Monoamine Oxidase Activity in Rat Erythrocytes: Evidence for its Localization in Reticulocyte Mitochondria[1]

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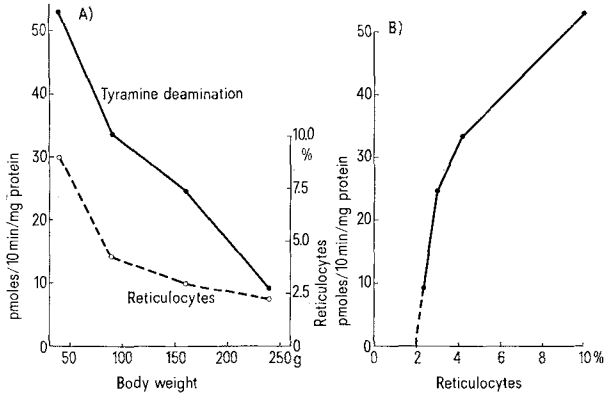
Summary. During growth of young rats, monoamine oxidase activity in erythrocyte membrane preparations decreases more rapidly than reticulocyte concentrations in the respective blood samples. Since reticulocytes lose their mitochondria prior to the substantia reticulo-filamentosa, the non-linear correlation between monoamine oxidase activity and reticulocyte counts indicates that erythrocyte monoamine oxidase is located in reticulocyte mitochondria.

During the course of studies designed to characterize the adrenergic β-receptor-effector system of circulating red blood cells[2–4], it was found that rat reticulocytes contain considerable monoamine oxidase activity which is readily inhibited by the typical monoamine oxidase inhibitor, pargyline[5]. This finding, in contrast to earlier investigations which had not revealed monoamine oxidase activity in red blood cells from several species [6,7], was obtained in animals in which marked reactive reticulocytosis had been produced by preatreatment with acetylphenylhydrazide.

It is known that hydrazines are in general irreversible inhibitors of monoamine oxidase activity[8]; this is also true for phenylhydrazine[9] (which is assumed to be the 'active moiety' of acetylphenylhydrazide[10–12]). It was of fundamental interest, therefore, to confirm the finding of monoamine oxidase activity in reticulocytes by use of a model reticulocytosis not involving toxic haemolysis. For this purpose, studies were carried out using young growing rats with a 'physiological reticulocytosis' due to the normally occurring dilution anaemia[13]; this experimental model has previously been used for studies of adenylate cyclase in red blood cells[14].

Methods. 4 groups of male Wistar rats with body weights of 40–50, 80–100, 150–170 and 230–250 g were used. Heparinized pooled blood from 20 to 60 animals was freed from leukocytes and platelets by filtration through cotton wool[15] and 4 washings with isotonic phosphate buffer, and erythrocyte membrane preparations were made, after DODGE et al.[16] as described previously[3,4]. Monoamine oxidase activity was determined by the method of WURTMAN and AXELROD[17] using ^{14}C-tyramine (spec. act. 13 mCi/mmole) as substrate. Reticulocytes were counted in blood smears stained with brilliant cresyl blue; protein was determined after RESCH et al.[18].

Results and discussion. As in previous investigations[13,14], with increasing body weight in young rats a steady decrease of reticulocyte counts was observed (see Figure); there was a concomitant, but not parallel, decrease of monoamine oxidase activity in the respective membrane preparations. While the physiological reticulocyte 'loss' can be described by first-order kinetics[14], there was an apparent zero-order decrease of monoamine oxidase activity (Figure A). Obviously, the enzyme activity is not – like adenylate cyclase[14] – present in all but only in part of the reticulocytes; this is even more clearly seen when monoamine oxidase activity is plotted against relative reticulocyte concentrations (Figure B): extrapolation of the nonlinear correlation function towards low MAO activities leads to an intersection on the abszissa at about 2% reticulocytes.



Monoamine oxidase activities (substrate concentration: 2×10^{-5} *M*) in membrane preparations (each point representing the mean of triplicate determinations) and reticulocyte counts in the respective blood samples pooled from rats grouped according to body weight (A); correlation between monoamine oxidase activities and reticulocyte counts (B).

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The observation that monoamine oxidase activity is present only in part of the circulating reticulocyte population can be explained by a more rapid rate of decrease of monoamine oxidase activity than of the substantia reticulo-filamentosa (which is responsible for the staining characteristics) during the course of red cell maturation. In this respect, monoamine oxidase obviously shares common characteristics with other enzymes, especially with the mitochondrial marker enzyme, cytochrome oxidase: it has been shown by ROSENTHAL et al.[19] that there is a similar nonlinear correlation between cytochrome oxidase activity and RNA content in rabbit reticulocytes. The latter finding is in agreement with the results of morphological investigations[20,21] which indicate a very rapid loss of mitochondria during the maturation of reticulocytes.

It may therefore be concluded that reticulocyte monoamine oxidase is a mitochondrial enzyme; this conclusion has recently been confirmed in preliminary experiments in which acetyl-phenylhydrazide was used as an inductor of reticulocytosis[22]. With respect to its substrate and inhibitor specificities[5,22], and also with respect to its mitochondrial location, the reticulocyte enzyme is classified as a 'classical'[6] monoamine oxidase.

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Anionic Activation of Human Salivary Amylase

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Summary. In all earlier studies on α-amylase, the influence of different ions were studied in phosphate buffer. The present report shows the effect of different ions individually with *Tris* and amino acid. Though it has been claimed recently that sodium ion is an activator of α-amylase, this study reconfirms that sodium ion does not activate human salivary amylase.

The activation of mammalian α-amylases by chloride ion, and less effectively by certain other monovalent anions has long been known[1,2], whereas microbial and diastatic α-amylases do not require chloride ion as activator[3]. The various aspects of salt activation of α-amylases mostly of pancreatic and salivary origin have been studied by different workers. MYRBÄCK[1] had pointed out that the pH optimum of the enzyme shifts from 6.0 to 6.9 on being activated in the presence of Cl^-. It is also reported that the pH optima shift occurs by the addition of other anions; a few of them activate the enzyme partially. BERNFELD et al.[4] showed that, after removal of such activators by exhaustive dialysis of both the enzyme and the substrate, the activity is reduced to 15% of its original value, which is entirely restored instantaneously by the inclusion of 0.01 *M* NaCl to the reaction mixture. Using various chlorides and other anions, they concluded that Cl^- is essential to mammalian α-amylases for full activation and the cation has got no role in the process. Calcium ion is believed to be firmly bound to the protein molecule and contributes to the structural stability against higher pH and proteolytic degradation[2]. In contrast to the above findings, recently it has been shown that sodium ion and not the chloride ion is sole activator for pancreatic α-amylases[5] of toad, reptile, pigeon and rat. However, the literature fails to provide enough information about the role of different activators independently, particularly anionic, since all of the earlier experiments were done with phosphate buffer which itself is likely to activate to some extent.

In order to study the activation by anions independently and by their influence on the shift of pH optimum, the present investigation was carried out using suitable buffers with and without phosphate at two different pH values viz. 6.0 and 6.9. This report simultaneously throws light upon certain observed discrepancies in earlier reports.

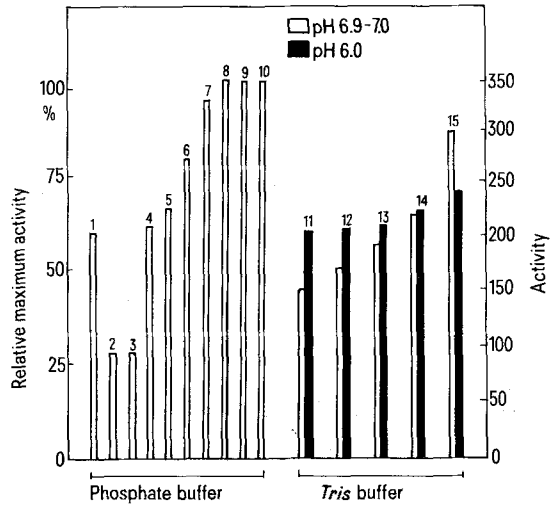

Fig. 1. Activation of α-amylase by ions (salts). Numbers 1 to 10 all in phosphate buffer. 1. buffer alone; 2. Na_2CO_3; 3. $CuCl_2$; 4. $NaNO_3$; 5. CH_3COONa; 6. NaBr; 7. $CsCl_2$; 8. NH_4Cl; 9. KCl; 10. NaCl. Numbers 11 to 15 all in *Tris* buffer. 11, aspartate; 12, acetate; 13, phosphate; 14, oxalate; 15, chloride.

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