812. Thin sections were cut on a LKB II ultramicrotome. Unstained and stained[17] sections were examined in a Hitachi HU IIB electron microscope.

Results. Our morphological observations are in agreement with the published results on the ultrastructure of epithelial cells of the gall-bladder[18, 19]. Intensively stained structures are found in the cytoplasm of epithelial cells of mice gall-bladder (Figure 1). These structures are single-membrane bound and have a round, oval or rod-like shape. The diameters of round organelles measure from 0.2 to 0.3 nm. The reaction product within the organelles shows a granular texture. No crystalloid core is found in the matrices. These organelles are found throughout cytoplasm of the epithelial cells. It is not possible to demonstrate a continuity of the limiting membranes of our organelles with the smooth surfaced endoplasmic reticulum. The membranes and crystes of mitochondria are stained. This is most probably due to peroxidatic activity of cytochrome oxidase[20] or cytochrome c[21]. The staining of some lysosomes of epithelial cells is probably due to peroxidatic activity of lipid peroxides[22] or hemes and metals[23].

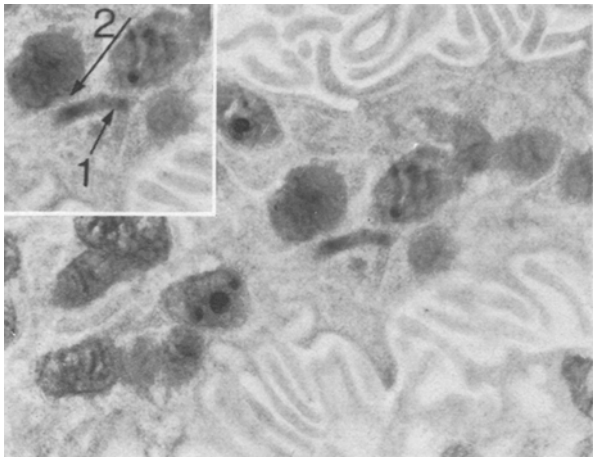

Fig. 3. Fragment of epithelial cell of mice gall-bladder. Peroxisome (1). Mitochondria (2). ×21,000.

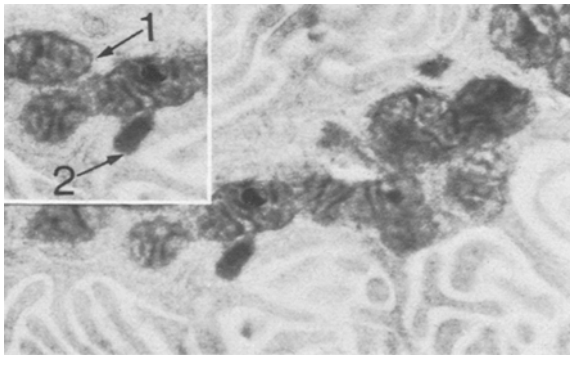

Fig. 4. Fragment of epithelial cell of mice gall-bladder. Mitochondria (1). Peroxisome (2). ×17,000.

No product reaction was observed in our organelles if the tissue was incubated in the medium without DAB or after heating. A very weak reaction remained if the tissue was incubated in DAB solutions without H_2O_2.

Discussion. The organelles described in the epithelial cells of mice gall-bladder are similar to peroxisomes found in many animal cells[7]. We think that our results point to the fact that the organelles described may be peroxisomes. The function of peroxisomes in epithelial cells of gall-bladder remains to be elucidated. The role of the organelles in animal cells remains obscure. But it is possible, as we suggested previously[24, 25], that one of their functions is antimicrobial. Probably, peroxisomes of gall-bladder epithelia are antimicrobial organelles.

Summary. Electron-cytochemically peroxysomes were found in the epithelial cells of mice gall-bladder. The possibility is discussed that these are antimicrobial organelles.

W. A. Fomina, V. V. Rogovine, L. A. Piruzyan, R. A. Muravieff and E. A. Podovinnikova

Institute of Chemical Physics, 117334 Moskwa (USSR), 19 February 1975.

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Ultrastructural Cytochemistry of Peroxidase and Acid Phosphatase in Mice Maturing Eosinophils

Materials and methods. Observations were made on eosinophils from mice bone marrow. Small pieces were fixed in 4% glutaraldehyde in 0.1 *M* cacodylate buffer, pH 7.4 for 5 h at 0–4 °C. After a wash in 0.1 *M* cacodylate buffer, pH 7.4 containing 7% sucrose for 18–20 h in cold, the specimens were incubated for peroxidase in the modified Graham-Karnovsky medium[1] for 10 min at 37 °C. The incubation medium composed of 0.01% H_2O_2, 7% sucrose and 20 mg 3, 3′-diaminobenzidine tetrachloride (DAB) in 10 ml 0.1 *M Tris*-buffer, pH 7.6. After the incubation, the specimens were washed in 7% sucrose for 5–7 min and postfixed in 2% O_5O_4 for 20 h, dehydrated through graded concentrations of acetone and embedded in Durcupan.

Controls for activity of peroxidase included: 1. omitting DAB from the incubation medium, 2. omitting H_2O_2 from the incubation medium (in this case the specimens were incubated for 10 min and 60 min), 3. preincubation with absolute acetone for 40 min – 24 h following the incubation in the complete medium and the medium without H_2O_2, 4. preincubation with the following inhibitors:

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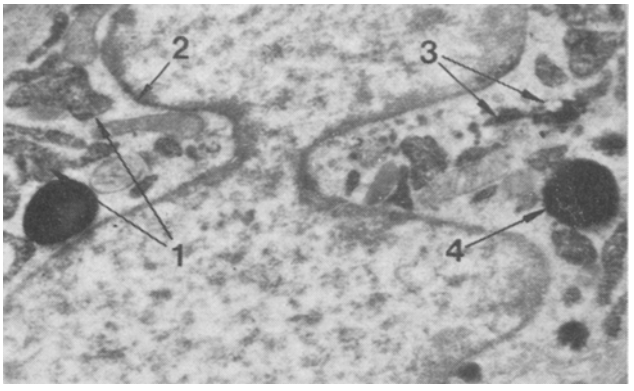

Fig. 1. Eosinophilic promielocyte. Electron-cytochemical reaction on peroxidase. The reaction product is present in wide canals and cysternes of endoplasmic reticulum (1), in perinuclear cysternes (2), in the Golgi apparatus (3), in immature specific granules (4). ×17,000.

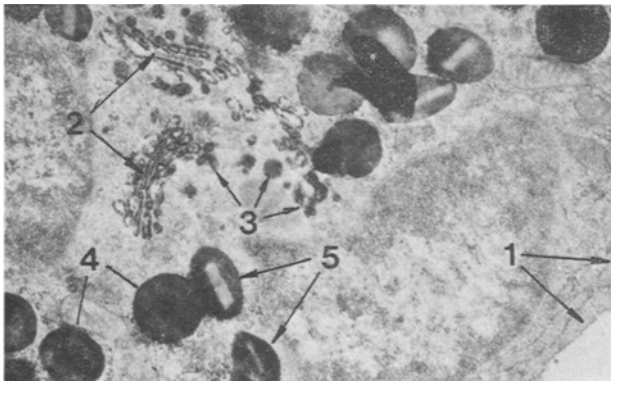

Fig. 2. Eosinophilic myelocyte. Electron-cytochemical reaction on peroxidase. Canals of endoplasmic reticulum are negative (1). Cyternes (2), small vesicles (3) of the Golgi apparatus contain the reaction product. Immature specific granules (4) are homogeneous reactive. In mature granules (5) the reaction product is present only in the matrix, the crystal being negative. ×21,000.

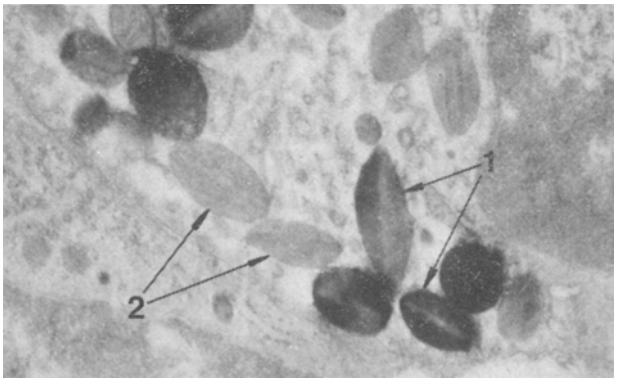

Fig. 3. Fragment of segmento-nuclear eosinophil. Electron-cytochemical reaction on peroxidase. The reaction product is seen in mature granules (1). Note negative granules (2) near some peroxidase. ×23,000.

10^{-2}–10^{-1} *M* KCN, 10^{-2}–10^{-1} *M* Na_3N, 10^{-2} *M* hydroxylamine for 30 min. Then, the specimens were incubated in the complete incubation media with the same concentration of each inhibitor added.

The method of simultaneous demonstration of peroxidase and acid phosphatase was reported[2]. Thin sections were cut on LKB ultramicrotome. Thin sections both unstained and stained lead citrate were examined in a Hitachi HU-IIB electron microscope.

Results. In various studies of development, eosinophils showed the peroxidase activity. In promyelocytes (Figure 1) the reaction product was localized in rough endoplasmic reticulum including the perinuclear space, elements of the Golgi apparatus and immature granules. The density of the reaction product increases from the endoplasmic reticulum to the granules. In myelocytes, the peroxidase reaction product disappears from the endoplasmic reticulum and remains in the Golgi elements and the granules (Figure 2). Crystal-containing mature granules appear in promyelocyte. It contains the reaction product in the granular matrices only, the crystal remaining clear.

In mature cells (Figure 3) the reaction is positive in the matrices of mature specific granules only, some granules are nonreactive others are only partially filled with the reaction product (Figure 3). Sometimes in mature cells immature granules are encountered.

No reaction product was detected in the sections incubated without DAB. The deletion of H_2O_2 from the incubation medium led to interesting results. A weak reaction remained in some granules. This reactivity increases with the prolongation of the incubation period up to 1 h and also at preincubation with absolute acetone during 40 min. 10^{-2}–10^{-1} *M* KCN and 10^{-2} *M* Na_3N decreases the peroxidase activity, but not fully. Only 10^{-1} *M* Na_3N and 10^{-1} *M* hydroxylamine inhibits the peroxidase activity completely.

In the simultaneous demonstration of peroxidase and acid phosphatase, the former was localized in specific granules in eosinophic myelocyte (Figure 4). Acid phosphatase is present in the matrix of some immature specific granules (Figure 4) and in the Golgi apparatus. In an immature granule on the background of a less dense sediment of peroxidase reaction product, more dense sediment of acid phosphatase reaction product is present especially on the periphery of the granule. Crystal-containing mature granules do not contain acid phosphatase. The acid phosphatase reaction product is fine-crystalline and very dense. The peroxidase reaction product is homogeneous and of moderate density. Therefore the reaction products of the two enzymes are distinct.

Discussion. In eosinophils from bone marrow of mice, the peroxidase activity is shown in the rough endoplasmic reticulum including the perinuclear space. the Golgi apparatus, the immature and mature specific granules. With the development of eosinophils the peroxidase gradually disappeared form the perinuclear space, the endoplasmic reticulum and the Golgi apparatus. In mature cells the peroxidase remains only in granules. It seems possible that with the development the elements of the apparatus, the synthesis of secretory protein may be reduced and the enzyme accumulated and stored in granules. This is in agreement with the general scheme of

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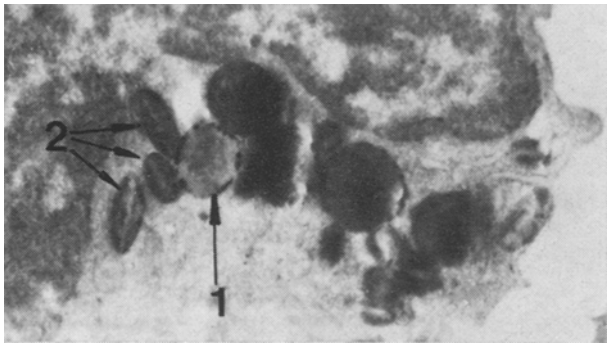

Fig. 4. Fragment of eosinophilic myelocyte. The double-reaction on peroxidase and acid phosphatase. Immature granule (1) is seen in which, on the background of a less dense reaction product on peroxidase near the periphery of the organelle, there is a more dense reaction product on acid phosphatase. Mature eosinophilic granules (2) contain a homogeneous reaction product on peroxidase. The crystal remains negative. ×14,500.

Jemieson and Palade[3,4] and also with autoradiographic data on the path of granule formation in granulocytes[5]. Our results on peroxidase localization in mice eosinophils coincide with the data obtained by other authors on rats, rabbits and guinea pigs[6–10].

We demonstrated a heterogeneous peroxidase reaction in specific granules. It is likely that this phenomenon dependends on the absence of granular permeability of DAB and H_2O_2. Cotran and Litt[7] showed a positive reaction in some granules on the incubation of guinea-pig eosinophils in a medium lacking H_2O_2. We also demonstrated a weak positive reaction on some granules on the incubation without exogenous H_2O_2. Preincubation with absolute acetone does not inhibit this reaction but, on the contrary, promotes it. This depends on a small destruction of membranes and an increase in substrate permeability. Perhaps eosinophilic granules are not homogeneous in regard to the presence of endogenous peroxides. The granules in which endogenous peroxide is present are more reactive and their role in cellular metabolies seems to be more important.

It is likely that the incubation with the omission of H_2O_2 permits the characterization of the granules not only on the basis of enzymatic activity when, as noted above, a certain heterogeneity in the activity in the matrix of granule was found, but also on the concentration of endogenous peroxides.

Previously[1], we presented the results on a simultaneous demonstration of peroxidase and acid phosphatase on developing mice neutrophils. Acid phosphatase was present in the Golgi apparatus and the peroxidase in azurophil granules. Our results obtained on eosinophils have confirmed our assumptions that the peroxidase-containing granules are not lysosomes, but special peroxidase-containing antimicrobial organelles[11,12].

Summary. The peroxidase and acid phosphatase activity in developing mice eosinophils has been demonstrated. Both peroxidase and acid phosphatase are localized in various cellular organelles.

V. V. Rogovine, R. A. Muravieff, W. M. Frolova, N. G. Geranina and L. A. Piruzyan

Department of Medical Biophysics, The Institute of Chemical Physics, The Academy of Sciences of the USSR, Moskwa 117334 (USSR), 29 November 1974.

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Role of Food Quality Versus Quantity in Determining the Developmental Fate of a Gall Midge Larva (*Heteropeza pygmaea*) and the Sex of its Paedogenetically-Produced Eggs

Depending on rearing conditions, the larval ovaries of the viviparous paedogenetic gall midge *Heteropeza pygmaea* can produce 2 types of eggs which both develop through embryogenesis in the haemocoele of the larvae[1–3]. These eggs can be male- or female-determined[4] and, in accordance with the sex of their progeny, the growing mother larvae with female and/or male embryos are called female-mothers, male-female-mothers or male-mothers.

For a long time the role of the nutritive conditions which determine the type of mother larvae and – indirectly – the sex of its eggs, has been discussed[1,5,6]. The larvae feed on fungus which in the laboratory is easy to grow on a malt-agar substrate. Since, under defined culture conditions for the fungus, male-mothers and male-female-mothers appeared only when the density of population of the feeding larvae in the Petri dishes was relatively high (and especially much higher than was required for the production of female-mothers[4,7]), it was assumed that the quantity of the food was the determining factor for the developmental fate of the larvae and for the sex of their progeny[1,8]. Looking for a method which would yield a reproducible high percentage of male-mothers and male-female-mothers in the cultures, we came upon a simple formula for the culture medium of both female-mothers and male-mothers or male-female-mothers respectively. In this culture method merely the age of the fungus is altered; the population density of the larvae is held constant.

Material and method. In this context we give only the above-mentioned formula for the cultivation of the fungus, which yielded either female-mothers or a high

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