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.

KENNETH R. WOODS†
ELIZABETH C. PAULSEN*
RALPH L. ENGLE, JR.,† JAMES H. PERT
Marine Biological Laboratory, Woods Hole, Massachusetts, and *Department of Medicine, New York Hospital–Cornell Medical Center, New York*

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* Present address: Department of Zoology, Rutgers University, New Brunswick, N.J.
† Lalor fellow, 1957.

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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.

Table 1. Effect of injections of ethanol, saline, and 3-amino-1,2,4-triazole (AT), alone or in various combinations, on liver catalase activity.

No. of animals	Injection			Interval between injection and sacrifice (hr)	Catalase activity ± S.D.† (mEq/mg of liver)
	Ethanol* (g/kg)	Saline	AT (g/kg)		
4	0	0	0		1.131 ± 0.120
3	1	0	0	2	1.068 ± 0.093
6	0	0	1	0.5	0.224 ± 0.183
4	0	0	1	1	0.097 ± 0.031
3	0	Amt. equal to that in 1 g/kg dose of ethanol	1	1 (after AT)	0.116 ± 0.026
6	1	0	1	1 (after AT)	0.982 ± 0.235

* In saline. † S.D. = standard deviation.

It was decided to determine whether the protective effect of ethanol was permanent or temporary.

In order to determine this, a group of animals was injected with ethanol followed in 1 hour by an injection of AT. Two blood samples were drawn from each rat, the first from 0 to 11 minutes following the AT injection, the second from 60 to 181 minutes following the withdrawal of the first sample. The rat was sacrificed at from 0 to 125 minutes following the withdrawal of the second blood sample. Immediately after the animal was sacrificed, a sample of liver was removed, and the catalase activity was determined. Since the animals were sacrificed at from 60 to 258 minutes following the AT injection, any significant change in catalase activity could not be due to the time interval between AT in-injection and sacrifice. It has been shown (*5*) that, in the rat, 60, 120, 180, and 240 minutes following AT injection, the catalase activity is 13.7, 14.9, 8.7, and 9.6 percent of control values, respectively. Marshall and Fritz (*9*) established that blood ethanol disappearance follows a straight line with time at all blood ethanol concentrations except at low levels (< 10 mg percent), at which levels disappearance follows an exponential curve.

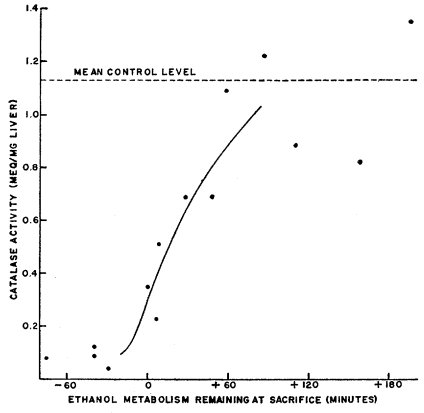

Fig. 1. Liver catalase activity in rats previously injected with ethanol and 3-amino-1,2,4-triazole and sacrificed with varying times of ethanol metabolism remaining.

In view of this, the approximate time at which all ethanol leaves the animal's body may be determined by preparing a graph of the blood ethanol concentrations with time and by extrapolating the blood ethanol line to zero concentration. This was done for each rat, and the number of minutes of ethanol metabolism remaining at the time of sacrifice was determined. If the animal was sacrificed before all ethanol had left its body, the number of minutes of ethanol metabolism remaining is designated by a plus sign. If the animal was sacrificed after all ethanol had left its body, the number of minutes of ethanol metabolism remaining is designated by a minus sign.

Figure 1 shows a plot of the catalase activity against the number of minutes of ethanol metabolism remaining at the time of sacrifice. It can be seen that, approximately 60 to 120 minutes before all ethanol disappears from the animal's body, the AT begins to take effect and the liver catalase activity begins to decrease. The activity falls rather rapidly from the point that represents 60 minutes before all ethanol has disappeared. Within 30 minutes after the disappearance of all ethanol, maximum depressing effects of AT are observed. Perhaps this lag in AT effect is partly or wholly the result of the lag in disappearance of ethanol when low concentrations of blood ethanol are reached. However, as Fig. 1 demonstrates, the ethanol effect is temporary; AT begins to take effect shortly before all ethanol has left the body, and maximum depressing effects are observed shortly after all ethanol has disappeared.

Recently Alexander (*10*) has shown that ethanol reverses the in vitro catalase-depressing effects of an ethanol-soluble boiled aqueous extract (BAE) of normal rat liver. He states that this extract continuously generates H_2O_2, which is decomposed predominantly to oxygen and water in the presence of catalase but with the formation of some inactive catalase-H_2O_2 complex II. This results in decreased catalase activity. When ethanol is present in the system, it removes the H_2O_2 formed and is itself peroxidized to acetaldehyde in the process, thus preventing the accumulation of complex II. In this manner, in the presence of ethanol and ethanol-soluble boiled aqueous extract, catalase activity remains normal. He concludes that the catalatic inhibition of this extract is due to autoxidation of ascorbic acid and sulfhydryl groups in the extract.

The efficiency of ethanol in protecting against the inhibition of AT suggests a mechanism similar to that described by Alexander with ethanol-soluble boiled aqueous extract. However, in the absence of information concerning autoxidation or metabolism of AT, definite conclusions cannot be drawn.

GEORGE H. NELSON
Department of Biochemistry, University of South Dakota Medical School, Vermillion

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Blocking by Picrotoxin of Peripheral Inhibition in Crayfish

In vertebrates, inhibition takes place within the central nervous system. But a crayfish "thinks in its claws" (*1*). More prosaically, in the Crustacea both excitation and inhibition take place in the peripheral muscle. Because these events are at the fringes of the crayfish body, it is easy to assay drugs for effects on the inhibitory process. The drug we found to block inhibition is picrotoxin (*2*) (which is known to pharmacology for setting off convulsions when injected into a mammal).

The preparation used was the claw of the crayfish, *Orconectes immunis*. The