of them in Figure 4 makes an asymmetrical synapse with a large cell of the medial nucleus; the whole bouton is swollen and filled with a coiled mass of neurofilaments. By invagination of developed glial processes, part of this bouton is detached from its contact region, and the postsynaptic membrane thickening remains in the corresponding site (Figure 4, large arrow). In the present study it was found that the boutons derived from SCT fibres largely formed asymmetrical synapses with small-sized dendrites, dendritic spines and cell bodies, and a few formed symmetrical synapses on the cell bodies with wide synaptic clefts.

According to recent electrophysiological studies, excitatory responses with long latencies were recorded in the medial and the interpositus nuclei following stimulation of peripheral limb nerves[9-11], cutaneous mechanoreceptors[9,10], and hindlimb muscle afferents[11], and those with short latencies were rarely recorded following stimulation of the ventrolateral funiculus at C3[10]. Therefore, it is concluded that major input to the cerebellar nuclei is derived from the lateral reticular nucleus or the inferior olive, rather than from the spinal cord. The present observations apparently show that the fibres from the spinal cord terminate directly upon the dendrites and somata of large cells in the cerebellar medial and interpositus nuclei. However, it is not possible to estimate exactly the number of SCT fibre terminals in these nuclei.

*Zusammenfassung.* Bei Katze und Kaninchen wurden Projektionen vom Rückenmark zu den Kleinhirnkernen elektronenmikroskopisch untersucht und nach Läsionen des Halsmarkes wurden viele degenerierte Endknöpfe der Kollateralen des Tractus spinocerebellares in den Nuclei medialis und interpositus gefunden.

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## Synthesis of Nuclear and Mitochondrial DNA in Rat Liver After Injection of the Carcinogenic Compound Diethylnitrosamine

Diethylnitrosamine (DENA) is carcinogenic in many animal species[1] and can induce liver tumors either after prolonged feeding, in the diet[2], or after single administration[3]. Early modifications of nuclear DNA (nDNA) synthesis in rat liver have been observed after injection of dimethylnitrosamine (DMNA)[4], and late effects of DENA on rat liver nDNA synthesis have been analyzed autoradiographically by RAJEWSKI[5]. Although the alkylation of nucleic acids by DENA in rat tissues is less severe than that of similar amounts of DMNA, the basic mechanism of action is the same in both cases and involves the appearance of N-7 alkylated guanine[6]. This similitude incited us to look for possible differences in the effects of DENA on the synthesis of rat liver nDNA and mitochondrial DNA (mtDNA)[7], the probability of which is strengthened by the observation of WUNDERLICH et al.[8] that liver mtDNA

[1] H. DRUCKREY, R. PREUSSMANN, S. IVANOVIC and D. Z. SCHMÄHL, Z. Krebsforsch. *69*, 103 (1967).
[2] D. SCHMÄHL, R. PREUSSMANN and H. HAMPERL, Naturwissenschaften *47*, 89 (1960).
[3] E. SCHERER and M. HOFFMANN, Eur. J. Cancer *7*, 369 (1971).
[4] B. W. STEWART and P. N. MAGEE, Biochem. J. *125*, 943 (1971).
[5] M. F. RAJEWSKI, Eur. J. Cancer *3*, 335 (1967).
[6] P. F. SWANN and P. N. MAGEE, Biochem. J. *125*, 841 (1971).
[7] M. CHÈVREMONT, in *Cell Growth and Cell Division*, Symposia of the International Society for Cell Biology ,(Ed. R. J. C. HARRIS; Academic Press, New York 1963), vol. 2, p. 323.

Specific radioactivity, in dpm/mg DNA, of nDNA and mtDNA after injection of DENA

| Time (h) after injection | nDNA | | | mtDNA | | |
|---|---|---|---|---|---|---|
| | DENA | controls | DENA/controls | DENA | controls | DENA/controls |
| 1 | 980 | 1800 | 0.55 | 89.150 | 52.500 | 1.70 |
| | 2300 | 5700 | 0.41 | 25.280 | 15.140 | 1.67 |
| 2 | 2170 | 4430 | 0.49 | 47.650 | 59.370 | 0.80 |
| | 715 | 1300 | 0.55 | 31.620 | 38.890 | 0.81 |
| 4 | 653 | 1970 | 0.33 | 46.140 | 33.570 | 1.37 |
| | 865 | 2360 | 0.36 | 49.506 | 39.470 | 1.25 |
| 6 | 644 | 913 | 0.70 | 32.940 | 43.610 | 0.75 |
| | 940 | 1100 | 0.92 | — | — | — |
| 12 | 630 | 3670 | 0.17 | 34.965 | 51.708 | 0.67 |
| | 1200 | 3560 | 0.34 | 12.590 | 37.780 | 0.33 |
| 24 | 5830 | 2770 | 2.1 | 11.230 | 59.000 | 0.19 |
| | 2320 | 1020 | 2.3 | 12.070 | 48.000 | 0.25 |
| 30 | 7650 | 1250 | 6.1 | — | — | — |
| | 4500 | 2180 | 2,0 | 7.400 | 24.760 | 0.30 |
| 48 | 48.890 | 1700 | 28.7 | 60.800 | 66.580 | 0.91 |
| | 20.230 | 4355 | 4.6 | — | — | — |

Results of 2 separate experiments; 5 rats are used per experiments.

shows a preferential alkylation as compared with nDNA after treatment of rats with a single injection of DMNA or N-methyl-N-nitrosourea[9].

*Material and methods.* Male Wistar rats weighing 180 to 200 g were used. They were starved 24 h before sacrifice. A dose of 100 mg/kg DENA was injected i.p. (the DENA purchased by Schuckardt was diluted with NaCl 0.15 *M* to a concentration of 40 mg/ml). Control rats were injected with a similar volume of NaCl 0.15 *M*. 1 h before sacrifice, the animals were given an i.p. injection of 20 µCi $^3$H-Thymidine at a specific radioactivity of 1 Ci/m*M*. After sacrifice, the liver was quickly removed, perfused with ice-cold 0.25 *M* sucrose pH7, blotted dry, weighed and homogenized in 10 vol. 0.25 *M* sucrose.

The separation of nuclei and mitochondria as well as the extraction of nDNA and mtDNA were carried out as previously described[10]. The radioactivity was measured in a Packard Tri Carb liquid scintillator, and the efficiency of counting determined by use of an internal standard. The amount of DNA was determined by the diphenylamine reaction according to BURTON[11]. The results (Table) are expressed in dpm/mg DNA.

*Results and discussion.* Relatively large differences may be observed in the specific radioactivities from one series of rats to the other, but the ratios of activities of injected group relative to control group were consistent from one series to the other for any given time, as well for nDNA as for mtDNA. The results of two different experiments are presented in the Table. For each experiment, 5 rats were used in order to have enough liver tissue for the isolation of mtDNA.

From the Table, it is seen that nDNA synthesis first passes through an inhibitory phase, then increases markedly 30 h, and still more 48 h, after injection of DENA. A similar increase, though appearing more rapidly, has been observed after injection of DMNA[4]. It shows the appearance of a regenerative process induced by the discrete necrotic lesions due to DENA injections. If the behaviour of liver nDNA synthesis in DENA-treated rats is reminiscent of what is observed after partial hepatectomy, it is however not the case for mtDNA synthesis. Whereas in regenerating liver, mtDNA synthesis continuously increases shortly after partial hepatectomy[12], the increase is small and transient after DENA injection and is followed by a rather pronounced decrease at the moment when nDNA synthesis begins to increase. Therefore, the regenerative processes induced by partial hepatectomy or by DENA injection are different as far as DNA synthesis is concerned. Nuclear DNA synthesis is more sensitive than mtDNA synthesis to DENA: inhibition is observed already 1 h after injection for the former, but only from the 6th h for the latter. It remains to be established whether differences in alkylation level between nDNA and mtDNA could account for the differences observed in the synthesis of liver nDNA and mtDNA in DENA-injected rats[13].

*Résumé.* L'injection de diéthylnitrosamine à des rats provoque, dans les heures qui suivent, une inhibition de synthèse plus forte au niveau du DNA nucléaire du foie qu'au niveau du DNA mitochondrial. A la phase d'inhibition succède une stimulation de synthèse de nDNA, avec retour à un niveau normal de la synthèse de mtDNA.

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[8] V. WUNDERLICH, I. TETZLAFF and A. GRAFFI, Chem. biol. Interactions *4*, 81 (1971–1972).
[9] V. WUNDERLICH, M. SCHÜTT, M. BÖTTGER and A. GRAFFI, Biochem. J. *118*, 99 (1970).
[10] L. BAUGNET-MAHIEU, C. BAES and R. GOUTIER, Biochem. Pharmac. *20*, 141 (1971).
[11] K. BURTON, Biochem. J. *62*, 315 (1956).
[12] S. NASS, Biochim. Biophys. Acta *145*, 60 (1967).
[13] Grants from the Fonds de la Recherche Scientifique Médicale and Fonds de la Recherche Fondamentale Collective are gratefully acknowledged. The authors thank Miss R. LEMESTRÉE for skillfull technical assistance.

## Lysozyme Activity in the Plaice (*Pleuronectes platessa* L.)

Lysozymes (EC 3.2.1.17) are widely distributed in nature[1]. Our examination of teleosts caught in British waters showed that lysozyme was consistently present throughout the year in the sera of flatfish; *Pleuronectes platessa* L., *Platichthys flesus* (L.), *Limanda limanda* (L.), *Scophthalmus maximus* (L.) and *Scophthalmus rhombus* (L.). With other orders it was variable in its occurrence, even among individuals of the same species, such as the haddock, *Melanogrammus aeglefinus* (L.), and was never present in detectable amounts in 30 specimens of cod (*Gadus morhua* L.) examined over a 2 year period. Lysozyme activity was measured by a turbidimetric assay[2], with hen egg white lysozyme (Armour, crystallized) as a standard.

Lysozyme, either alone or in conjunction with complement and antibody is thought to contribute to bacteriolytic mechanisms in vertebrates, including fish[3]. For this reason the present study is concerned with the nature and cellular origin of lysozyme in the tissues and body fluids of the plaice. Previous work had shown that lysozyme was present not only in the plasma, but also the cutaneous mucus[4].

After centrifugation of heparinized plaice blood, a fraction containing erythrocytes and no detectable white cells was obtained, together with another fraction containing white cells contaminated with a small proportion of erythrocytes. The 2 fractions were washed and lysed. Lysozyme activity could only be detected in the white cell fraction. Since the erythrocyte fraction was inactive it was concluded that the enzyme was exclusively associated with the white cells. Neutrophils and monocytes, together with lymphocytes and thrombocytes have been described in plaice blood[5], although the white cells represent less than 2% of the total blood cells and are comprised of 81% lymphocytes, 18% neutrophils and less than 1% monocytes (D. A. CONROY, personal communication).

[1] P. JOLLÈS, Angew. Chem. int. Edn. *8*, 227 (1969).
[2] G. LITWACK, Proc. Soc. exp. Biol. Med. *89*, 401 (1955).
[3] V. L. VLADIMIROV, Bull. Off. int. Epizoot. *69*, 1365 (1968).
[4] T. C. FLETCHER and P. T. GRANT, Biochem. J. *106*, 12P (1968).
[5] C. S. WARDLE, J. mar. biol. Ass. U.K. *51*, 977 (1971).