A Biochemical Approach for the Study of Uveitis by Protein and LDH-Isoenzyme Analysis of Serum and Aqueous Humor (33899)

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Determination of enzymatic activities in tissues and fluids has been a useful method for studying metabolic changes in physiologic and pathologic conditions. Analysis of isoenzymes provides additional information because they may indicate the source of origin of such changes. Lactate dehydrogenase (LDH) is present in all tissues of the body including the eye and its humors. Two sensitive agar gel electrophoretic methods for the determination of serum proteins and LDH-isoenzymes we recently reported (1, 2) have been adapted for studies in ocular tissues and aqueous humor. In the present study, basic biochemical information is presented on proteins and LDH-isoenzymes of serum and aqueous humor from normal subjects and from patients with uveitis.

Materials and Methods. Serum proteins and LDH-isoenzymes were determined by the agar gel electrophoretic techniques previously reported (1, 2). Total LDH was determined on serum and tissue extracts spectrophotometrically by the method described in Bergmeyer (3). Fresh ocular tissues were excised from a human eye 6 hr postmortem. These tissues were rinsed in saline, blotted and weighed. Ten percent tissue homogenates were prepared in a glass homogenizer with buffered saline pH 7.5. The homogenates were then centrifuged at 15,000 rpm for 10 min and the supernatants were analyzed for total LDH and isoenzymes.

Aqueous humor from human eyes was obtained by the following paracentesis technique. The eye was locally anesthetized by 1 drop of 1% proparacaine hydrochloride. The paracentesis needle attached to a capillary tube previously described (4), was used. The cornea was perforated temporally 1 mm above the limbus as shown in Fig. 1. The

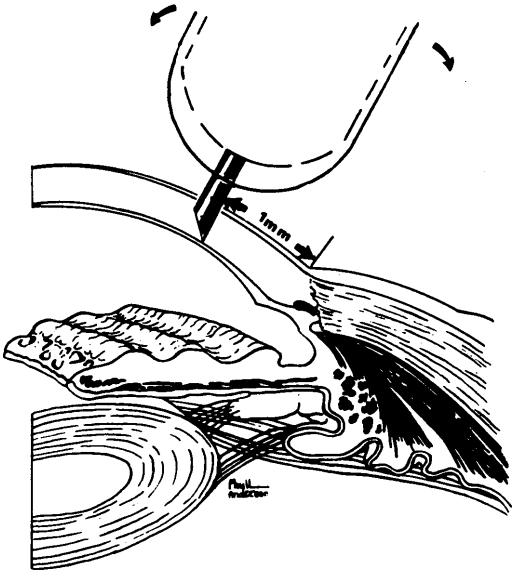

FIG. 1. Paracentesis of the cornea with a microneedle 1 mm above the limbus.

capillary tube was allowed to fill with aqueous humor to the 0.2-ml mark by intraocular pressure and capillary action. By this microtechnique, trauma to the cornea and contamination of the aqueous humor was minimized. The aqueous humor thus obtained was centrifuged by a microfuge and analyzed within 1 hr.

For the determination of LDH-isoenzymes in aqueous humor and comparison of the densities of the zones of various samples, the technique used for serum was adjusted and standardized as follows: Ten μl of aqueous humor were placed in the slot of the agar slide, electrophoresis was accomplished in 12 min and the slides were incubated with the developing mixture for 4 hr.

The total protein in the aqueous humor was estimated as follows: On a slide covered

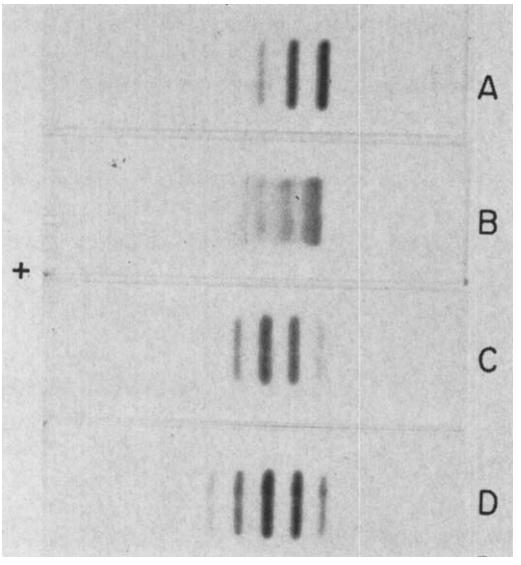

FIG. 2. Normal human patterns, by agar gel electrophoresis, of: (A), serum proteins; (B), serum LDH-isoenzymes; and (C), aqueous humor LDH-isoenzymes; (D1), aqueous protein zone: (D2), standard protein zone.

with agar gel prepared similarly as for serum protein electrophoresis (1), two slots were made. In one slot 10 μl of aqueous humor was placed, and in the other 10 μl of a standard protein solution diluted to contain a close approximation of the protein concentration in the aqueous humor. The slides were covered with a new layer of agar gel 1 mm thick and then they were fixed and stained for proteins as previously described for serum proteins (1). The density of the zones was measured in a densitometer, and the protein concentration in the unknown was estimated from the standard. By these techniques both proteins and LDH-isoenzymes can be determined simultaneously in serum and aqueous humor from the same patient.

Results. In Fig. 2 are shown the normal patterns of serum proteins and LDH-isoenzymes, the aqueous humor LDH-isoenzymes as well as the total protein zones of the aqueous humor and the standard protein samples. The LDH-isoenzyme pattern of the aqueous humor is entirely different from that of serum from the same individual. While the serum protein zones are clearly delineated on the microscopic slide, the concentration of proteins in the normal aqueous humor is very small and only the total protein can be detected in a single zone. The calculated aqueous humor protein in this example was 20 mg/100 ml. This illustrates that proteins, LDH, and isoenzymes can be determined simultaneously in the serum and aqueous humor of the same subject.

Figure 3 shows the isoenzyme patterns of the various ocular tissues surrounding the aqueous humor. In these tissues the cathodal isoenzymes 5, 4, and 3 predominate in general, although similarities and differences exist among their patterns.

Figures 2 and 3 show that the aqueous isoenzyme patterns are closely related to those of the ocular tissues and they are entirely different from the serum isoenzyme patterns. These observations point to the fact that analysis of aqueous LDH-isoenzymes is a useful approach in the investigation of physiologic and pathologic processes in the eye. Increased activity of LDH or change of the isoenzyme pattern in the aqueous humor may reflect metabolic alterations or injury of the tissues surrounding it, or increased permeability from blood capillaries.

Since metabolites can permeate from the vitreous into the aqueous humor and the vit-

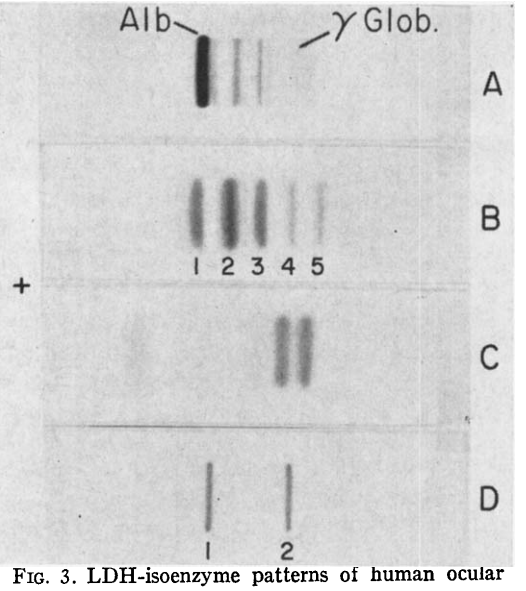

FIG. 3. LDH-isoenzyme patterns of human ocular tissues by agar gel electrophoresis: (A), cornea; (B), lens; (C), iris; and (D), ciliary body.

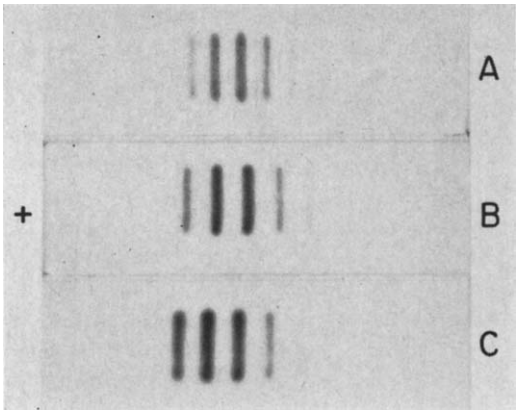

FIG. 4. LDH-isoenzyme patterns of human ocular tissues by agar gel electrophoresis: (A), vitreous humor; (B), retina; and (C), choroid.

reous is surrounded by the retina, the isoenzyme patterns of some posterior tissues of the eye were obtained and are shown in Fig. 4.

The above-mentioned techniques have been applied to the investigation of normal and pathologic cases. Some preliminary results are illustrated by the following examples. In 6 cases diagnosed as toxoplasmosis by clinical and serologic criteria, the isoenzyme-5 predominated in the aqueous humor and in 6 cases of "histoplasmic choroiditis" the aqueous isoenzyme patterns were similar to the normal. In order to seek an explanation of this difference, a case of severe active toxoplasmic uveitis was investigated. In this case, increased LDH activity and protein concentration was found in the aqueous humor. The serum and aqueous protein and isoenzyme patterns are shown in Fig. 5. The protein patterns of serum and aqueous humor are qualitatively similar indicating clearly serum penetration into the aqueous humor. The isoenzymes 1, 2, and 3 in the aqueous probably originated from serum, since they are not detectable in the normal aqueous and their pattern in this case is similar to those of the serum. The increased activity of isoenzyme-5, however, does not appear to originate from serum. A possible explanation of the origin of isoenzyme-5 is either from leukocytes, which are characterized by a preponderance of isoenzyme-5, and are often observed in aqueous and vitreous humors of uveitis cases or from the ocular tissues rich in isoenzyme-5.

Discussion. Following the development of the agar gel electrophoretic techniques for the determination of proteins and LDH-isoenzymes, they were applied in the analysis of serums from patients with uveitis. The serum isoenzymes in these patients were either normal or showed abnormalities incident to other clinical conditions not associated with uveitis. The protein patterns were either normal or showed the characteristic pattern of increased γ-globulin as in chronic infection. It was reasoned that the metabolic changes occurring in the eye as a result of uveitis, are of small magnitude to influence significantly the large serum metabolic pool. For this reason, these techniques were adapted for analysis of isoenzymes and proteins in the aqueous, which represents primarily, the dynamic equilibrium of constituents of the tissues surrounding it.

The proteins in the aqueous, although in small concentration, appear to be qualitatively similar to those of serum [Francois *et al.* (5)] from where they probably originate. The predominance of isoenzymes-4 and 5 in the aqueous indicates that they originate

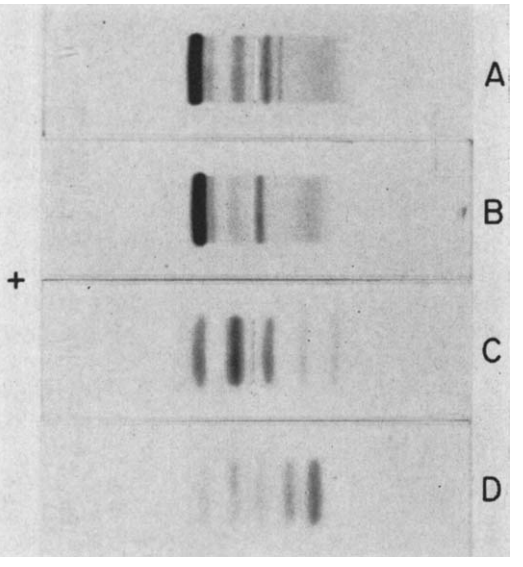

FIG. 5. Patterns from a toxoplasmic uveitis case, by agar gel electrophoresis of: (A), serum proteins; (B), aqueous proteins; (C), serum LDH-isoenzymes; and (D), aqueous LDH-isoenzymes.

from the surrounding tissues rather than from the serum. Thus, determination of LDH-isoenzymes in the aqueous may detect metabolic changes of ocular tissues.

The combined determination of proteins and isoenzymes in the serum and aqueous of patients not only may be useful for diagnostic purposes, but it has more general interest since it offers the possibility of testing permeability and other physiologic functions in the eye.

Summary. Proteins and LDH-isoenzymes were determined simultaneously in human serum and aqueous humor by an agar gel electrophoretic technique. The isoenzyme patterns of ocular tissues were obtained. Normal and uveitis cases were studied. The results showed that in uveitis, the protein concentration and isoenzyme activity in the aqueous humor increased; the protein pattern in the aqueous was similar to that of the serum indicating serum protein penetration into the aqueous, while the isoenzyme pattern of the aqueous showed an influence from both the serum and the ocular tissues. The combined determination of proteins and LDH-isoenzymes in serum and aqueous may be useful as a diagnostic aid, for permeability studies in the eye and for detection of metabolic changes of ocular tissues.

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