react when sections were incubated in the 'direct-colouring' medium, although AChE in the synaptic areas of the cerebellar islands and cell membranes was clearly demonstrable. If these sections were subsequently immersed in ammonium sulphide solution, they assumed the typical appearance of those incubated in the GEREBZTOFF medium, with intracellular deposits of copper sulphide. It appears therefore that ferricyanide ion may be unable to cross the surface membrane of some of these cells and so produce intracellularly the brown copper ferrocyanide compound. Ferrocyanide ion, iontophoresed from glass micropipettes, has been used as an intracellular marker to define cells in the central nervous system of the snail[6]; the cell membrane apparently acts as an effective barrier to its diffusion.

Isolation of areas of the vermal cortex by extensive undercutting did not greatly alter the staining ability of cells and synaptic glomerular areas in the granular layer.

Differences in the histochemically demonstrable amounts of AChE in the molecular layer were apparent when the two staining techniques were compared (Figure A and B), a considerably greater reaction taking place when the 'direct-colouring' technique was employed. Manometric determinations of the AChE content of micro-dissected samples of molecular and granular layers of the cat[7] have revealed that their enzyme content is comparable, and it appears that the direct-colouring technique yields a more accurate representation of the amount of enzyme present in this layer. It has not been possible with either method to demonstrate an association between AChE and neuronal structures in the molecular layer of adult cats. Undercutting the cerebellar cortex does not alter the staining reaction in this layer.

Purkinje cells, which stain weakly in adult cats, exhibit higher levels of AChE in young cats.

Staining with 'direct-colouring' method has revealed the presence of many AChE containing fibres in the cerebellar white matter, a fact which had not been as apparent in sections stained with the GEREBZTOFF technique. Some of these fibres can be followed down to the middle and inferior peduncles, and are probably the terminal portions of mossy afferent fibres. Others may

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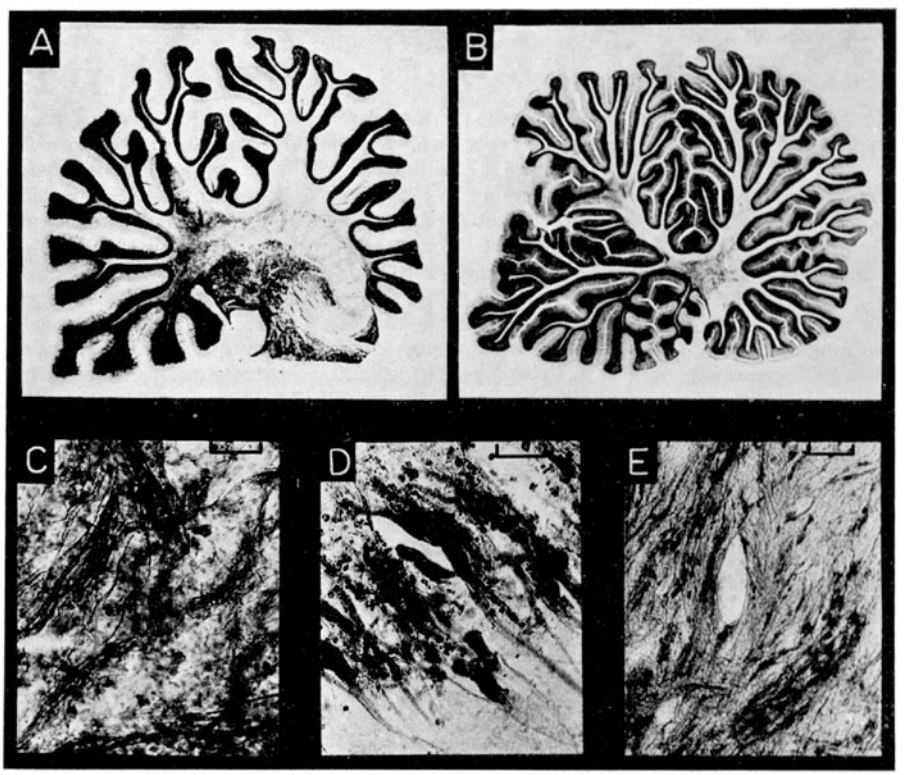

Frozen (30 μ) sections of cat cerebellar cortex and peduncles. A, sagittal section through cerebellum and superior peduncle incubated in GEREBZTOFF medium. B, sagittal, mid-line section through cerebellum incubated in direct-colouring medium. C, D, E, photomicrographs of distended fibres in operated cats. C, fibres in superior peduncle on cerebellar side of lesion. Scale 50 μ. D, distended terminals of fibres in middle peduncle on brain-stem side of lesion. Scale 50 μ. E, fibres in inferior peduncle on brain-stem side of lesion. Scale 100 μ.

constitute association pathways within the cerebellar cortex. After undercutting of the cortex, stained fibres on both sides of the lesion exhibit the distended appearance peculiar to interrupted axons (see below). The white matter of the posterior vermal cortex stains more densely than in other areas (Figure A).

Acetylcholinesterase staining fibres are present in all three cerebellar peduncles; those in the middle peduncle, which stains the most densely, being present in the highest concentration. The polarity of cholinesterase containing fibres was clarified by the results from cats in which peduncular transections were made. This technique of tract interruption, with a subsequent swelling of the axons on the side of the lesion nearest to the parent neurone and simultaneous intensification of staining in cholinesterase containing neurones has been extensively discussed[8,9] and utilized for determinations of the polarity of nervous pathways[10]. The stained fibres in the middle peduncle (Figure D) were identified as afferents to the cerebellum; those in the superior peduncle (Figure C) were mainly efferents with a small minority of afferent fibres; and the inferior peduncle contained both afferent (Figure E) and efferent fibres. Many stained distended fibres in the superior peduncle could be traced back to their origin from cells in the deep nuclei, which also contained AChE (Figure A).

Since the cerebellar peduncles of mammals contain relatively large amounts of choline acetylase[2,3] it is reasonable to postulate that the stained afferent fibres in the middle and inferior peduncles are cholinergic. These axons would terminate as mossy fibres in the granule cell layer. The absence of stained fibres in the molecular layer makes it unlikely that they terminate as climbing fibres. Efferent fibres from the cerebellum may also be cholinergic, since the superior peduncle contains both choline acetylase[2,3] and cholinesterase staining fibres.

The persistence of AChE staining cells, fibres and synaptic glomerular areas in isolated areas of the cerebellar cortex suggests that there may be cholinergic interneuronal circuits within this region. It is of considerable interest that undercutting the vermal cortex of the cerebellum of rats in which few, if any, cells in the granular layer stain with an intensity comparable to those in the cat, leads to a disappearance of AChE in the glomeruli within two days[11]. Since the cerebellar peduncles of the rat stain in a comparable manner to those of the cat, it is reasonable to assume that the mossy afferents are cholinergic. There is, however, no comparable evidence to support the existence of a cholinergic interneuronal system in the vermal cortex of this species.

Résumé. La distribution de l'acétylcholinestérase dans l'écorce, les noyaux centraux et les pédoncules du cervelet du chat a été étudiée à l'aide d'une technique histochimique. Les pédoncules de quatre chats ont été coupés et on a isolé l'écorce cérébelleuse d'un autre groupe de quatre chats. Ces expériences durèrent quelques jours.

Les fibres moussues des pédoncules cérébelleux moyen et inférieur contiennent de l'acétylcholinestérase. Les cellules des noyaux cérébelleux centraux et leurs fibres dans les pédoncules cérébelleux supérieur et inférieur sont aussi colorées par l'acétylcholinestérase.

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La formule chromosomique de *Sorex minutus* L. (Mammalia-Insectivora)

Les chromosomes des Soricidés n'ont fait l'objet que d'un très petit nombre d'investigations. Chez les musaraignes du genre *Sorex*, seules les formules chromosomiques de deux espèces ont été décrites. *Sorex araneus* L. a été étudié par BOVEY[1] qui relève, chez deux mâles, la présence de 23 chromosomes dont un trivalent sexuel. La nature de ce complexe qui répond au schéma X-Y_1Y_2 est établie par SHARMAN[2] après examen de caryotypes femelles, dotés de 22 chromosomes. De plus cet auteur note une variation de type robertsonien du nombre autosomique. Ce polymorphisme chromosomique a été étudié par la suite par FORD, HAMERTON et SHARMAN[3], FORD et HAMERTON[4], MEYLAN[5], MATTHEY et MEYLAN[6] et MEYLAN[7]. Il ressort de ces travaux qu'il existe en fait deux «espèces» distinctes qui ne peuvent être séparées actuellement que par l'examen de leurs caryotypes. L'une, monomorphe, possède 23 chromosomes et un NF (nombre fondamental = nombre de bras principaux chez la femelle) de 42, l'autre, polymorphe, est caractérisée par un nombre diploide variant de 21 à 31 pour un NF constant, égal à 40. En 1964, HALKKA et SKARÉN[8] ont étudié la formule chromosomique de *Sorex unguiculatus* Dobson. Malheureusement, la qualité des figures observées n'a pas permis à ces auteurs de donner avec certitude le nombre diploide de cette espèce qu'ils admettent être de 41, avec un NF voisin de 71. La Figure 3 publiée par les auteurs finlandais montrant un X-Y typique à la métaphase I, le nombre diploide doit être pair, 40 ou 42, et non impair, ce qui impliquerait l'existence d'un trivalent sexuel.

Au printemps 1961, j'ai capturé vivant 6 *Sorex minutus* L. adultes, 5 ♂♂ et 1 ♀, à Troistorrents et à Champéry dans le Val d'Illiez (Alpes valaisannes). Des préparations par écrasement, sans choc colchicinique préalable, ont été effectuées à partir de la rate et des gonades selon la méthode que j'ai décrite en 1964[7]. Les peaux et les crânes de ces animaux sont conservés au Musée zoologique de Lausanne (5) et dans ma collection privée (1).

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