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.

THELMA C. FLETCHER and ANN WHITE

Natural Environment Research Council, Institute of Marine Biochemistry, St. Fittick's Road, Aberdeen, AB1 3RA (Great Britain), 12 April 1973.

[15] F. W. KRAUS and J. MESTECKY, Archs oral Biol. *16*, 781 (1971)
[16] Y. GHOOS and G. VANTRAPPEN, Histochem. J. *3*, 175 (1971).

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

[1] M. POTTER, in *Methods in Cancer Research* (Ed. H. BUSCH; Academic Press, New York 1967), vol. 2, p. 105.
[2] A. R. WILLIAMSON and B. A. ASKONAS, Archs Biochem. Biophys. *125*, 401 (1968).
[3] D. BURK and M. WOODS, Radiation Res. Suppl. *3*, 212 (1963).
[4] L. BENADE, T. HOWARD and D. BURK, Oncology *23*, 33 (1969).
[5] H. J. COHEN and M. KERN, Biochim. biophys. Acta *188*, 255 (1969).
[6] E. HELMREICH, M. KERN and H. N. EISEN, J. Biol. Chem. *236*, 464 (1961).
[7] R. M. SWENSON and M. KERN, Proc. natn. Acad. Sci. *57*, 417 (1967).
[8] M. KERN, E. HELMREICH and H. N. EISEN, Proc. natn. Acad. Sci. *45*, 862 (1959).

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.

Table II. Effect of catalase and other protein additions on the production of immunoglobulin by MOPC 46 tumor cells

Experiment	Cells/flask	Glucose	Additions	Radioactivity in immunoglobulin (cpm/flask)
1	1×10^8	—	none	200
	1×10^8	—	Catalase	7.440
	1×10^8	+	none	9.390
	1×10^8	+	Catalase	9.740
	1×10^8	—	Horseradish peroxidase	2.620
	1×10^8	—	Bovine globulin	40
	1×10^8	—	Mouse globulin	300
	1×10^8	—	Egg albumin	150
	1×10^8	—	Boiled cells	230
2	1×10^8	—	none	300
	1×10^8	—	Catalase (10 μg)	4.610
	1×10^8	—	Catalase (1 μg)	1.920
	1×10^8	—	Catalase (100 μg-boiled)	20
3	1×10^8	—	none	50
	1×10^8	—	Catalase (10 μg)	3.560
	1×10^8	—	Catalase (10 μg) + + ATA	50
4	5×10^8	—	none	7.520
	5×10^8	—	Catalase	15.690
5	5×10^8	—	none	10.330
	5×10^8	—	ATA	9.990

The incubation and assay conditions are as described in the legend to Table I. Except where indicated otherwise, further additions were made per flask as follows: Catalase, 1,000 U (0.1 mg protein); bovine γ-glubolin, 1.0 mg; egg albumin, 1.0 mg; and horseradish peroxidase, 270 U (0.075 mg protein); ATA, 10 mg. 5×10^8 MOPC 46 cells (heated for 15 min in boiling water bath) and mouse γ-glubolin, 1.0 mg, were added as indicated.

from a 5-fold increase in cell concentration. In other experiments, galactose, mannose, pyruvate and α-ketoglutarate also significantly increased the incorporation in substitution for glucose.

The addition of medium, derived from the incubation of 5×10^8 cells for 2 h, to a fresh preparation of 1×10^8 cells was without stimulatory effect. The addition of 4×10^8 rabbit lymph node cells could stimulate incorporation of radioactivity by 1×10^8 MOPC 46 cells specifically into mouse gammaglobulin, from 600 to 3800 cpm/flask, but killed cells did not.

The decreased incorporation of radioactivity into immunoglobulin by 1×10^8 cells in the absence of glucose could be partially prevented by the presence of catalase during the incubation (Table II). Horseradish peroxidase mimicked the effect. Other proteins at even higher concentrations were devoid of this capacity (Table II, experiment 1). The effect was eliminated by heat inactivation of the enzyme or by the presence of the specific catalase inhibitor, 3-amino-1,2,4-triazole (ATA)[9], in nontoxic amounts[4] (Table II, experiments 2 and 3), stressing the importance of enzymatic activity. Catalase also stimulated incorporation by 5×10^8 MOPC 46 cells, indicating the insufficiency of the intracellular mechanism for coping with peroxides even at this cell concentration (Table II, experiment 4). Measurement of the catalase content of these cells[10] revealed that 5×10^8 MOPC 46 cells contain less than 10 units of catalase while the amount of ATA added was sufficient to inhibit ten times that amount. However, the addition of ATA had no effect on the radioactive incorporation by 5×10^8 cells (Table II, experiment 5); the importance of catalase as the mechanism for peroxide detoxification by these cells is thus questionable.

MOPC 46 cells, in the presence of glucose, were able to cope with endogenously-generated peroxides since addition of catalase was without effect (Table II, experiment 1). Glutathione peroxidase has been implicated in glucose-dependent peroxide detoxification[11]. The determination of glutathione peroxidase activity in MOPC 46 tumor tissue, prepared and analyzed by the method of HOCHSTEIN[12], showed enzyme activity of 0.024 μmole NADH oxidized per min/43 μg crude enzyme extract, greater than that described for glutathione peroxidase-rich rat liver by HOCHSTEIN[12].

The data presented here demonstrate that at least some of the effects of substrate and cell concentration on cells in vitro can be studied by examining the synthesis and secretion of Ig. The inhibition of incorporation of radioactivity into Ig by MOPC 46 cells, in the absence of glucose or other suitable substrate is due, at least in part, to the endogenous production of peroxides, including hydrogen peroxide[13,14]. Thus, the addition of peroxide detoxifiers may be helpful in obtaining increased radioactive precursor incorporation in studies requiring the limitation of glucose.

[9] E. MARGOLIASH and A. NOVOGRODSKY, Biochem. J. *68*, 468 (1958).
[10] R. F. BEERS, JR. and I. W. SIZER, J. biol. Chem. *195*, 133 (1952).
[11] G. COHEN and P. HOCHSTEIN, Biochemistry *2*, 1420 (1963).
[12] P. HOCHSTEIN and H. UTLEY, Molec. Pharmac. *4*, 574 (1968).
[13] P. NICHOLLS and G. R. SCHONBAUM, in *The Enzymes* (Ed. P. D. BOYER, H. LARDY and K. MYRBACH; Academic Press, New York 1963), vol. 2, p. 147.
[14] J. F. THOMPSON, Radiation Res. Suppl. *3*, 93 (1963).
[15] G. C. MILLS, Archs Biochem. Biophys. *86*, 1 (1960).
[16] O. WARBURG, in *New Methods in Cell Physiology* (Interscience Publishers, New York 1962), p. 511, 515.
[17] This work was performed during a Staff Associateship at the N.I.H.-N.I.A.M.D.

It has been suggested that glutathione peroxidase rather than catalase is the principal defense against peroxide toxicity in some cells[11, 12, 15]. The above studies have demonstrated significant levels of catalase[12]. The enhanced peroxide detoxification capability of the MOPC 46 cells in the presence of glucose could be related to the generation of reduced cofactors for glutathione peroxidase during glucose metabolism. Thus these results are consistent with the view that catalase may not provide the major mechanism for peroxide detoxification by the MOPC 46 tumor cell and other neoplastic cells[16], and that glutathione peroxidase offers a likely alternative mechanism[17].

Résumé. Des péroxides détoxifiants peuvent empêcher la réduction de la production des immunoglobulines par les cellules de la tumeur MOPC 46 en l'absence de glucose. Le contenu en glutathion de péroxydase de ces cellules est élevé tandis que s'abaisse le contenu en catalase.

H. J. Cohen[17]

Department of Medicine, Duke University Medical Center, Durham (North Carolina 27710, USA),
12 October 1973.

The Distribution of Inorganic Phosphate in Blood Conserved at 4°C in Acid-Citrate-Dextrose with Adenosine and Persantin®

During the conservation of blood in acid-citrate-dextrose (ACD) solution at 4 °C, there occurs a relatively fast decomposition of phosphate compounds, especially 2, 3-diphosphoglycerate (2, 3-DPG). This fact is reflected in the increasing concentration of inorganic phosphate (P_i) inside the erythrocytes and its gradual penetration outside the red cell[1–3]. As we have stated before, the addition of adenosine in combination with persantin to the conserving medium causes the slower rate of decomposition of 2, 3-DPG and slower increase of P_i inside the red cell[2]. Similarly, Gibson et al.[4], who incubated blood at 37 °C, stated the slower increase of P_i in the presence of adenosine and persantin. Gerlach et al.[5] have found that persantin considerably reduced the rate of phosphate influx and efflux in the erythrocytes incubated at 37 °C, the efflux being more strongly influenced than the influx. Persantin retards also the adenosine transport across the red cell membrane and inhibits adenosine deaminase in whole blood[6]. The rate of the P_i increase in blood con-

[1] G. R. Bartlett and H. N. Barnet, J. clin. Invest. *39*, 56 (1960).
[2] B. Zachara, Acta haemat. *48*, 164 (1972).
[3] H.-G. Böer, P. Stehle, H. Roigas and S. Rapaport, Folia haemat. *90*, 265 (1968).
[4] J. G. Gibson II and F. J. Lionetti, Transfusion *6*, 427 (1966).
[5] E. Gerlach, B. Deuticke and J. Duhm, Pflügers Arch. ges. Physiol. *280*, 243 (1964).
[6] R. D. Bunag, C. R. Douglas, S. Imai and R. M. Berne, Circulation Res. *15*, 83 (1964).

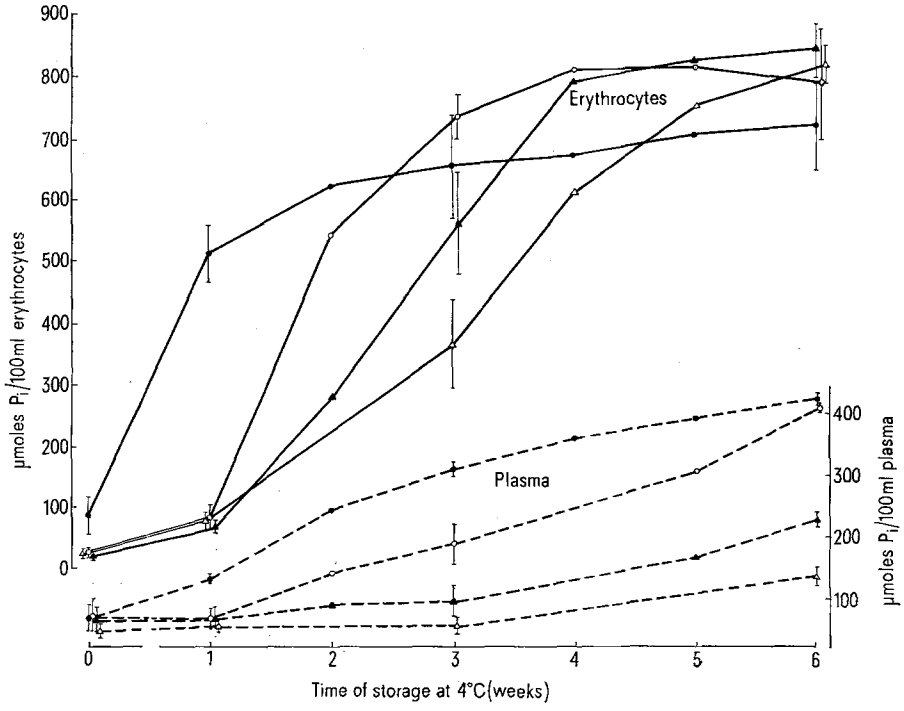

Fig. 1. The content of P_i in erythrocytes (—) and in plasma (- - -) in blood conserved at 4 °C in ACD (●), ACD-A (○), and in ACD-A-P ($\Delta = 1 \times 10^{-4}\,M$; $\square = 4 \times 10^{-4}\,M$). The content of P_i is expressed in µmoles per 100 ml of packed red cells (mean ± S.D. of 3 experiments) and per 100 ml of plasma (mean ± S.D. of 6 experiments).