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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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.

Since the intravenous administration of 6-chromanol of hexahydrocoenzyme Q_4, an effective antioxidant, both before and after carbon tetrachloride intoxication, markedly inhibited carbon tetrachloride–induced fatty infiltration and mortality (*7*), the influence of coenzyme Q_4 on concentrations of liver triglyceride and liver lipid peroxide in both control and ethanol-treated rats was determined.

Male or female rats, previously maintained on a Purina laboratory chow diet, were fasted for 8 hours before receiving, by oral intubation, a single dose of ethanol (0.6 g/100 g of body weight as a 50 percent solution, weight to volume). Control rats received an equivalent volume of either saline or glucose which was isocaloric to the administered ethanol.

Coenzyme Q_4 (50 mg/100 g), in the form of a specially prepared glycerol-phospholipid emulsion (*7*), was administered 24 and 0 hours before the oral intubation of ethanol. Control rats received a glycerol-phospholipid-corn oil emulsion which was also prepared by using high-frequency sound with 5 percent glucose to give, as in the case of the coenzyme Q_4 preparation, an emulsion with a mean particle size of approximately 2 μ. Both emulsions were administered intravenously in a volume of 0.5 ml/100 g. Twenty-four hours after oral intubation, the rats were anesthetized with ether, livers and adipose tissue were removed, and lipids were extracted and purified (*8*). Peroxides were determined iodimetrically on the purified lipid extracts by a modified Wheeler procedure (*9*).

During the peroxide determinations lipid extract samples were flushed with oxygen-free nitrogen to prevent the possible artifactual induction of lipid peroxides (*10*). All experimental and control samples were determined simultaneously. Because of the observations of Woodford *et al.* (*10*), the livers and adipose tissue were removed, weighed, and placed in distilled chloroform as rapidly as possible to avoid exposure to air. Samples were minced under chloroform prior to chloroform-methanol extraction to further avoid possible changes induced by exposure to air during preparation of tissue.

Liver triglycerides were determined (*11*) and total lipids were measured gravimetrically. All analyses were conducted in duplicate, and the data were analyzed by use of the *t*-test for difference between means, with a 95 percent confidence limit.

In agreement with previous observations (*2*), a significant increase occurred in the weight of the liver in ethanol-treated rats (Table 1). This increase was not associated with any change in body weight, as initial and final body weights were comparable in all groups.

In comparison with either saline- or glucose-treated rats, the rats that received ethanol alone manifested a significant increase in both liver triglyceride and peroxide concentrations (Table 1). The total liver content of lipoperoxides increased from 5.6 and 7.0 microequivalents (μeq) in the saline- and glucose-treated rats, respectively, to 16.2 μeq in the ethanol-treated group, a mean increase of 150 percent.

Glucose-treated rats that received coenzyme Q_4 had concentrations of liver triglyceride and peroxide comparable to those in the saline-treated group (Table 2). Ethanol administration increased concentration of liver triglyceride by 80 percent and induced a mean increase of 73 percent in lipid peroxide concentrations. Administration of coenzyme Q_4 to animals that received ethanol significantly inhibited the ethanol-induced increase in the concentration of liver triglyceride and completely prevented the previously observed increment in lipid peroxides.

The concentration of peroxide in adipose lipids was significantly greater than that in livers of groups treated with saline, glucose plus Q_4, or ethanol plus Q_4. However, in contrast to the increment in liver lipid peroxides in the ethanol-treated group, the adipose peroxide concentration was not altered from control values after administration of ethanol.

The data demonstrate that administration of ethanol not only induces accumulation of triglyceride in liver, but also enhances peroxidation of liver lipids in vivo. Coenzyme Q_4, which had been demonstrated to inhibit accumulation of liver triglycerides and to protect rats from lethal exposure to carbon tetrachloride (*7*), significantly inhibited both accumulation of triglycerides and peroxidation of liver lipids. The finding that adipose lipids from ethanol-treated rats showed no alteration in concentration of peroxide while liver lipids manifested enhanced peroxidation indicates the specificity of the process; it also denotes that observed changes in liver lipid peroxides were not of an artifactual nature. Specificity is noted in the effectiveness of coenzyme Q_4 to inhibit the fatty infiltration induced by ethanol and carbon tetrachloride, but not the fatty liver induced by ethionine (*7*).

Table 1. Influence of administration of ethanol to male rats on concentration of liver triglyceride and peroxidation of liver lipids. Values are expressed as means ± standard errors.

Rats (No.)	Liver weight (g)	Liver triglyceride (mg/g)	Peroxide (μeq per gram of lipid)
	Saline		
8	7.4 ± 0.2	8.3 ± 0.8	15.8 ± 2.1
	Glucose		
5	7.0 ± 0.2	10.4 ± 1.8	19.0 ± 4.6
	Ethanol		
8	8.6 ± 0.4	17.6 ± 2.0	30.4 ± 2.8

Waravdekar *et al.* (*12*) have demonstrated that peroxides obtained by oxidation of highly unsaturated fatty acids by ultraviolet radiation have toxic effects on liver cells. These studies further stress the importance of ethanol-induced in vivo peroxidation as a potential factor in the pathogenesis of hepatic injury.

Additional studies have demonstrated that ferrous ion–induced peroxidation of lipids leads to swelling, lysis, and disintegration of isolated rat liver mitochondria, with loss of proteins and lipids (*13*), but these effects are abolished by antioxidants. It is significant that swelling and fusion of mitochondria, with degeneration and dissolution, occurred in vivo in ethanol-treated rats (*14*) under experimental conditions similar to those we employed. Mitochondrial changes were also associated with alterations in the endoplasmic reticulum of hepatocytes in rats that were given an acutely intoxicating dose of

Table 2. Influence of intravenous administration of coenzyme Q_4 on the concentration of liver lipid and peroxidation of lipids in ethanol-treated female rats. Values are expressed as means ± standard errors.

Rats (No.)	Liver triglyceride (mg/g)	Peroxide (μeq per gram of liver lipid)	Peroxide (μeq per gram of adipose lipid)
	Saline		
6	16.0 ± 1.9	33.6 ± 2.1	55.3 ± 9.7
	Glucose + Q_4		
6	12.2 ± 2.5	27.9 ± 3.3	60.8 ± 8.6
	Ethanol		
6	35.8 ± 4.3	58.0 ± 1.7	57.6 ± 9.7
	Ethanol + Q_4		
5	20.7 ± 2.9	23.7 ± 2.6	65.5 ± 10.2

ethanol, and both structural alterations were prevented by administration of antioxidant. Since mitochondria and endoplasmic reticulum are rich in unsaturated fatty acids and since they have lipid peroxidation catalysts, they are prime sites for peroxidation of lipids (*15*). Damage to enzymes and proteins by free radical intermediates of lipid peroxidation (*15*) can also be a significant deteriorative reaction in ethanol-induced liver injury.

The present studies indicate that enhanced peroxidation of liver lipids after administration of ethanol is a factor in the pathogenesis of liver injury. The observed protective action of antioxidants is probably due to inhibition of free-radical formation, with the resulting prevention of lipid peroxidation damage to membranous organelles, as well as possible prevention of reactions of peroxidized lipid with protein (*15*).

Our study denotes that antioxidant capacity or potential of the liver cell may be an important factor in determining the degree and nature of hepatic injury after exposure to certain chemicals, which conceivably stress antioxidant balance. Our results and the results of others (*4–7*) suggest an intimate relation between liver injury induced by ethanol and lipid peroxidation. This finding, in conjunction with the observations that antioxidants prevent ethanol-induced fatty infiltration, ethanol-lipid–induced hypertriglyceridemia, and carbon tetrachloride–induced fatty infiltration, necrosis, and lethality (*5–7*), indicate the potential importance of lipid peroxidation in pathogenesis of liver injury from certain chemicals. These findings contribute not only to a new concept of liver injury but also to the further consideration of antioxidants in the prevention (*4–6*) and treatment (*7*) of experimentally induced liver injury.

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Visual Acuity in a Stumptail Macaque

Abstract. *Visual acuity in a normal stumptail macaque is 1.4 minutes of arc—similar to man's. Destruction of the fovea by photocoagulation decreased acuity to 9 minutes of arc. These facts suggest that the fovea in the macaque has the same physiological role in visual acuity as in man.*

The inferred function of the fovea in primates other than man has been based primarily on analysis of anatomical similarities (*1*). In man the average visual acuity is approximately 1 minute of arc (20/20) under a wide range of photopic lighting conditions (*2*). When the fovea is not functional, acuity declines to 10 minutes of arc (20/200) (*3*). In the tree shrew (*4*), the basis for visual acuity does not appear to change noticeably throughout large portions of the retina. Our earlier work (*5*) on the pigeon eye showed similar results, in that massive lesions in the foveal region did not produce decrements in visual performance. In these animals at least, the different areas of the retina and the fovea do not seem to function in the same way as in man.

Various workers (*6*) assessed the roles of the various elements of the monkey's visual system. Although they agreed on a maximal visual acuity of approximately 1 minute of arc, they did not directly evaluate the function of the fovea. Jacobson *et al.* were unable to find changes in the electroretinogram after macular destruction in a series of monkey eyes (*7*). We have attempted to measure visual acuity in a monkey eye before and after foveal destruction.

A stumptail macaque, *Macaca speciosa,* was housed in a cage (0.9 by 1.2 by 2.1 m) that also served as the test chamber. The response panel contained a peephole through which the monkey could see the visual-acuity target, a white Landolt C on a black background; luminance of the target was determined with a Macbeth photometer.

The monkey was required to press a lever located below the peephole to turn on the light source that transilluminated the Landolt C. The light was programmed to come on after a variable number of lever presses, the average number being 15. The gap in the C faced either left or right, its direction being programmed in accordance with a modified random series (*8*). Once the target was illuminated, the monkey was required to choose a lever indicating the direction of the opening in the C; if he pressed the appropriate lever six times, a correct choice was recorded; if he responded on the inappropriate lever four times, an incorrect choice. A food pellet was delivered for every second correct choice. On every correct choice a buzzer sounded for 2 seconds. Both the size and distance of the Landolt C were varied to obtain as wide a range of visual angles as possible.

A ruby-laser photocoagulater was used to destroy the fovea (*9*). The collimated laser beam was focused on the fovea by the cornea and lens of the eye; neither cornea nor lens absorbed sufficient energy from the unfocused beam to injure them. In each eye a 1-mm lesion was placed in the center of the macula (see cover). Postmortem histological examination showed that the fovea in each eye had been destroyed, while the retina and other tissues appeared normal (*10*).

Data collected prior to any retinal

Table 1. Behavioral data before (intact) and after (destroyed) binocular destruction of monkey foveas. Criterion for acuity threshold was 70-percent correct.

Intact			Destroyed		
Visual angle (min)	Correct (%)	S.E.	Visual angle (min)	Correct (%)	S.E.
1.2	56	5.0	2.9	53	2.3
1.4	74	6.2	5.7	56	2.6
1.9	76	4.3	6.9	56	3.0
2.4	95	2.4	7.8	64	2.1
3.4	93	2.6	9.0	72	3.3
			13.8	90	1.9