NATURE OF THE NADI REACTION IN GRANULOCYTES

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Although the Nadi reaction is one of the oldest histochemical methods, the exact nature of this histochemical reaction in the granulocytes of the blood is still unsettled. Different authors explain m-Nadi-reactions in granulocytes by the presence of peroxidase or of phenoloxidase, by the peroxodation of fats or again take the view that, while its exact nature is obscure, it does not involve oxidase or peroxidase [3, 4, 10, 11, 12, 13]. Pearce [14] is of the opinion that g-Nadi-reactions are undoubtedly connected with cytochromoxidase activity. Cytochromoxidase has been stated to be present in all kinds of leukocytes on the basis of investigations carried out with the g-Nadi-reaction [7]. There are reports [3, 4] that the intensity of the reaction for cytochromoxidase increases with maturation of the granulopoietic elements and is maximum in mature granulocytes.

The author is not at all convinced that Pearce's theory that the g-Nadi-reaction is connected with cytochromoxidase can be applied to the granulocytes of the blood. Granulocytes differ from other cells in the human body in that they contain peroxidase, the substance having been isolated and studied biochemically [1, 2, 5]. Peroxidase of granulocytes oxidizes p-phenylene-diamine, derivatives of which are used in the nadi reaction. The reaction is known to develop in the presence of a catalyst without the addition of hydrogen peroxide as the latter is formed as a result of an auto-oxidation reaction [1]. It would therefore, appear to be incorrect to say that a positive g-Nadi-reaction in a system containing peroxidase is the result of cytochromoxidase action.

An attempt was made to determine the nature of the factors responsible for m- and g-Nadi reactions in the granulocytes of the blood.

METHOD

Recent modifications of the Nadi reaction, developed by Burstone [8, 9] and Bilski [6], in which p-methoxy-p-amino-diphenylamine or diethyl-p-phenylene-diamine replaces dimethyl-p-phenylene-diamine, were used in the investigation. Blood films from healthy subjects were used in the fresh state for g-nadi reactions and after fixation in 10% formalin containing 1% calcium chloride for m-reactions. Potassium or sodium cyanide (M 10^{-3}), sodium diethyldithiocarbamate (M/5000 - M/3000) and a catalase preparation (0.1 mg/100 ml mixture)* were added to the incubation medium and any resultant changes in the Nadi reactions were noted. In one experiment incubation was carried out in the dark, with the incubation mixture saturated with CO_2. Some films were also heated for 10 min in normal saline at 60°C and 90°C.

RESULTS

Both Burstone and Bilski methods produced essentially the same morphological picture - the appearance of large numbers of colored granules† in the cytoplasm of neutrophil and eosinophil granulocytes. No basophil cells were

* The catalase preparation was kindly placed at our disposal by S. I. Krainev.

† A dye of the type of indophenol blue is formed in Nadi reactions with substances of the type of p-phenylene-diamine.

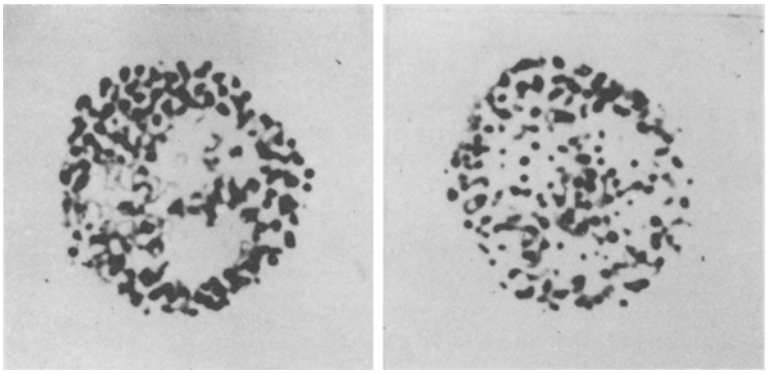

Fig. 1. Neutrophil granulocytes in fresh blood film. In this and in Fig. 2. Burstone Nadi reactions with p[methoxy-p-aminodiphenylamine. 2600 ×.

identified. As it was difficult to estimate activity in eosinophils in all experiments, what follows refers to neutrophil granulocytes only. No essential differences of any kind between fixed and fresh films, i.e., between m- and g-Nadi reactions, were observed in respect of morphology or distribution of the dye granules (Fig. 1 and 2). In both cases the dye granules were evenly distributed through the cytoplasm, leaving the nucleus free.

Saturation of the incubation mixture with CO_2 and the addition of diethyldithiocarbamate had no effect on the results of Nadi reactions in either fixed or fresh films. Addition of cyanide or catalase suppressed m- and g-Nadi reactions in granulocytes when the incubation period was only long enough to produce clear morphological results in parallel experiments of ordinary type.

Prolongation of the incubation time led to staining of granulocytes in both m- and g-Nadi reactions even with catalase or cyanide in the incubation mixture. This may be illustrated by the results of one experiment. With the p-methoxy-p-aminodiphenylamine of Burstone, distinct staining of the granulocytes in fixed preparations developed after 42 min whereas, when catalase had been added, the first signs of staining were seen after 90 min and staining of comparable intensity after 150 min. Addition of cyanide to the incubation mixture retarded the development of color by 45 min. Similar results were obtained with fresh preparations.

Heating fixed and fresh films in saline at 60°C did not result in suppression of m- and g-Nadi reactions in granulocytes but heating at 90°C abolished reaction.

These observations indicate the m- and g-Nadi reactions are morphologically the same. Nor did the inhibitors or other factors reveal any differences between the 2 types. This suggests that the same factors are responsible for both. The experimental results provide no support for the explanation of dye granule formation in the cytoplasm of granulocytes by cytochromoxidase action as the distribution of granules after fixation with formalin, which destroys cytochromoxidase irreversibly [14], was the same as in fresh films. Nadi reactions in granulocytes were nor suppressed by heating at 60°C or by saturation of the incubation mixture with CO_2, both of which inactivate cytochromoxidase

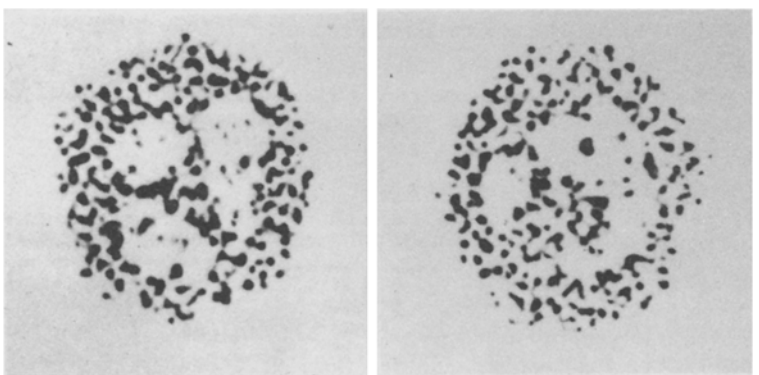

Fig. 2. Neutrophil granulocytes in fixed blood film.

[15]. The failure of diethyldithiocarbamate to exhibit reaction indicates that phenoloxidase is not responsible as diethyldithiocarbamate inhibits the activity of copper-containing enzymes, of which phenoloxidase is one [2]. The failure of diethyldithiocarbamate to produce an inhibitory effect is also evidence against the participation of cytochromooxidase in the Nadi-reactions of granulocytes as the oxidation of diethyldithiocarbamate by cytochromoxidase results in the formation of substances which suppress the activity of the enzyme [2].

The inhibition of Nadi reactions by catalase and cyanide would indicate that peroxidase is concerned in these reactions as its activity is suppressed both by the presence of cyanide and in the absence of hydrogen peroxide, which is destroyed by catalase [5]. It is more difficult to explain the nature of the "residual" Nadi reaction in granulocytes seen in the presence of cyanide and catalase. It may be that the "residual" reaction in the presence of catalase can be explained by the persistence of very small quantities of hydrogen peroxide and the similar effect in the presence of cyanide, by its incomplete inhibitory effect. It is not impossible, however, that dyes of indophenol blue type may be synthesized by the action of some one of the cyanide-resistant flavoprotein terminal oxidases [2]. These enzymes are probably present in granulocytes as they form hydrogen peroxide, which is the substrate for peroxidase. A correlation between content of flavoprotein terminal oxidases and peroxidase activity has been reported [2].

The fact must be considered that the "residual" Nadi reaction, left unsuppressed by cyanide and catalase, is only seen when the incubation period is prolonged considerably. With ordinary incubation periods, adequate for the development of good morphological results, the effect of the factor resistant to cyanide and catalase is not in evidence. This suggests strongly that m- and g-Nadi reactions in granulocytes are mainly the result of peroxidase activity. Indirect confirmation of this is afforded by the fact that reactions for peroxidase and the m- and g-Nadi reactions yield, in fact, identical results in the granulocytic series [3, 4].

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