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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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).

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It has not yet been possible to isolate individual classes of cells from the heterogeneous white cell population. However, the production of peritoneal exudate cells, 18 h after the i.p. injection of shellfish glycogen[6], yielded a reasonably homogeneous population of cells which, morphologically, most resembled the blood neutrophils. These cells contained higher levels of lysozyme than were found in the plasma. 48 h after glycogen injection, the majority of the exudate cells were monocytic, but when harvested and lysed, were also found to contain lysozyme at concentrations similar to those found in the earlier granulocyte population. It is probable that both cell types contribute to the activity of the plasma, since there is an increase in the numbers of both monocytes and neutrophils in the blood, following the i.v. injection of latex beads (0.091 µm diameter), and a 50% increase in the serum lysozyme levels 5 h after the injection. The clearance of colloidal carbon from plaice blood showed the kidney and spleen to be the principal phagocytic organs, but not the liver. This may account for the absence of lysozyme from the liver homogenates.

Reported increases in the lysozyme levels of immunized fish[3,7] could reflect changes in the white cell population during the development of the immune response. Various infections of fish also result in changes in the leukocyte numbers[8], leading to variations in the blood lysozyme levels, which may in turn be diagnostic of the disease state of the fish.

A fraction containing all the lysozyme activity could be isolated from plaice serum by gel filtration on Sephadex G-75 (Figure). The elution volume was identical with hen egg lysozyme, suggesting a similar molecular weight of between 14,000 and 15,000. The isolated plaice serum lysozyme had a pH optimum of 5.4 ($I = 0.1$) and exhibited the properties attributed to a lysozyme[9], including weak chitinase (EC 3.2.1.14) activity. After electrophoresis on cellulose acetate at pH 8.6, the lytic activity of the basic lysozyme could be demonstrated[10] in the cathodal region, but its migration at only half the speed of the hen egg lysozyme probably reflects a difference in their amino acid composition.

While fractionating the plaice serum on Sephadex G-75, the last peak eluting from the column was found to contain a high level of chitinase activity, distinct from that associated with the lysozyme fraction. A similar fractionation of cod serum, which contained no lysozyme, also exhibited chitinase activity in a low molecular weight fraction, when assayed with colloidal chitin as substrate[11]. Unlike the lysozyme, the chitinase activity appears to be related to diet, since the chitinase activity was rapidly lost from the sera of cod and plaice when they were not feeding.

Although a leukocytic origin for plaice plasma lysozyme would be in agreement with the mammalian condition[12] the origin of the secretory lysozyme may be different. In the plaice skin, it has been possible to demonstrate histochemically[13], distinct, lysozyme containing cells, absent from cod epidermis, which do not appear to be leukocytes and which occur both in the basal layer of the epidermis and also migrating towards the surface (C. K. MURRAY, personal communication). Whether these cells are the only source of secretory lysozyme in the plaice, is not known. Eosinophilic granular cells, of unknown function, have been described in plaice epidermis[14] and whether these are related to the lysozyme containing

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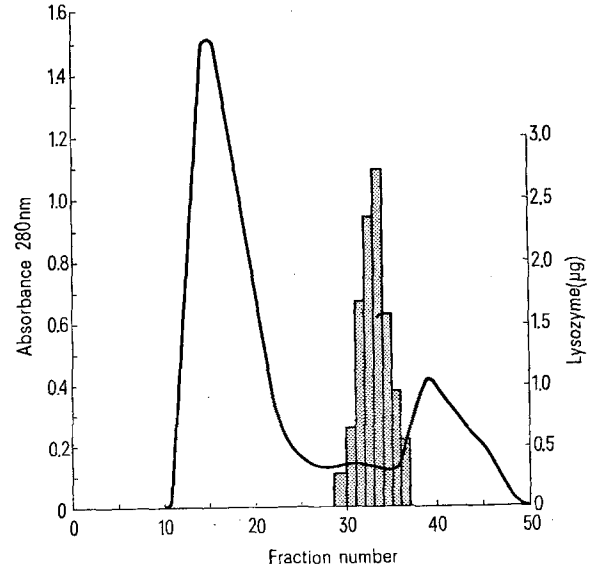
Lysozyme content of tissues from *Pleuronectes platessa* L.

No. of samples	Tissue	µg lysozyme/mg protein (mean ± s.e.m.)
188	Serum	0.149 ± 0.005
29	Plasma	0.114 ± 0.01
12	Lymph	0.231 ± 0.035
36	Peritoneal exudate cells (18 h)	0.449 ± 0.089
12	Mucus	0.322 ± 0.09
13	Kidney	0.278 ± 0.043
10	Spleen	0.146 ± 0.029
15	Stomach	0.192 ± 0.037
10	Gill lamellae	N.D.[a]
15	Intestine	N.D.[a]
10	Liver	N.D.[a]

[a] N.D. indicates lysozyme activity not detectable. Peritoneal cells were harvested in 1.1% NaCl (18 h after glycogen injection) and the packed cells, together with the other organs, were homogenized in water with an Ultra-Turrax. This was followed by 4 cycles of freezing and thawing and after centrifugation at 3000 g for 10 min, the supernatants were assayed. Mucus was dialysed against water at 4 °C for 18 h and concentrated by freeze-drying, before assay.



Gel filtration on Sephadex G-75 of 3 ml of plaice serum. The shaded area indicates fractions containing lysozyme. Fractions (3 ml) were eluted at 9 ml/h from a column 90 × 1.5 cm, with 0.06 *M* phosphate buffer pH 6.0 containing 0.05 *M* NaCl, at 4 °C.

cells remains to be established. Lysozyme activity was not associated with the goblet cells, so that mixing between the mucus and lysozyme probably only occurs after discharge. It was not possible to demonstrate lysozyme activity in the detached, mucus-free epidermis by the turbidimetric assay, which may reflect the loss of lysozyme containing cells into the mucus during collection. Lysozyme was also present in the gill mucus but could not be demonstrated in the gill lamellae once the mucus had been removed. The origin of lysozyme in mammalian secretions has not yet been established, although the demonstration of lysozyme in distinct epithelial cells of the human parotid gland[15] and in Paneth cells of mice[16] would suggest local synthesis rather than derivation from the plasma, and may be analogous to the plaice epidermis.

Zusammenfassung. Die Untersuchung der Lysozymverteilung im Gewebe des Knochenfischs, *Pleuronectes platessa* L., ergab, dass das Plasma-Lysozym von Leukozyten abstammt, das Hautschleim-Lysozym dagegen von spezifischen Epidermiszellen.

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Peroxide Detoxification Affecting the Production of Immunoglobulin by Mouse Myeloma Tumor Cells in vitro

The synthesis and secretion of immunoglobulins (Ig) is a major characteristic of mouse myeloma tumors. Moreover, cells derived from such tumors have been shown to be capable of producing Ig in vitro[1,2]. The toxic effect of peroxide on tumor cells, when the former is added to cells or generated by radiation or chemical means, has been well documented[3,4]. Evidence is presented here for the toxic effect of endogenously generated peroxides on Ig production of mouse myeloma cells in vitro.

MOPC 46 myeloma tumors were carried in BALB/c mice by serial transplantation[1] and cell suspensions prepared and incubated with radioactive precursors (^{3}H-leucine) as previously described[5–8]. Specific anti-mouse γ-globulin and anti-rabbit γ-globulin antisera were produced and the specific coprecipitation of labeled Ig with the appropriate controls were performed as previously described[5–8]. This technique detects Ig with a high degree of accuracy and specificity[5,7].

The effects of glucose and cell density on the incorporation of radioactivity into Ig by MOPC 46 tumor cells are shown in Table I. Glucose stimulated incorporation to a greater extent at low cell concentration than at higher cell concentration. In the absence of glucose, a disproportionate increment in Ig production resulted

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Table I. Effect of cell density on the production of immunoglobulin by MOPC 46 tumor cells

Experiment	Cells/flask	Incubation with glucose	Incubation without glucose
		Radioactivity in immunoglobulin (cpm/flask)	
1	1×10^8	11.970	370
	5×10^8	27.390	6.380
		Radioactivity in Immunoglobulin 10^6 Cells	
		cpm	cpm
2	1×10^6	67	ND[a]
	5×10^6	162	ND[a]
	1×10^7	261	ND[a]
	5×10^7	285	4
	1×10^8	270	6
	2×10^8	186	15
	3×10^8	124	28
	4×10^8	104	41
	5×10^8	92	36

[a] ND, not determined.
Duplicate 30 ml beakers containing the desired cell number and 30 μc ^{3}H-leucine in 3.0 ml of incubation medium were incubated at 37 °C for 2 h. 1 flask received 30 μl of 1 *M* glucose to yield a final concentration of 10^{-2} glucose, while the duplicate flask received 30 μl water. After incubation, the contents of the beakers were centrifuged at $1{,}350\times g$ for 5 min, the extracellular medium transferred to a tube containing 100 μmoles of unlabeled leucine in 2 ml 0.1 *M Tris* HCl pH 7.4, and centrifuged at $9{,}000\times g$ for 5 min. Radioactivity specifically coprecipitable as mouse immunoglobulin was determined as described.