for alkaline phosphatase. They noted that free formaldehyde, when present, inhibited the oxidation, but alkaline phosphatase in formalin-fixed tissues remained active for this substrate. It would appear that this type of substrate may soon be applicable for determination of alkaline and acid phosphatase of serum in the clinical laboratory.

Alkaline phosphatase is generally regarded as a microsomal enzyme and the optimum pH varies between 8.5 and 10.0. Our histochemical reactions correlated with previous reports concerning optimum pH and distribution and activity in homogenate data[16-18].

In regard to acid phosphatase, granules and droplet formation occurred at the site of the enzyme activity which is generally considered to be lysosomal. Cold acetone fixation was found to be the best method for tissue preparation for histochemical demonstration of the enzymes[19].

Résumé. Le principe indigogénique à la détection histochimique des phosphates alcalins et acides a été appliqué. Ces substrates ont l'avantage de permettre une localisation précise de l'enzyme, sans ou avec minime diffusion. C'est aussi une méthode simple et directe pour la mise en évidence de ces enzymes sans passer par une réaction couplée. Un bisindigo très insoluble a lieu apparaît aux points d'activité enzymatique. Le 5-bromo-4-chloro-composé donne un indigo bleu-vert tandis que le 5-bromo-6-chloro-composé donne un indigo magenta.

P. L. Wolf, J. P. Horwitz, J. Vazquez, J. Chua, M. S. Y. Pak, and Elisabeth von der Muehll

Department of Pathology, Wayne State University School of Medicine and the Detroit Institute of Cancer Research, Detroit (Michigan, USA), 2nd June 1966.

[16] M. S. Burstone, *Enzyme Histochemistry and its Application in the Study of Neoplasms* (Academic Press, New York and London 1962), p. 191.
[17] R. K. Morton, Biochem. J. *55*, 786 (1953).
[18] R. K. Morton, Biochem. J. *57*, 595 (1954).
[19] This investigation was supported in part by Public Health Service Research Grants Nos. CA-02624 and CA-02903 from the National Cancer Institute and in part by an institutional grant to the Detroit Institute of Cancer Research from the United Foundation of Greater Detroit allocated through the Michigan Cancer Foundation and the Detroit General Hospital Research Corporation.

Ribonuclease Activity in Leucocytes of Hyperimmunized Rabbits

Ribonuclease has been described in rabbit polymorphonuclear leucocytes[1,2] and the enzyme is associated with specific granules. These specific granules have been described by Cohn and Hirsch[3] as lysosomal in nature. Following a cytochemical technique described by Atwal et al.[4], while studying the enzymatic properties of the specific granules of PMN leucocytes of Swiss mice, a semiquantitative survey was attempted on ribonuclease positive granules of rabbit PMN leucocytes in which earlier biochemical investigations indicated a high concentration of ribonuclease.

2 rabbits (1 of the animals was inoculated with an attenuated strain of *Coxiella burneti* for hyperimmune Q serum) were bled from the ear vein. The air-dried coverslip preparations were fixed in cold formalin vapors, and ribonuclease activity was measured cytochemically as described by Atwal et al.[4]. The leucocytes of these animals showed a distinct cytochemical dichotomy by showing a difference in the number of positive reactive granules. The vaccinated rabbit showed a higher number of PMN leucocytes, and every cell had more positive granules than the leucocytes of the normal rabbit (Figure 1). The positive granules were distributed throughout the whole extent of the cytoplasm, while in normal rabbits these granules were limited to the perinuclear zone of the cytoplasm (Figure 2). The routine hematological data indicated a distinct leucocytic reaction in the vaccinated rabbit. The cytoenzymatic dimorphism, although shown in 2 animals, was consistent in 3 successive experiments. This observation prompted a further study of enzymatic concentration to see if this phenomenon of increased ribonuclease activity had a correlation with the course of immunological response of the vaccinated animals. The cytochemical behavior of 5-nucleotidase activity in rabbit leucocytes during experimental infection with mixtures and single strains of various staphylococci etc., has been described by Szmigielski et al.[5]. They observed significant changes in 5-nucleotidase activity of infected animals in polymorphonuclear leucocytes in peripheral blood. During the present study a similar situation occurred when maximum enzyme activity was observed on the tenth day following the first inoculation. This coincided with the first peak phase in the antibody titer. Then followed a decline in the enzyme activity in PMN leucocytes. It again reached its previous high concentration level on the 28th day. This second rise in enzyme activity coincided with the second peak phase of the antibody concentration in the plasma[6]. It is speculated that the function of intracellular ribonuclease is to degrade the unwanted RNA[7]. Rabbit leucocytes have been described as having a rapid turnover in unstable RNA[8].

The question is: Why would the leucocytes show increased ribonuclease when the animal is in the process of active antibody formation? Are the circulating PMN leucocytes actively functional as phagocytes? The increased lysosomal ribonuclease indicated that this might be the case. The digestive enzymes of lysosomes and their role in the formation of phagosomes for the engulfed

[1] J. M. Barnes, Brit. J. exp. Path. *21*, 261 (1940).
[2] R. J. Dubos and C. M. MacLeod, J. exp. Med. *67*, 791 (1938).
[3] Z. A. Cohn and J. G. Hirsch, J. exp. Med. *112*, 983 (1960).
[4] O. S. Atwal, J. B. Enright and F. L. Frye, Proc. Soc. exp. Biol. Med. *115*, 744 (1964).
[5] S. Szmigielski, J. Jeljaszewig and J. Wilczzuski, Pathologia Microbiol. *29*, 186 (1966).
[6] D. Behymmer, personal communications.
[7] J. Utsunomiya and J. R. Roth, J. biophys. biochem. Cytol. *29*, 395 (1966).
[8] P. A. Marks, C. Willson, J. Kruh and F. Gros, Biochem. biophys. Res. Commun. *8*, 9 (1962).

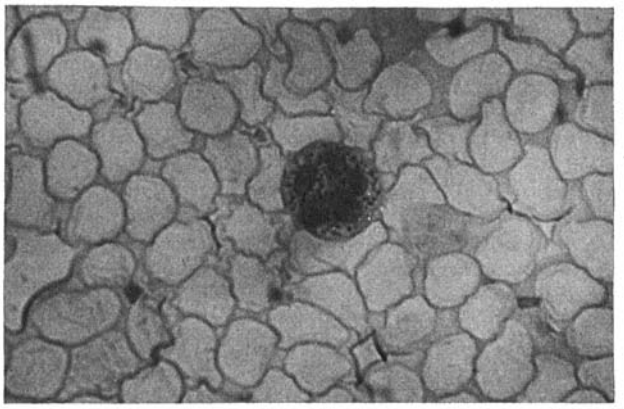

Fig. 1. Ribonuclease activity of polymorphonuclear leucocytes from hyperimmunized rabbit. The enzyme-positive granules are scattered throughout the whole extent of the cytoplasm. × 800.

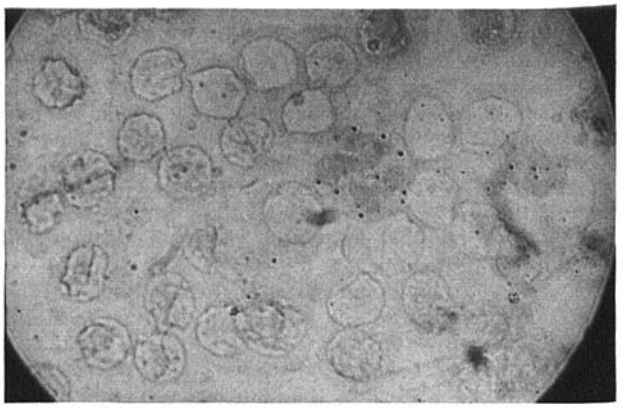

Fig. 2. Ribonuclease activity of polymorphonuclear leucocytes from normal rabbit. Note a few positive granules in the perinuclear zone. × 800.

particles of antigen is a well-known concept[9]. There are proposals that neutrophils engulf and perhaps digest particulates as chylomicrons in the blood, thus serving a function in clearing lipemic serum and metabolizing lipids[10]. The PMN leucocytes perhaps function as agents involved in the alteration of the antigen and transfer of information to immunological competent cells, which in this case are probably circulating cells. Such a role has been associated with the macrophage system[9]. COHN[11] suggests the possibility of macrophage ribonucleic acid in some way transferring information to competent lymphoid cells. HULLIGER and SORKIN[12], while investigating antibody formation in the circulation, showed changes in the composition of peripheral blood cells of hyperimmunized rabbits with a high number of PMN leucocytes at a stage when antibody synthesis in blood and thoracic duct cells was fairly active.

In a recent report CLINE[13] indicated a profound effect of phagocytosis on the several aspects of RNA metabolism. He postulates that particle ingestion induces an accelerated rate of destruction of pre-existing RNA and an increased rate of RNA synthesis. It is possible then, that the circulating PMN leucocytes of rabbit in the event of an infection are involved in the engulfing of the antigen. And during the process of phagocytosis the induced destruction of pre-existing RNA and increased RNA metabolism would need the increased concentration of intracellularly located ribonuclease to degrade this RNA. It may also be speculated that the rapidly proliferating PMN leucocytes contain much less ribonuclease inhibitor than the normal animal cells. UTSUNOMIYA[7] conceives of such a possibility of lower concentration of ribonuclease inhibitor in hepatic proliferating cells.

More extensive work is required to understand the dynamics of leucocytic enzymes in a wide variety of infections, especially before such conclusions are established.

Résumé. Les leucocytes du sang périphérique de lapins hyper-immunisés contre *Coxiella burneti* ont été comparé avec des leucocytes de lapins normaux. Par la méthode cytochimique on a pu constater chez les animaux hyper-immunisés un accroissement du taux des leucocytes polynucléaires, l'enzyme ayant une activité accrue ce qui s'accorde avec un titre élevé d'anticorps dans le plasma. L'auteur cherche à déterminer la signification de cette augmentation de l'enzyme chez les animaux hyper-immunisés et de son rapport avec la réaction immunogène.

O. S. ATWAL

Department of Anatomy, University of California, Davis (California, USA), 12th September 1966.

[9] J. P. THIERY, J. Microscopis *3*, 77 (1962).
[10] G. IZAK, A. DEVRIES and C. GALEWSKI, J. Lab. clin. Med. *55*, 564 (1960).
[11] Z. A. COHN, in *The Inflammatory Process* (Ed. V. W. ZWEIFACH, L. GRANT and R. T. MCCLUSKY; Academic Press, New York 1965), p. 347.
[12] L. HULLIGER and E. SORKIN, Immunology *9*, 291 (1965).
[13] M. J. CLINE, Blood *27*, 752 (1966).

Effects of the Inhibitor of Xanthine Dehydrogenase, 4-Hydroxypyrazolo(3,4 d)pyrimidine (or HPP) on the Red Eye Pigments of *Drosophila melanogaster*

The mutants *ry* and *ma-l* of *D. melanogaster* are deficient in xanthine dehydrogenase (XDH)[1-3]. In consequence they do not form the reaction products isoxanthopterin and uric acid, and accumulate the corresponding substrates 2-amino-4-hydroxypteridine and hypoxanthine[4-6]. There is also a partial loss of the red pteridines of the eye; the eye colours being a dark red-brown (yellow-orange in the double mutant *st ry* or *ma-l;st*) in contrast to the bright red of normal flies[7,8].

Phenocopies of the *ma-l* and *ry* – or, correspondingly, of the *st ry* and *ma-l;st* – eye colour mutants have been obtained when wild-type or *st* flies were grown on a medium containing the inhibitor HPP[9-11].

Because at least 3 substances (neo-, iso- and drosopterin) contribute to the red colour of the eye in *Drosophila*[12,13], we proceeded to examine their modifications in the phenocopies produced by HPP as compared to the normal flies.