Multiple Forms of Esterases from Human Erythrocytes.* (26939)

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Different forms of enzymes which hydrolyze carboxyl ester linkages (esterases) have been described from the blood plasma of man and other vertebrates(1-4), and several forms of esterases have also been reported from mouse red cell hemolysates(4). The present communication describes a system in which multiple forms of human red cell esterases can be resolved by zone electrophoresis, and presents evidence which suggests that some of the observed variations in these enzymic forms are under genetic control.

Methods. Erythrocyte hemolysates were prepared by lysing the washed, packed red cells with one volume of distilled water and extracting with 0.4 volume of toluene. Aliquots of the hemolysates were then subjected to electrophoresis in a vertical starch gel system similar to that developed by Smithies(5). Satisfactory resolution of the enzyme patterns (zymograms) during electrophoresis was produced with a bridge buffer solution (pH 8.0) containing 0.3 *M* H_3BO_3 and 0.05 *M* NaOH and a gel buffer (pH 8.6) containing 0.02 *M* H_3BO_3 and 0.0046 *M* NaOH. Electrophoresis was carried out with a gradient potential of about 7.0 volts/cm (current: 5MA/gel) for 18-20 hours at 3-5°C. After electrophoresis, the zymograms were visualized on the gels with Blue RR salt as the dye coupler and the acetate, propionate, and butyrate esters of α-naphthol as substrates. The dye-coupling procedure and inhibition study method were essentially those described by Markert and Hunter(6).

Characterization of the esterase forms. Nine bands of enzyme activity have now been resolved by this system, a composite of which is diagrammed in Fig. 1. Bands A_1 through A_8 showed the highest hydrolytic activity with the shorter acyl chain esters in the order: α-naphthyl acetate > α-naphthyl propionate > α-naphthyl butyrate, whereas the reverse was true with band *B*. The most consistently observable bands in their order of prominence were: A_4, *B*, A_7, A_8, and A_6; bands A_1, A_2, A_3 and A_5 were regularly seen only in fresh, undiluted hemolysates. Band A_8 was the slowest of the prominent esterase forms to develop with α-naphthyl acetate. Band A_7 was characteristically diffuse.

Further characterization of band *B* was demonstrated by the fact that its rate of anodal migration, relative to the *A* esterases, was increased by addition of NaCl to the bridge buffer.

It was not possible to inhibit any of the *A* esterases with eserine sulfate (10^{-2} *M*), diisopropylfluorophosphate (10^{-3} *M*), and B.W. 284C51j dibromide (10^{-4} *M*). The *B* esterase was inhibited by diisopropylfluorophosphate (10^{-4} *M*) but not eserine (10^{-2} *M*) or B.W. 284C51j dibromide (10^{-4} *M*).

On the basis of these results, the *A* esterases could be classified as arylesterases and the *B* esterase as an aliesterase according to the terminology applied by Augustinsson(1) to plasma esterases. The cholinesterase of the red blood cell could not be demonstrated by the system used in the present study.

Some diminution of enzyme activity as well as formation of methemoglobin (MetHb) was characteristic of certain hemolysates stored at −10°C. However, no effect on electrophoretic mobility of esterase bands was observed in the zymograms of these stored hemolysates.

Possible acquired variations. What appeared to be a decrease in intensity and an increase in migration rate of the A_4 and A_7 esterase components was noted in 2 cases of patients suffering from cirrhosis of the liver with associated anemia (Fig. 2:b). A similar alteration was seen in a jaundiced child with acute hemolytic anemia (reticulocyte count: 35.5%); however, the apparently altered A_4 band migrated slightly faster than in the above cases and showed no decrease in in-

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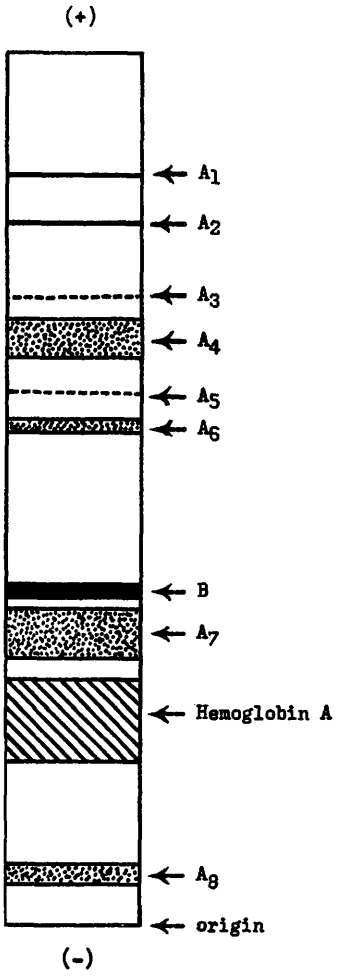

FIG. 1. Composite diagram of multiple esterase forms from normal human erythrocyte hemolysates.

tensity. When this patient was seen again the reticulocyte count had returned to 3.5%, and no alteration in bands A_4 or A_7 was observed (Fig. 2:d,e).

Clearly, these esterase forms can be affected by environmental factors which may be induced by certain clinical states. The nature of these alterations is presently under investigation.

Genetic variation. A survey of red cell hemolysates from adult, male, monozygotic twins was undertaken for the purpose of detecting phenotypic differences in esterase patterns which might be under genetic control. During this survey, one set of twins was found to show what appeared to be a decreased activity and doubling of the A_4 and A_6 components (Fig. 4: d, e). The involvement of adjacent bands suggests a structural relationship among bands A_3 through A_6.

Fresh blood samples were subsequently obtained from the mother, father, and one sister of the twin propositi. The same variation was found in the mother and the twins, while the sister and father showed the normal pattern (Fig. 4). The medical histories of the mother and twins were felt to be noncontributory.

This family is now under investigation and a complete report is forthcoming.

Summary. A system is described in which multiple bands of esterase activity can be resolved from human red cell hemolysates by zone electrophoresis in starch gel. Variations from the normal pattern were observed in certain patients with liver disorders and associated anemias. Another atypical esterase

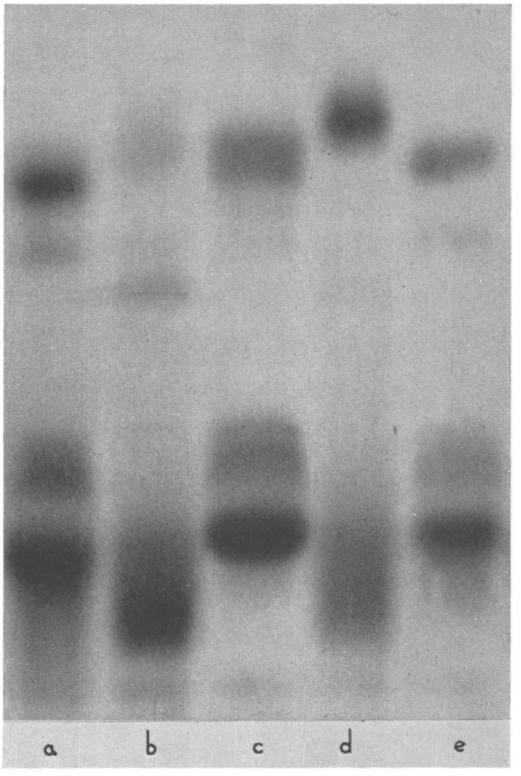

FIG. 2. Zymograms (substrate: α-naphthyl acetate) of normal and altered esterase forms from human erythrocyte hemolysates: normal subject (a); patient with cirrhosis of liver and anemia (b); individual *b* from Fig. 4 (c); patient with jaundice and hemolytic anemia (d), and same subject after treatment (e).

pattern was found in a pair of monozygotic twins and their mother indicating that the observed variation is under genetic control.

It has now been demonstrated that band A_4 is composed of two distinct bands. In the genetically determined variation the slower band of this doublet migrates at the same rate as in the normal pattern whereas the advanced band migrates faster than normal. A new band has also been located between bands A_4 and A_5 which shows decreased activity in the atypical condition under genetic control.

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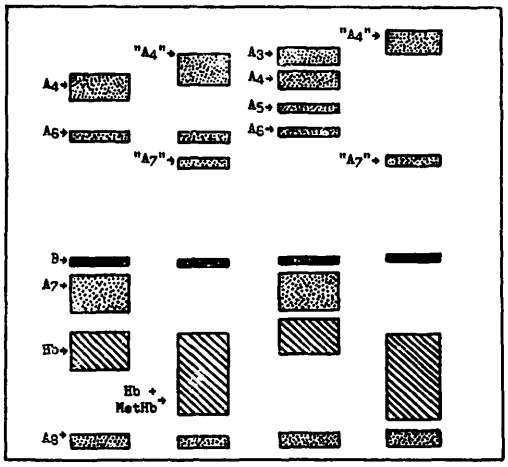

FIG. 3. Diagram of Fig. 2. Location of band B is indicated even though it is not well defined.

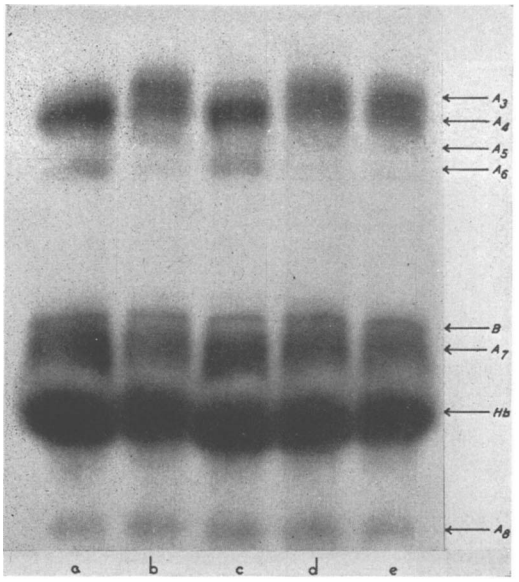

FIG. 4. Zymograms exhibiting genetic segregation of normal and atypical esterase forms from the family study: father (a); mother (b); daughter (c); and identical twin sons (d, e).

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Effects of 5-Br-Deoxyuridine and 5-F-Deoxyuridine on Nucleic Acid Metabolism by Rabbit Bone Marrow Cells.* (26940)

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A series of investigations has demonstrated that 5-bromodeoxyuridine (5-Br-deoxyuridine) may be utilized in lieu of thymidine for deoxyribonucleic acid (DNA) biosynthesis in animal and microbial systems(1-5). The anti-metabolite is not only incorporated into DNA but also blocks the incorporation of thymidine and thymidine precursors into DNA(6-10). 5-fluorodeoxyuridine (5-F-deoxyuridine) is known to inhibit formation of DNA thymine and specifically interferes with

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