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.

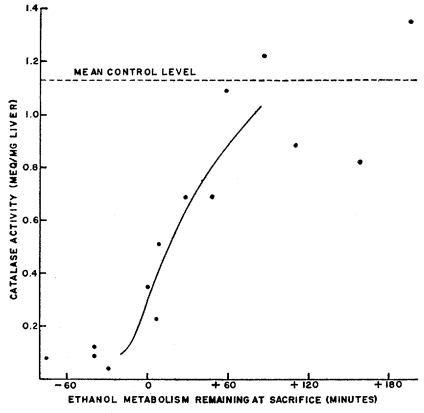

Fig. 1. Liver catalase activity in rats previously injected with ethanol and 3-amino-1,2,4-triazole and sacrificed with varying times of ethanol metabolism remaining.

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.

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7 November 1957

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

amputated claw was arranged so that the finger's movement was recorded on a revolving drum. The closer muscle was cut from the finger, and the excitatory and inhibitory nerves to the opener muscle were dissected free and placed on separate stimulating electrodes. The claw was perfused with a buffered modification of van Harreveld's crayfish Ringer's solution (*3*). Picrotoxin, when used, was dissolved in the Ringer's solution.

One way in which we recorded inhibition was by stimulating the excitatory nerve tetanically until a contraction was well developed and by then briefly stimulating the inhibitory nerve as well. During the stimulation of the inhibitory nerve, the contraction decreased (Fig. 1, IA). After the claw had been perfused with picrotoxin solution (Fig. 1, IB), the contraction was no longer affected by stimulation of the inhibitory nerve.

Inhibition was also seen when the excitatory nerve and the inhibitory nerve were stimulated together at the outset (Fig. 1, IIA). With this method there was either no contraction, showing that the inhibition was complete (as in the illustration), or the muscle contracted at a rate slower than normal, showing that the inhibition was partial. The extent of the inhibition depended on the ratio of the frequency of inhibitory nerve stimulation to the frequency of excitatory nerve stimulation (*4*). After perfusion with picrotoxin, the muscle responded to concurrent stimulation of the excitatory and inhibitory nerves as if the excitatory nerve alone had been stimulated (Fig. 1, IIB). Picrotoxin had blocked inhibition.

The extent to which inhibition was blocked depended on the concentration

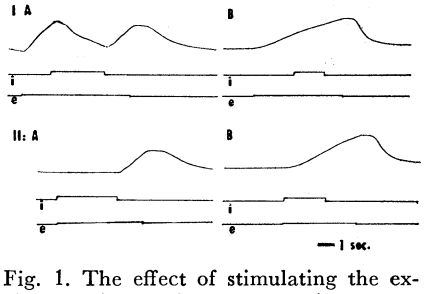

Fig. 1. The effect of stimulating the excitatory (e) and inhibitory (i) nerves running to the opener muscle of the crayfish claw. An upward deflection of the signal trace indicates that the nerve was stimulated at a rate of 60 stimulations per second. (IA) Stimulation of the inhibitory nerve during the contraction caused by stimulating the excitatory nerve; (IB) same as IA, but 30 minutes after the perfusion of $10^{-4}M$ picrotoxin; (IIA) initial simultaneous stimulation of the excitatory and of the inhibitory nerves; (IIB) same as IIA, but after the perfusion of picrotoxin.

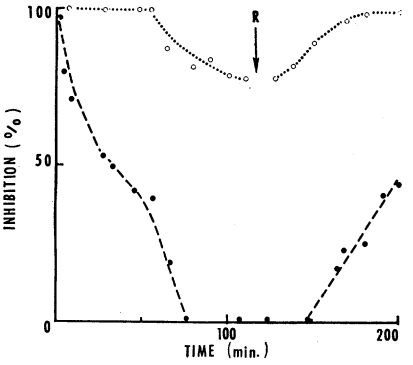

Fig. 2. The percentage inhibition at intervals following the onset of perfusion of $10^{-4}M$ picrotoxin. The excitatory nerve was stimulated at a rate of 60 stimulations per second. The inhibitory nerve was simultaneously stimulated at a rate of 21 stimulations per second (dotted line) or at 150 stimulations per second (broken line). At the time indicated by *R*, perfusion of picrotoxin was stopped and perfusion with Ringer's solution was begun.

of picrotoxin. Picrotoxin ($10^{-6}M$) had almost no effect, while higher concentrations progressively decreased the effect of inhibitory nerve stimulation. The action of picrotoxin was almost independent of *p*H over the range tested (from *p*H 6.3 to *p*H 7.7).

The time course of the action of picrotoxin was determined by stimulating both the excitatory and inhibitory nerves at intervals after the onset of picrotoxin perfusion. The percentage inhibition was measured from the record (*5*). One experiment was plotted as shown in Fig. 2. Originally the contraction was completely inhibited when the inhibitory nerve was stimulated at rates of either 21 or 150 stimulations per second. Soon after picrotoxin was introduced, inhibitory nerve stimulation at a rate of 21 per second produced no inhibition, while stimulation at 150 per second gave only partial inhibition. The graph also shows that the effect of picrotoxin was eliminated by washing the drug out of the claw.

The mechanism of picrotoxin action was then investigated. It was evident that picrotoxin does not act by selectively blocking the conduction of nerve impulses along the inhibitory nerve, for inhibition was normal after the nerve had been soaked in picrotoxin, so long as the muscle was free of the drug. And the excitatory mechanism was not interfered with, for it remained normal long after the muscle and the nerves were exposed to picrotoxin.

The analysis may be carried further by assuming that the inhibitory nerve releases an inhibitory transmitter at its ending. Picrotoxin might block inhibition by slowing the release of the inhibitory transmitter or by affecting some later step in the inhibitory mechanism—for example, by blocking the combination of the inhibitory transmitter with a receptor molecule. It seems unlikely that picrotoxin slows the release of the inhibitory transmitter, because Florey, McLennan, and Elliott have shown that the crayfish stretch receptor and the crayfish muscle can be inhibited by a chemical from the mammalian brain, and that this chemical's inhibitory action on the stretch receptor is blocked by picrotoxin (*6*). And our data are also consistent with the idea that picrotoxin blocks the combination of the inhibitory transmitter with a receptor molecule.

It may be concluded that picrotoxin reversibly blocks peripheral inhibition in crayfish muscle. Probably picrotoxin joins with the receptor molecule and thereby blocks the action of the inhibitory transmitter; that is, picrotoxin appears to have a "curarelike" action on this inhibitory synapse. Perhaps the convulsions and central nervous system excitation produced when picrotoxin is injected into a mammal are caused by a similar mechanism.

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3. This modification was made by adding 55 ml of 0.2*M* trismaleate buffer to 945 ml of crayfish perfusion fluid. The crayfish perfusion fluid was the Ringer's solution of van Harreveld [*Proc. Soc. Exptl. Biol. Med.* 34, 428 (1936)] with the $NaHCO_3$ omitted.
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5. The percentage inhibition is defined as:
$$\frac{C_e - C_{ei}}{C_e} \times 100$$
where C_e is the initial rate of contraction with stimulation of the excitatory nerve alone and C_{ei} is the initial rate of contraction when both the excitatory nerve and the inhibitory nerve are stimulated.
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