Localization of xanthine dehydrogenase in rat serum by paper electrophoresis

BLAUCH, KOCH AND HANKE[1] reported that rat blood serum when incubated with xanthine or hypoxanthine causes an increase in uric acid due to the presence of xanthine oxidase. However, as far as we know, no quantitative data for xanthine oxidase (XO) or xanthine dehydrogenase (XD) activities in rat blood serum have been published up to now. Using the manometric method of AXELROD AND ELVEHJEM, modified as previously reported[2] for XO activity and the colorimetric method of VILLELA[3] for XD activity, we were able to confirm the presence of XO and XD activities in blood serum of normal rats[4].

Therefore, we were interested to know the distribution of this enzyme in the protein fractions of rat serum. Preliminary experiments with 50% ammonium sulfate precipitation and subsequent dialysis against water showed that all of the enzymic activity was confined to the globulin fractions of the serum.

For a more detailed study, paper electrophoresis was performed in an apparatus previously described[5,6]. Electrophoretic experiments were carried out at 25° C for 16 hours with a potential of about 250 volts. Paper strips (10 cm × 57 cm) were soaked with phosphate buffer pH 7.4, and 0.01 ml of serum for protein and 0.2 ml (10 adjacent spots with 0.02 ml each) for enzyme detection were applied to the same strip. The proteins were revealed by the dye elution method already described[6], using a 2% w/v solution of sodium carbonate in 50% v/v methyl alcohol instead of sodium hydroxide solution.

The barbital buffer pH 8.6 usually employed for electrophoretic separations was substituted for the phosphate buffer, since we verified that with the former the enzyme activity was inhibited in 50%. The possibility that XD could be related to the lipoprotein fractions of rat serum was tested by staining the strips of the same serum with Sudan black according to the method of SWAHN[7]. The protein and lipoprotein patterns are shown in Fig. 1.

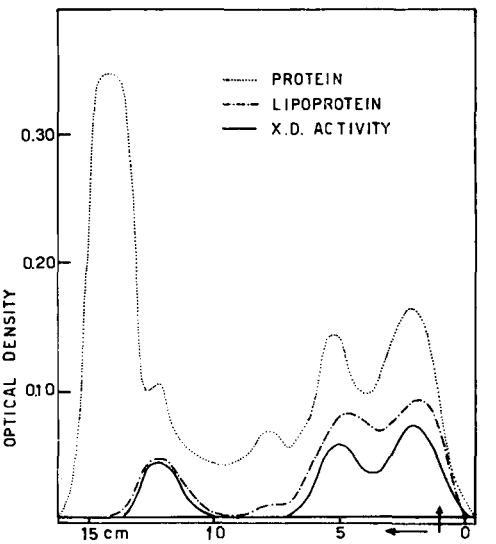

Fig. 1. Paper electrophoresis of proteins, lipoproteins and distribution of xanthine dehydrogenase in rat blood serum. The arrows indicate the site of application of the serum and the direction of the migration.

The enzyme detection was made by cutting the paper strips in parallel segments of 1.5 cm, and placing each in a Thunberg tube containing 5 ml of phosphate buffer solution pH 7.4. After the air was evacuated, 0.1 ml of sodium xanthine and 0.3 ml triphenyltetrazolium chloride were tipped in. The tubes remained for 18 hours in a water bath at 37° C. Control blanks were run simultaneously and formazan produced was extracted with petroleum ether after acidification with acetic acid. The color was read at 480 mμ in a Unicam spectrophotometer. The results are shown in Fig. 1. The manometric technique is not feasible because the amount of serum is too small to measure the XO activity.

Fig. 1 indicates that: (a) The XD activity is localized in the globulin fractions corresponding to the mobility of α_1, β and γ globulins. (b) The albumin fraction appeared to be devoid of any XD activity. (c) The lipoprotein fractions presented the same mobilities as those showing XD activity.

These results show that XD activity of rat blood serum is distributed in the globulin fractions and possibly bound to the lipoproteins.

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