stimulation of steroidogenesis without blocking this enzyme activation (*1*). The possibility that the ACTH stimulation of steroidogenesis is mediated through the activation of protein synthesis by cyclic-AMP, therefore, is not incompatible with our findings (*3*).

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Endrin: Use of Concentration in Blood To Diagnose Acute Toxicity to Fish

Abstract. *Channel catfish,* Ictalurus punctatus *(Rafinesque), were exposed to continuously renewed solutions of endrin in water. Analyses of the fish blood by gas chromatography revealed a well-defined threshold concentration of endrin in the blood, approximately 0.30 microgram per gram, that, if exceeded, results in death. Fish exposed to lethal concentrations of endrin in water for periods of time insufficient to cause death had blood-endrin concentrations markedly lower than those that died from exposure to the same water. There was little overlap in range of endrin concentration in blood between dead and living exposed fish.*

Probably no other single group of chemicals has been accused more frequently of causing fish kills than the modern pesticides. There is a serious need for techniques that will give additional proof that pesticides are the cause of death of fish or wild animals. During the 1963–64 investigations of large fish kills on the lower Mississippi River, a direct relationship was observed between endrin concentration in blood and intoxication of channel catfish, *Ictalurus punctatus* (Rafinesque). These observations, plus additional ones obtained more recently, are presented in this report.

The concentrations of pesticides in various organs of animals have been measured frequently, but the purpose was often for survey data and not for toxicological studies. Among those who have tried to relate concentrations in tissue with intoxication, Brown *et al.* reported that in the blood of man and dogs there is a critical concentration of dieldrin above which clinical signs of intoxication appear (*1*); and Burdick *et al.* reported a relatively close relationship between the DDT content of eggs and fry mortality in lake trout (*2*).

Dale *et al.* found a relationship between the degree of illness and the concentration of DDT in rat brains, and they also noted a sharp rise in the concentration of DDT in plasma after administration of the insecticide (*3*). The plasma concentration, however, remained approximately constant from the slight tremor stage through convulsions and death. In our efforts to detect acute endrin toxicity, we used the concentration of endrin present in the blood of fish that had been exposed to endrin dispersed in water. We reasoned that blood was the vehicle by which endrin reached the target organ and that the concentration of endrin in the blood should be related to intoxication. This work was begun before the paper by Brown *et al.* (*1*) was available.

Channel catfish, weighing from 200 to 500 g, were exposed to 15 different lethal and sublethal concentrations of endrin in water. The test water was renewed continuously by a serial dilution apparatus (*4*) from a water-supply system similar to the one described by Lemke and Mount (*5*). Renewal of the endrin was necessary to maintain known and constant concentrations. Tap water was passed through a 1.6-m^3 activated carbon filter as a safety precaution to remove traces of pesticides and then to a 375,000-liter pond reservoir where it was detained 60 to 90 days before use. This detention period minimized daily variations in chemical characteristics. Tests were made at 15° and 22°C ± 1°C in stainless-steel troughs 30 by 30 by 180 cm. All water lines and valves were made of plastic or stainless steel; after the introduction of endrin, contact of the test water with plastic was minimized, for many plastics readily sorb endrin. Three fish were placed in each trough, and the rates of water flow were controlled so as to give approximately 1 ml of water per 2 g of fish per minute. Flow rates were not adjusted during a given test when one or two fish died.

Fish were obtained from four locations (one in Arkansas and three in Ohio); before exposure, all contained less than 0.03 μg/g of endrin in the blood. Blood was obtained by drying and severing the caudal peduncle and allowing the blood to drip into an acetone-rinsed vial; the sample was then kept frozen until analysis. Fish were removed at the time of death; those that died during the night were discarded.

The blood samples were assayed by the procedure described by Schafer, Busch, and Campbell (*6*). If less than a 10-g sample of blood was used (usually 2 to 3 g was available), the sample weight was adjusted to 10 g with distilled water. Reagent blanks were consistently negative for endrin or other interferences. After saponification of the blood sample with alcoholic KOH, the chlorinated hydrocarbons were extracted into hexane. Measured volumes of the hexane extract were assayed with a gas chromatograph equipped with an electron-capture detector. Chromatograms of samples were compared with chromatograms of standard solutions of recrystallized analytical reference standard endrin (Shell). Some of the sam-

ples were analyzed on a column like that described by Schafer *et al.* (*6*). The other samples were assayed on columns prepared in the following manner: 2.0 g of GE XE-60 and 0.5 g of Epon 1001 were dissolved in 30 ml of acetone, 30 g of 30/60 mesh Johns-Manville Chromosorb T was stirred into the solution, and the mixture was dried with a suction filter and packed into an aluminum tube 150 cm long with outside diameter of 3 mm. The column was conditioned with a gas flow at 200°C for 48 hours and then maintained at 185°C with a flow rate of 50 ml of nitrogen per minute. Seventeen blood samples, fortified with endrin, were interspersed with the other samples to determine the accuracy and precision of the method. The results indicated an average of 96 percent recovery and a standard deviation of 9.6 percent.

The presence of endrin was verified in approximately one-fifth of the blood samples by thin-layer chromatography. Samples of water from the test chambers were shaken with a known amount of hexane (usually 10 ml) for 4 minutes in a separatory funnel. The hexane extract was concentrated, if necessary, and a measured volume was injected into the gas chromatograph. Recovery studies were made with each set of water samples, and the results were within the range reported above for the blood samples.

The endrin concentration in the test water was as much as 75 percent less than the introduced concentration because of uptake by the fish and adsorption on various surfaces. Measured endrin concentrations as low as 0.25 to 0.30 μg/liter were found to be acutely toxic to the catfish in 10 days or less.

Figure 1 depicts the relationship of the concentration of endrin in the blood to the exposure time and lethality. In approximately 90 percent of the 95 exposed fish that were analyzed, the endrin concentration in the blood of fish killed by endrin poisoning was greater than the concentration of endrin in the blood of fish that were exposed to endrin in the water but not killed. In nonlethal exposures to endrin, lasting as long as 44 days, the concentration of endrin in the blood was not as high as it was in fish killed by endrin. For example, four fish were exposed to 0.1 μg/liter and four fish to 0.2 μg/liter for 44 days. Although those in 0.2 μg/liter convulsed frequently when disturbed and those in 0.1 μg/liter were very nervous, the mean blood concentration of each group was 0.18 and 0.25 μg/g, with ranges of 0.11 to 0.25 μg/g and 0.20 to 0.28 μg/g, respectively. These results indicate that endrin is not stored in blood. Even in water containing concentrations of endrin that killed some fish and not others (partial kill resulted either from insufficient time or because of individual variation), there was a distinct difference between the concentration of endrin in the blood of fish that were killed and those that were exposed but not killed. Figure 1 shows some values for fish that survived exposure for 4, 21, and 44 days in water containing endrin concentrations that killed other fish in the same tank.

The results of this study indicate that the concentrations of endrin in the circulating blood of channel catfish is related to intoxication and can be used as a diagnostic tool to determine the occurrence of endrin poisoning. No fish survived blood concentrations exceeding 0.28 μg/g and no fish died with less than 0.23 μg/g. Only 5 of 62 fish killed by endrin had less than 0.28 μg/g of endrin in the blood. If continuous exposure to endrin concentrations is not lethal in 44 days, longer exposure does not usually result in death (*7*). This method, therefore, should be applicable to fish kills resulting from endrin toxicity. Other studies (not yet published) have demonstrated that the critical blood concentration of endrin (approximately 0.3 μg/g) for channel catfish is higher than for some other species of fish. Since the ranges of endrin concentration in the blood for lethal and nonlethal exposures overlapped in only 10 percent of the individuals, the detection of endrin-caused mortality is positive even with limited sample size.

This method for detecting endrin mortality is especially practical because one needs to know neither the concentration of endrin in the water nor the exposure time in order to detect lethal toxicity. As is shown in Fig. 1, only the mean for fish killed in 24 hours or less is greater than 0.6 μg/g and no mean is less than 0.4 μg/g; hence the threshold concentration in the blood is essentially independent of both time of exposure and concentration of endrin in the water. While this may seem unusual at first glance, there is no obvious reason why such factors should alter the threshold concentration within the body unless acclimation occurs.

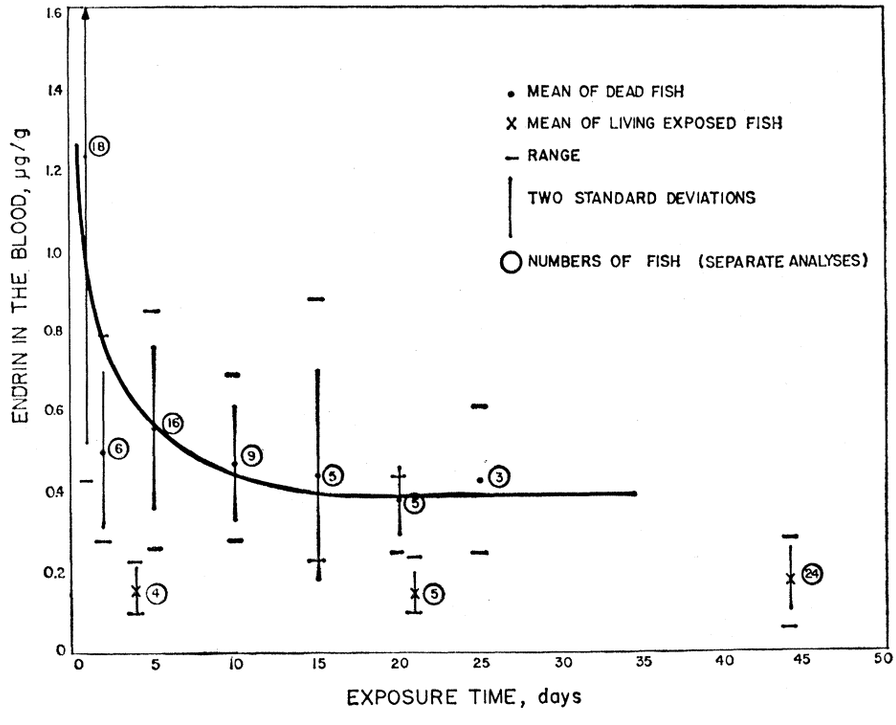

Fig. 1. Effect of time of exposure to endrin dissolved in water on the concentration of endrin in the blood of channel catfish. Numbers in circles indicate the number of fish (separate analyses) in each group. Results for dying fish are grouped by 5-day intervals except those less than 1 day, 1 to 2 days, and 2 to 5 days.

This study and previously cited literature suggest that analysis of blood for chlorinated hydrocarbon insecticides might be used as a clinical tool as well. The data of Dale *et al.* (*3*), however, suggest that, in mammals, it may not be possible to distinguish between a dose causing death and a dose producing only tremors.

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24 March 1966

Adenovirus in Blood Clots from Cases of Infectious Hepatitis

Abstract. *Adenovirus type 5 was isolated from blood clots from 27 of 30 sporadic cases of infectious hepatitis. Only one isolation of virus, also adenovirus 5, was made from blood clots from 70 persons with no known contact with infectious hepatitis.*

Investigation of the etiology of infectious hepatitis in Arizona included efforts to isolate viruses from blood clots. Blood was collected in vacuum tubes without preservative or anticoagulant from sporadic cases and from family contacts. Specimens were refrigerated at approximately 8°C until processing, which was completed within 10 hours of collection. Serums were centrifuged at 1500 rev/min for 20 minutes and decanted from the clots. The clots were frozen and thawed rapidly three times in dry ice–alcohol bath, and 0.2 ml of the lysed clots was inoculated into each of two or four tubes of tissue culture of human embryonic lung cells (*1*). Tissue culture was grown in M-199 medium containing 15 percent calf serum and maintained in this medium containing 2 percent calf serum. Cultures were incubated in stationary racks at 37° to 39°C. On the initial passages, medium was changed 24 hours after inoculation to avoid toxic effects. A minimum of five blind passages was made at 5-day intervals before specimens were considered free of virus. For each passage, cultures were frozen and thawed rapidly three times, and 0.2 ml of the pooled material was inoculated into each tube of tissue culture. Cytopathogenic effects frequently were observed on the second passage, but usually complete destruction of the cell sheet was not obtained until fourth or fifth passage.

From 8 June to 29 December 1965, viruses were isolated during the acute phase of disease from 27 of 30 sporadic cases of infectious hepatitis. Age of patients ranged from 2 to 45 years. Viruses also were recovered from all of 12 family contacts of two cases, three of whom subsequently developed infectious hepatitis. These patients and contacts all lived in the vicinity of Phoenix, Arizona, but they were not concentrated in any particular areas.

Because of the regularity with which viruses were isolated from persons with infectious hepatitis, efforts were made to determine the prevalence of virus among people without signs or symptoms of infectious hepatitis. Blood specimens were obtained between 3 December and 29 December 1965 from 70 persons with no known contact with infectious hepatitis. These persons were matched with the age of patients as closely as possible. Examination of the specimens as described above resulted in one isolation of virus. During the same interval, viruses were isolated from all of the five sporadic cases available for study.

The viruses isolated from cases or contacts and the single virus isolated from the control group produced adenovirus type cytopathology and appeared to be adenovirus type 5 on the basis of serum neutralization tests made in human embryonic lung tissue culture. For these tests, 100 $TCID_{50}$ (tissue culture infective dose, 50 percent effective) was neutralized with dilutions of commercially prepared type-specific antiserums containing at least ten antibody units.

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4 April 1966

Peroxidation of Liver Lipids in the Pathogenesis of the Ethanol-Induced Fatty Liver

Abstract. *Administration of an acutely intoxicating dose of ethanol produced significant increases in the concentration of liver triglyceride and enhanced the peroxidation of liver lipids in rats. Adipose triglyceride and lipid peroxide concentrations were unaltered. Coenzyme Q_4, an effective antioxidant, significantly inhibited accumulation of liver triglyceride following ethanol intoxication and prevented the peroxidation of liver lipids. These results, which demonstrate the selective ability of ethanol to induce peroxidation of liver lipids, together with the effectiveness of antioxidants, support the previously proposed hypothesis that peroxidation of liver lipids following ethanol intoxication is a factor in the pathogenesis of ethanol-induced liver injury.*

Previous studies have demonstrated the development of actute fatty infiltration of the liver, caused by an accumulation of triglyceride following oral administration of a single intoxicating dose of ethanol (*1–3*) or alcoholic beverages (*3*) to normal rats. Administration of an antioxidant prior to, or simultaneous with, ethanol was associated with inhibition of acute fatty liver induced by ethanol (*4–5*). Hypertriglyceridemia induced by simultaneous administration of ethanol and triglyceride was prevented by antioxidants (*4*); and accumulation of triglyceride in liver, as well as necrosis and mortality, following a lethal dose of carbon tetrachloride was also inhibited by either the intraperitoneal, oral, or intravenous administration of antioxidants (*5–7*). Results of these studies suggested the hypothesis that antioxidants protected ethanol-treated and carbon tetrachloride–exposed rats by inhibiting the formation of lipid peroxides, lipohydroperoxides, or other complexes (*4–7*).

To test this hypothesis, total lipids, triglycerides, and peroxide concentrations of liver and adipose tissue were measured following administration of an acutely intoxicating dose of ethanol.