Starch Gel Electrophoresis of Some Invertebrate Sera

An interest in the phylogenic emergence of characteristic serum proteins has prompted us to examine the electrophoretic properties of the blood of several invertebrate forms (*1*.)

The method of zone electrophoresis in starch gel described by O. Smithies (*2*) was used to separate serum proteins of fourteen species of decapod Crustacea, two species of Arachnoidea, and two species of Mollusca. Sera from 6 to 12 different specimens of each form were examined. Soluble starch was produced by treatment of potato starch with acetone-HCl (*3*) at 36.1°C for 70 minutes (*4*). Gels were prepared from this soluble starch in 0.030*M* sodium borate buffer of *p*H 9.05, and electrophoresis was carried out at 10°C for 16 hours with a potential of 4.5 v/cm. Members of each species were collected from restricted habitats, with the exception of the green crab. A specimen from waters near Scotland (*5*) gave electrophoretic patterns identical to those of specimens from the Woods Hole region. Within a given species, differences in sex, size, or stages of the molt cycle produced no significant difference in the electrophoretic patterns.

Representative patterns for each form included in the present series are illustrated in Fig. 1; that for a normal human serum is shown at the bottom for comparison and orientation. The cathodic region into which gamma globulins usually migrate is at the left of the origin, while in the human pattern albumin occupies the most prominent and extensive zone in the anodic region. Among the invertebrates studied, there is no indication that serum proteins having electrophoretic properties similar to those of mammalian gamma globulins occur in the serum. Figure 1 also indicates that the serum proteins of these invertebrates are highly species specific above the generic level, although similarities between closely related forms are observed.

From the Arthropoda we have studied, the three species of the fiddler crab (*Uca*) have nearly identical serum electrophoretic patterns. The two species of hermit crab (*Pagurus*) also give patterns which are quite alike. In some cases such similarities might be extended to groupings of families, as demonstrated by the patterns obtained for the calico crab (*Ovalipes*) when compared with those obtained for the green crab (*Carcinides*). An exception, however, is the blue crab (*Callinectes*), the third representative of the family Portunidae which was sampled. This species has practically no similarity to the other two with respect to electrophoretic pattern. This indicates the need for caution in interpreting the significance of the observations thus far obtained in invertebrates correlating morphology with serum protein patterns. A further example of the need for caution in applying this technique to taxonomic problems is indicated by comparing the pattern produced by serum of the lobster (family Homaridae) with the pattern obtained from the serum of the mole shrimp, *Emerita talpoida* (family Hippidae). The two patterns are nearly identical even though the forms are taxonomically in different families.

Comparing the two representatives of

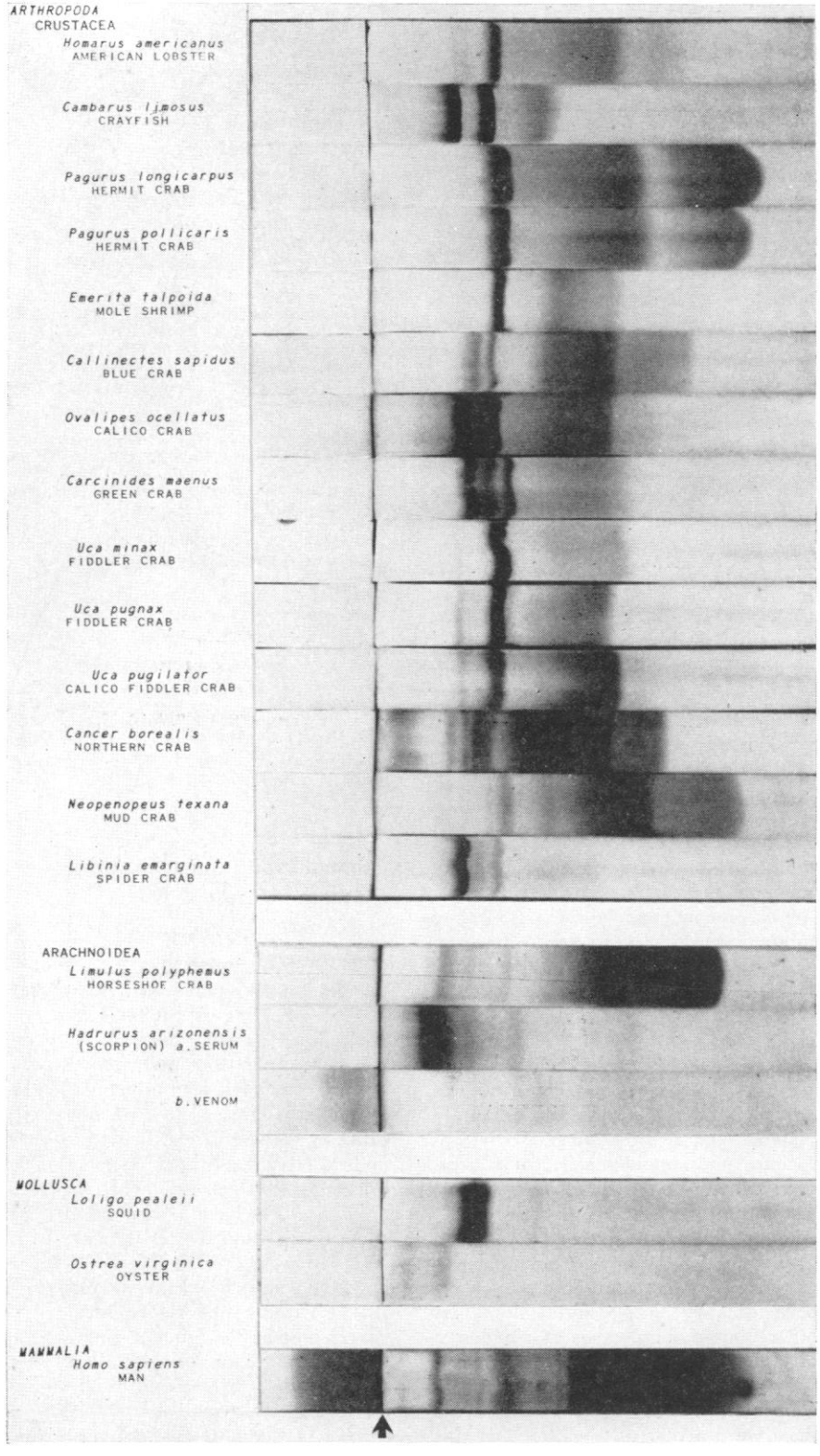

Fig. 1. Starch gel electrophoretic patterns of hemocyanin-containing sera, scorpion venom, oyster serum, and normal human serum. The position at which the samples were applied is indicated by the arrow below the figure. The cathodic area is to the left of the origin, and the anodic area is toward the right.

Arachnoidea cited in Fig. 1, we find that the horseshoe crab (*Limulus*) has quite a different serum protein composition from that of its nearest living relative, the scorpion (*Hadruras arizonensis*) (*6*). Scorpion venom produced a pattern quite unlike scorpion serum, its proteins migrating toward the cathode in a fashion similar to that found in electrophoresis of human gamma globulin.

From the Mollusca we have studied, a species of Cephalopoda and one of Gastropoda are presented here. Serum from the squid (*Loligo pealii*) yielded two major components of moderate mobility, while that from the oyster gave two slow components. No respiratory proteins have been found in oyster blood, and the bands which developed are probably due to other types of serum proteins, the concentrations of which are relatively low.

Hemocyanin is the principal serum protein of many invertebrates, and, in the case of the spiny lobster (*Panulirus interruptus*), it forms a single boundary upon electrophoresis in the Tiselius apparatus (*7*). The boundary method has also revealed the presence of other proteins amounting to less than 3 percent of the total protein. The dense bands found in patterns obtained by zone electrophoresis in starch gel are thus probably due to hemocyanin, even though more bands than one are frequently found. Studies with the ultracentrifuge of the *p*H stabilities of hemocyanins from several different species have demonstrated that these proteins aggregate reversibly near their isoelectric points and yield products of high sedimentation constants ($S_{20}>22$) (*8*). Molecular sieve properties of starch gel might be expected to prevent the admission of such large particles into this medium, as is demonstrated by the failure of some macroglobulins ($S_{20}>19$) to migrate into such gels (*9*). However, since the *p*H of the gel buffer is usually above the alkaline limits of the stability range for the aggregated molecular species, the dissociated hemocyanins ($S_{20}\cong 17$) are observed. The appearance of more than one dense band in these electrophoretic patterns indicates that, at alkaline *p*H, hemocyanins usually dissociate into nonidentical constituents even though the dissociation products have ultracentrifugal homogeneity (*8*). The possibility arises that the copper-containing protein moiety responsible for enzymatic oxygen transport may have been isolated electrophoretically from other protein components of a polymer of higher molecular weight. This possibility should be tested by location of the copper in the electrophoretic patterns since we are presently confronted with the alternative that hemocyanins may distribute copper between more than one dissociation product.

Despite the few exceptions which might prove to have important biological significance, the results of these studies indicate that the electrophoretic patterns of the serum proteins of closely related invertebrates have a remarkable degree of specificity. These findings suggest that starch gel electrophoresis of serum proteins may be useful in certain racial studies, taxonomic problems, and considerations of biochemical individuality.

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References and Notes

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5. Serological Museum sample No. 57-2786 was kindly contributed by Dr. Alan Boyden, Department of Zoology, Rutgers University, New Brunswick, N.J.
6. We are indebted to Dr. Herbert L. Stahnke, Poisonous Animal Research Laboratory Arizona State College, Tempe, for sending us live scorpions in the mail.
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Ethanol Protection against the Catalase-Depressing Effect of 3-Amino-1,2,4-Triazole

In 1941 Greenstein *et al.* (*1*) reported that the liver catalase activity of rats and mice bearing extrahepatic tumors is markedly lowered. Nakakara and Fukuoka (*2*) in 1948 reported the separation of tumor fractions which, when injected into mice, lowered the liver catalase activity. In extension of this finding, Greenfield and Meister (*3*) have been able to isolate fractions of various tumors which have a marked in vivo effect on the lowering of the liver catalase activity in mice. Recently Heim *et al.* (*4*) have been able to reproduce all the catalase changes occurring in a cancer host by the use of 3-amino-1,2,4-triazole (AT) in a normal animal. They reported that 3 hours after an intraperitoneal injection of AT, in a dose of 1 g/kg of body weight, the liver catalase activity of rats is reduced to 10.9 percent of control values. This depressing effect on catalase activity gradually subsides, and 72 hours after the injection of AT the activity returns to 84.6 percent of control values.

Nelson *et al.* (*5*), while studying the effects of AT on ethanol metabolism, discovered that ethanol, if injected prior to AT, protected the dog from the catalase-depressing effect of AT. One experiment with the rat indicated that the effect was not species specific. It is the purpose of this report to establish the protective action of ethanol in the rat and to report this phenomenon in more detail.

Adult male Sprague-Dawley rats were used throughout the experiments. The animals were allowed free access to Purina laboratory chow and water until the day of the experiment; on this day, food and water were withheld. 3-Amino-1,2,4-triazole (*6*) was injected intraperitoneally as a 10 percent aqueous solution in a dose of 1 g/kg. Ethanol was injected intraperitoneally in a dose of 1 g/kg as a 20 percent (wt./vol.) solution in isotonic saline. Ethanol was injected 1 hour prior to the injection of AT. When isotonic saline was substituted for the ethanol injection, it too was injected 1 hour prior to the injection of AT. At a designated time after the injection, the animal was sacrificed by a blow on the head. A small specimen of liver was removed, washed free of blood, blotted dry, weighed, diluted with nine times its weight of 0.01*M* potassium phosphate buffer (*p*H 6.8), and homogenized in a chilled glass tissue grinder. The 1:10 homogenate was diluted with phosphate buffer to give a final dilution of 1:500. Catalase activity was determined by a modification of the assay procedure of Feinstein (*7*), perborate substrate being used, and is expressed as milliequivalents of sodium perborate destroyed per milligram of liver. Blood ethanol determinations were carried out by the method of Aull *et al.* (*8*).

From the summary of results given in Table 1 it can be seen that, 1 hour following the injection of AT, the catalase activity is reduced to 8.6 percent of control values. At ½ hour after the injection of AT, the catalase activity is 19.8 percent of control values. There appears to be quite a variation in the activity ½ hour following the AT injection, as is shown in the comparatively large standard deviation. It is also shown that, if the animal is pretreated with ethanol (1 g/kg) 1 hour prior to the injection of AT, the catalase activity 1 hour following the AT injection is reduced only slightly if at all. Ethanol per se does not affect the catalase activity of the control animals. If the animal is pretreated with a dose of saline (in an amount equivalent to that of saline in a dose of ethanol), no protective action against AT effects is seen.