result in sufficiently high plasma concentrations to exert a direct effect on hepatic glycogen metabolism.

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Lactic Dehydrogenase Isozymes and Ageing of Erythrocytes

DURING the life span of erythrocytes several of their enzymes undergo some modification[1–3]. Lactic dehydrogenase (LDH) is of special interest, because of the knowledge we have of its isozyme composition[4,5]. To investigate whether ageing affects each isozyme to the same degree, the electrophoretic pattern of LDH isozymes of young and old red cells were compared.

Young and old red cells from rabbits were separated by centrifugation in the presence of bovine serum albumin. In this procedure the upper layer consists of young cells, whereas the lower layer contains the heavier, older red cells. In one experiment, leucocytes (the density of which is similar to that of young red cells) were eliminated by agglutinating the red cells with phyto-agglutinins followed by slow centrifugation to remove the leucocytes. The clotted red cells were then hæmolysed after washing.

Both layers of hæmolysed cells were submitted to starch-gel electrophoresis, in borate buffer according to Smithies[7]. Isozymes were visualized by nitro blue tetrazolium salts and phenazine methosulphate, according to Markert[8] and Nachlas[9].

Under these conditions, two main anodic bands were found for both old and young red cells; they moved faster than hæmoglobin, and the faster one was the broader: these fractions seem to correspond to fractions 4 and 5.

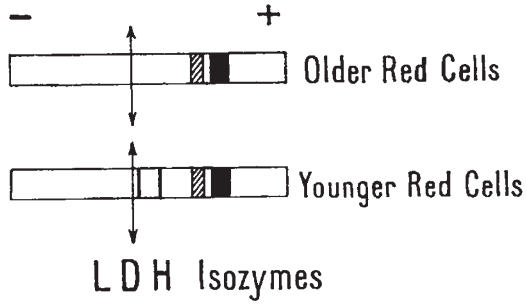

Fig. 1. LDH isozymes in older and younger red cells

In younger cells, two additional faint bands were observed; these were also anodic, but slower than the main bands. The migration rate of one of them was close to that of hæmoglobin. The same pattern was seen in the experiment where white cells were removed by centrifugation after agglutinating the red cells. Vesell[5] has pointed out that in many preparations of human hæmolysates, he found a faint supplementary band of slower mobility.

It seems that this band, which does not always occur, corresponds to the bands which we see only in younger cells. Cahn and Kaplan[10] have proposed that the various bands of lactic dehydrogenase are hybrids of four subunits of two different types. We have seen that there are two bands found in younger red cells which do not appear in older ones; it seems that these isozymes are preferentially inactivated or destroyed during the life span of the red cells.

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Electrophoresis of an Insect Inorganic Pyrophosphatase

IN our work on the properties of inorganic pyrophosphatase in the boll weevil, *Anthonomus grandis* Boheman[1], we used the enzyme present both in whole homogenates and in an acetone powder. We have since attempted electrophoresis of the enzyme to determine whether activity was present in one or more resolvable components.

All preparations and electrophoretic studies were made at 2° C. Approximately 1,000 frozen adult weevils were homogenized with cold acetone, and an acetone powder was prepared. After removal of the solvent and drying *in vacuo* in the refrigerator, the dry filter cake was extracted with demineralized water for 0·5 h and filtered through glass wool. The filtrate was centrifuged (20,000*g*) to remove particulate debris and other matter, and the clear supernatant was lyophilized. The lyophilized powder was used as the enzyme source.

Separation of the soluble components was achieved by paper electrophoresis, by means of a Durrum-type hanging papor strip electrophoresis cell (Spinco electrophoresis system).

The following conditions were found to give excellent separation in 4 h at 2° C: Buffer, veronal–HCl *p*H 8, 0·02 M; buffer volume, 1,100 ml. (550 ml. each in the (+) and (−) compartments); 3·5 m.amp, at 100 V; sample sizes, 1–3 mg of lyophilized powder in 10 μl.