steps, suggests that the proportion of strongly bound extra histones is in fact far less than this[6].

Résumé. La déoxyribonucléoprotéine (DNP) du thymus de veau peut lier de l'histone supplémentaire en solution physiologique. La plupart de cette protéine est très faiblement liée et s'est degagée par HCl de 3,3 m*M* ou moins. On suggère ainsi que l'histone supplémentaire soit combinée aux groupes carboxyl des histones originales du DNP. Néanmoins, environ 10% de la protamine, ajouté pareillement au DNP, fut liée fortement ce qui suggère qu'elle peut atteindre les groupes phosphates libres sur l'ADN. La protamine apparemment n'a pas deplacée les histones lorsqu'elle fut liée au DNP.

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The H_2O_2-Production by Polymorphonuclear Leucocytes During Phagocytosis

In guinea-pig[1–6] as well as in human[7] polymorphonuclear (PMN) leucocytes, an increased rate of oxidation of $NADPH_2$ by a granule bound KCN-insensitive oxidase has been demonstrated during phagocytosis.

A H_2O_2-production associated with the stimulation of respiration in phagocytosing PMN leucocytes has been argued on the basis of an increased oxidation of formate[8–12], which is known to be oxidized by peroxidase or catalase in the presence of a H_2O_2-producing system[13]. In the present study, direct evidences for H_2O_2-accumulation during phagocytosis have been provided. Guinea-pig leucocytes (95% PMN) were obtained from peritoneal exudates and human blood leucocytes (70% PMN) were separated from red cells by dextran sedimentation[14]. When needed the leucocyte suspensions were purified from residual erythrocytes by means of a rapid hypotonic haemolysis[15]. The results presented here refer to the experiments with human PMN leucocytes. Quite similar results have been obtained by using guinea-pig leucocytes. Phagocytosis was performed by adding bacteria (*Bacillus subtilis*, filamentous forms, killed by heating 30 min at 120 °C, opsonized with fresh homologous serum 30 min at 37 °C and washed in saline) to a leucocyte suspension in calcium-free Krebs-Ringer phosphate solution incubated at 38 °C in a closed plastic chamber equipped with a Clark oxygen electrode (Yellow Spring Instr. Co., Ohio, USA). Experiments of phagocytosis were also performed in shaken Warburg vessels at 38 °C for 15 min and thereafter the leucocyte-bacteria mixtures were transferred into the plastic chamber. The H_2O_2 has been measured as oxygen liberated after adding catalase (Sigma).

The membrane coating the platinum surface of the electrode was shown to be impermeable to H_2O_2 according to the procedure described by Rorth and Jensen[16]. It has been preliminarily shown that small amounts of H_2O_2 were quantitatively recovered as O_2 liberated by catalase added in excess (Figure 1) and that endogenous catalase of intact leucocytes, measured as O_2-production from added H_2O_2, is almost completely inhibited by 2 m*M* KCN (Figure 1).

The addition of catalase to PMN leucocytes during phagocytosis in the presence of 2 m*M* KCN causes a liberation of oxygen and a modification of the rate of the oxygen uptake, indicating that an appreciable amount of H_2O_2 was accumulated (Figure 2 a, b). When catalase is added to PMN leucocytes during phagocytosis in the absence of KCN, the liberation of oxygen is hardly detectable (Figure 2 dotted traces).

In other experiments leucocyte-bacteria mixtures were incubated 15 min at 38 °C with or without KCN and then transferred to the plastic chamber for the measurements of H_2O_2 as indicated above. Figure 3 shows that after 15 min of phagocytosis an accumulation of H_2O_2 takes place only when KCN is present. These findings directly indicate that a H_2O_2-forming respiratory system is involved in the stimulated respiration of phagocytosing PMN leucocytes.

An approximate calculation of the ratio oxygen consumed/oxygen liberated after catalase, shows that in the early stage after addition of KCN nearly all the oxygen is consumed with stoichiometric accumulation of H_2O_2. The values from the experiment of Figure 2b are: 0.2 μ atoms of oxygen consumed and 0.08 μmoles of H_2O_2 decomposed by catalase. In the long term experiments, when the measure of H_2O_2 is performed several min after addition of 2 m*M* KCN (Figure 2a) or after 15 min of preliminary incubation of phagocytosing PMN leucocytes at 38 °C (Figure 3), the amount of oxygen liberated by

[1] F. Rossi and M. Zatti, Experientia *20*, 21 (1964).
[2] F. Rossi and M. Zatti, Br. J. exp. Path. *45*, 548 (1964).
[3] M. Zatti and F. Rossi, Biochim. biophys. Acta *99*, 557 (1965).
[4] F. Rossi and M. Zatti, Biochim. biophys. Acta *113*, 395 (1966).
[5] F. Rossi and M. Zatti, Biochim. biophys. Acta *121*, 110 (1966).
[6] M. Zatti and F. Rossi, Experientia *22*, 758 (1966).
[7] M. Zatti and F. Rossi, unpublished.
[8] G. Y. N. Iyer, M. F. Islam and J. H. Quastel, Nature *192*, 535 (1961).
[9] G. Y. N. Iyer and J. H. Quastel, Can. J. Biochem. Physiol. *41*, 427 (1963).
[10] J. Roberts and J. H. Quastel, Nature *202*, 85 (1964).
[11] R. J. Selvaray and A. J. Sbarra, Nature *211*, 1272 (1966).
[12] B. Holmes, A. R. Page and R. A. Good, J. clin. Invest. *46*, 1422 (1967).
[13] B. Chance, J. biol. Chem. *182*, 649 (1950).
[14] M. Zatti and F. Rossi, Ital. J. Biochem. *10*, 19 (1961).
[15] R. Wagner and A. Yourk, Archs Biochem. Biophys. *39*, 174 (1952).
[16] M. Rorth and P. K. Jensen, Biochim. biophys. Acta *139*, 171 (1967).

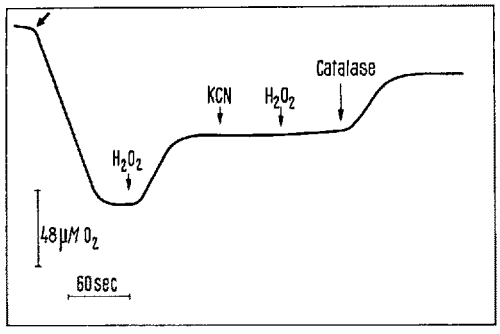

Fig. 1. Oxygen trace of H_2O_2-decomposition by catalase in a system of 2.0 ml containing 2×10^7 human PMN leucocytes in calcium-free Krebs-Ringer phosphate solution. At the points indicated the addition was made as follows: 2 µl of 0.1 *M* H_2O_2; 4 µl of 1 *M* KCN; 25 µl of catalase at 2.16×10^5 Sigma U/ml. The initial decrease of oxygen tension was obtained by gassing with nitrogen (thick arrow). Temperature 38 °C.

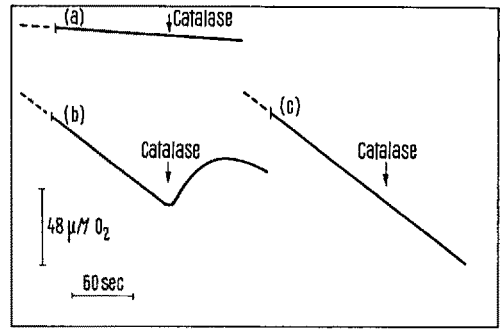

Fig. 3. Measure of H_2O_2 accumulated by 3×10^7 human PMN leucocytes at rest and after 15 min of phagocytosis. The leucocytes/*B. subtilis* mixtures (1:50) were incubated 15 min in shaken Warburg vessels at 38 °C and then transferred to a closed plastic chamber (see text). (a) Resting leucocytes with and without 2 m*M* KCN; (b) leucocytes phagocytosing in the presence of 2 m*M* KCN; (c) leucocytes phagocytosing without inhibitors. The dotted lines represent the 15 min of preincubation.

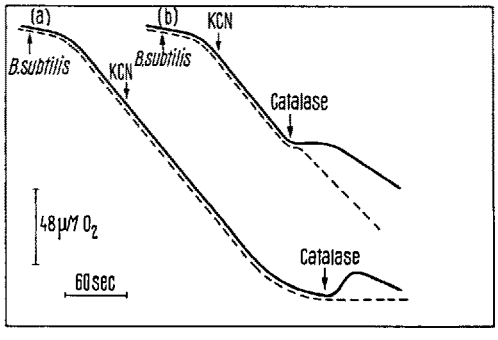

Fig. 2. Measure of H_2O_2 produced by human PMN leucocytes during phagocytosis with (———) and without (– – – – –) 2 m*M* KCN. For conditions see Figure 1. *B. subtilis*, 1×10^9.

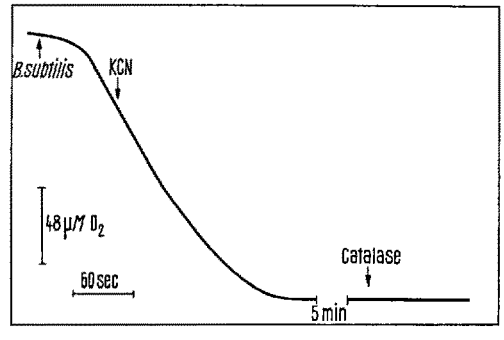

Fig. 4. Measure of H_2O_2 accumulated by 2×10^7 human PMN leucocytes during phagocytosis in the presence of 2 m*M* KCN. Catalase was added 6 min since the system was anaerobic. For conditions see Figures 1 and 2.

catalase is much less than that expected if all H_2O_2 were accumulated, and it can be non-detectable when catalase is added after the system was anaerobic for a few min (Figure 4). This could be due to utilization of H_2O_2 taking place even in the presence of KCN.

When phagocytosis occurs in absence of KCN, the amount of H_2O_2 accumulated by PMN leucocytes, as displayed by the procedure employed, is minimal. In normal conditions of phagocytosis the peroxide must be almost completely utilized as it is formed. The mechanism involved in this utilization is not known as yet, but the present results seem to indicate that it can be active even in the presence of 2 m*M* KCN (in spite of a partial sensitivity to the inhibitor). PMN leucocytes contain[11–13,20–23] H_2O_2-destroying systems, i.e. catalase and myeloperoxidase, and it has been suggested that myeloperoxidase is of great significance in protecting cells from H_2O_2[23]. Myeloperoxidase and H_2O_2 could be important factors for the bactericidal activity of PMN leucocytes[9,11,12,17–19,24,25].

Riassunto. Si può dimostrare un accumulo di H_2O_2 in leucociti polinucleati di essudato di cavia o di sangue umano durante la fagocitosi in presenza di KCN 2 m*M*. La produzione di H_2O_2 e l'attività della perossidasi si considerano importanti per l'azione battericida.

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[17] B. HOLMES, P. G. QUIE, D. B. WINDHORST and R. A. GOOD, Lancet *1*, 1225 (1966).
[18] P. G. QUIE, J. G. WHITE, B. HOLMES and R. A. GOOD, J. clin. Invest. *46*, 668 (1967).
[19] R. L. BAEHNER and D. G. NATHAN, Science *155*, 835 (1967).
[20] R. E. VERKAUTEREN and A. GILLS-VAN MAELE, Enzymologia *24*, 25 (1962).
[21] K. AGNER, Acta physiol. scand. *2*, Suppl. *8*, 1 (1941).
[22] J. SCHULTZ and K. KAMINKEN, Archs Biochem. Biophys. *96*, 465 (1962).
[23] J. M. RECHCIGL and W. H. EVANS, Nature *199*, 1001 (1963).
[24] S. J. KLEBANOFF, W. H. CLEM and R. G. LUEBKE, Biochim. biophys. Acta *117*, 63 (1966).
[25] R. J. SELVARAY and A. J. SBARRA, J. Bact. *94*, 149 (1967).