Table 1. Activity of serum creatine phosphokinase in three subjects after 1, 2, 3, and 4 weeks of ethanol administration. Normal activity, < 4.5 units.

Subject	Activity				
	Control	1 wk	2 wk	3 wk	4 wk
J.C.	1.5	2.0	2.0	6.8	11.8
F.B.	0.9	1.0	4.6	5.8	5.8
D.M.	1.8	1.9	2.2	5.4	5.6

was given as a 15 percent solution in fruit juices every 3 hours.

This is an amount of ethanol commonly consumed daily by chronic alcoholics. We have previously demonstrated that the fatty liver and increased serum transaminase activity produced by this amount of alcohol is rapidly reversible upon discontinuation of alcohol ingestion. At no time did the subjects display evidence of gross inebriation, such as ataxia or slurred speech. The diet was supplemented with generous amounts of a multivitamin preparation, minerals, and folic acid, as previously described (*10*). The activity of serum creatine phosphokinase was determined weekly by the method of Siegel and Cohen (*11*). Surgical biopsy specimens were obtained at various times from the deltoid muscle in two subjects and from the gastrocnemius muscle in one (Table 1). Ethanol was withheld for 12 to 18 hours prior to the biopsy. The muscle was resected after maintenance of isometric tension in a specially designed double clamp, fixed in 4 percent, cacodylate-buffered formaldehyde, post-fixed in 1 percent OsO_4, and processed for light and electron microscopy (*12*). The ultrastructural features were compared with those of a large number of normal muscle biopsy specimens obtained in other studies.

In all subjects, the activity of serum creatine phosphokinase rose during the period of ethanol administration (Table 1), suggesting that damage to muscle had occurred. The activity of the enzyme had returned to normal in specimens obtained 2 weeks after cessation of ethanol consumption. Light microscopy showed no abnormalities in any of the biopsy specimens. By contrast, striking deviations from normal were observed by electron microscopy in all specimens obtained after 28 days of ethanol ingestion; changes were more severe in subjects J.C. and F.B. (Figs. 1–3). The muscles displayed pronounced intracellular edema. The interfibrillar spaces were widened and contained glycogen, lipid droplets, deranged elements of sarcoplasmic reticulum, and irregular mitochondria, many of which were enlarged. Glycogen was increased in the I band between actin filaments. The sarcoplasmic reticulum was generally dilated and vesicular and often contained small osmiophilic particles. The basic sarcomere architecture was, however, not altered. In subject F.B., the specimen taken 6 months after ethanol intake appeared normal. In subject D. M., the control biopsy specimen, obtained before ethanol administration, appeared normal.

The results of our study indicate that chronic ingestion of ethanol for 28 days, independent of nutritional or other factors, leads to increased serum creatine phosphokinase activity and striking ultrastructural changes in skeletal muscle. The conspicuous separation of intracellular organelles we observed has been noted in rats fed ethanol in drinking water (*4*), and may be the earliest change which occurs. The dilatation of the sarcoplasmic reticulum, mitochondrial enlargement, and increased fat are similar to ultrastructural lesions produced in the liver of rats (*13*) and human volunteers (*10, 14*) by chronic administration of ethanol. Clinical evidence of muscular disease is noted only in a minority of chronic alcoholics. By the same token, the increase in serum creatine phosphokinase activity after controlled ethanol administration appears to be variable, since in another study a number of alcoholic subjects did not display increased activity of this enzyme after 2 to 3 weeks of ethanol consumption (*15*).

Injurious effects of ethanol in an organ such as the liver may be caused by changes induced by the metabolism of ethanol (*16*) or by a direct action of ethanol itself (*17*). In view of the fact that no appreciable metabolism of ethanol occurs in muscle, the muscular damage associated with chronic ethanol intake may be a direct effect of this compound. Alternately, ethanol may injure muscle by interfering with carbohydrate metabolism in that tissue (*4*). Since cardiac muscle shares many characteristics with skeletal muscle, the effects demonstrated in skeletal muscle may also be present in the heart and may play a role in alcoholic cardiomyopathy.

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Ethyl Mercury p-Toluene Sulfonanilide: Lethal and Reproductive Effects on Pheasants

Abstract. *Ethyl mercury* p-*toluene sulfonanilide (active ingredient of Ceresan M) at a dietary concentration of 30 parts per million (12.5 parts of mercury per million) was lethal to adult ring-necked pheasants. Egg production and survival of third-week embryos were sharply reduced when breeders were maintained on feed containing 10 parts of this compound per million (4.2 parts of mercury per million).*

The recent discovery of mercury pollution in many American and Canadian ecosystems, as in Sweden and Japan a decade earlier, has precipitated a need to evaluate the potential hazard to wild animals as well as to man. Industrial

discharge of mercury and widespread use of mercurial fungicides in agriculture have led to high residues in fish and in various birds and their eggs. In 1969 the hunting seasons on ring-necked pheasant (*Phasianus colchicus*) and Hungarian partridge (*Perdix perdix*) were closed in Alberta, Canada, because mercury levels exceeded the maximum approved for human consumption (*1*). Levels of mercury found in eggs of wild mallard (*Anas platyrhynchos*), pied-billed grebe (*Podilymbus podiceps*), and the common tern (*Sterna hirundo*) (*2*) were within the range found in eggs of experimental ring-necked pheasant that experienced reproductive impairment when fed grain treated with methyl mercury dicyandiamide (*3*).

Controlled studies of the lethal and reproductive effects of mercury compounds on birds are limited. Methyl mercury dicyandiamide has been studied with pheasants (*3*) and chickens (*4, 5*), ethyl mercury *p*-toluene sulfonanilide with chickens (*6*), and ethyl mercury phosphate with pheasants (*7*). Adult ring-necked pheasants maintained on wheat dressed commercially with methylmercuric dicyandiamide [Hg content, 21 parts per million (ppm)] died between 29 and 61 days; egg production remained normal when hens received this diet for 3, 6, or 9 days, and hatchability increased during the 3-day feeding period but decreased when birds were fed for 9 days (*3*). Survival and egg production remained normal when chickens received grain treated with methyl mercuric dicyandiamide (Hg content of grain, 6 ppm) as 75 percent of an 8-week diet (Hg content of total diet, 4.5 ppm) (*4*). However, this compound reduced egg production and hatchability, and induced torticollis and ataxia in some hens when presented on grain (Hg content, 18.4 ppm) composing 25 percent of an 88-day diet (Hg content of total diet, 4.6 ppm) (*5*). Six-week-old White Leghorns given encapsulated ethyl mercury *p*-toluene sulfonanilide at 5, 10, or 20 mg/kg per day (Hg equivalent, 2, 4, or 8 mg/kg per day) died within an average of 28, 11, or 12 days (*6*). No apparent effect on adult mortality and egg production resulted 9 months after pheasants received a 13-day diet of sprouting corn treated with ethyl mercury phosphate before planting (*7*). Corn was treated at the manufacturer's "recommended rate," but mercury content of the diet was not reported.

We report here the lethal and reproductive effects of dietary ethyl mercury *p*-toluene sulfonanilide on ring-necked pheasants. The chemical was obtained as the active ingredient in the commercial fungicide Ceresan M (*8*). We first measured the dietary lethality to adult pheasants (December 1968). Birds were kept out of doors in wire-floored pens. Attached wooden enclosures provided shelter and housed food and water, which were constantly available. Toxicant in the form of Ceresan M was added with corn oil to turkey breeder-mash and blended with a commercial food mixer. Toxicant plus oil (oil only in control diets) constituted 1 percent of the diet. Oil was added to improve toxicant dispersion and adherence to feed.

Survival of pheasants fed diets containing 10, 30, 90, or 270 ppm of the ethyl mercury compound (Hg equivalent, 4.2, 12.5, 37.4, or 112 ppm) was compared with survival of birds fed control diet. We sought at least one concentration that would be sublethal when administered continuously. Treatments were randomized among five pens; ten (five hens and five cocks) 7-month-old birds were randomly assigned to each pen.

All birds fed diets containing the compound at 30, 90, or 270 ppm died. Losses of birds fed diets with the compound at 10 ppm were comparable to those of controls. Median survival times were: 270 ppm, 15 days (range 7 to 19); 90 ppm, 28 days (range 25 to 33);

Table 1. Reproductive success among penned ring-necked pheasants maintained from the first through the second laying season on feed containing 10 parts* of ethyl mercury *p*-toluene sulfonanilide† per million (mercury equivalent, 4.2 ppm).

Parameter	Year	Laying period and treatment					
		First 4 weeks		Second 4 weeks		Seasonal summary (8 weeks)	
		Control	Mercurial (10 ppm)	Control	Mercurial (10 ppm)	Control	Mercurial (10 ppm)
Pens of birds	1969	4	4	4	4	4	4
	1970	4	4	4	4	4	4
Hen-periods (4 weeks each)	1969	23.6	17.7	23.0	15.0	46.6	32.7
	1970	19.1	14.0	18.0	14.0	37.1	28.0
Total eggs laid	1969	525	61	564	191	1089	252
	1970	390	73	332	66	722	139
Eggs laid per 4-week hen-period	1969	22.3	3.4‡	24.5	12.7‡	23.4	7.7‡
	1970	20.4	5.2‡	18.4	4.7‡	19.5	5.0‡
Eggs cracked of eggs laid (%)	1969	2.4	3.8	7.8	5.9	5.2	5.4
	1970	6.5	11.5	5.4	22.3	6.0	16.6
Eggs embryonated of eggs set (%)	1969	76	61	74	64	75	63
	1970	75	47	73	55	74	51
Live 3-week embryos of embryonated eggs (%)	1969	93	64‡	98	81§	95	77‡
	1970	89	47‡	97	48‡	93	47‡
Normal hatchlings of live 3-week embryos (%)	1969	65	45	87	84	76	76
	1970	78	44	80	50	79	47
14-day survivors of normal hatchlings (%)	1969	99.6	100	98.6	98.7	99	99
	1970	98.0	88	97	100	98	94
14-day survivors of embryonated eggs (%)	1969	60	27	84	65	72	56
	1970	68	12‡	74	20‡	71	16‡
14-day survivors per 4-week hen-period	1969	9.2	0.6‡	12.9	4.7§	11.0	2.4‡
	1970	9.0	0.4‡	8.2	0.4‡	8.6	0.4‡

* Treatment was started 52 days before the onset of laying; the initial rate of 25 ppm was reduced to 20 ppm after 22 days and to 10 ppm 40 days later. † Active ingredient in Ceresan M. ‡ Difference from control highly significant ($P \leq .01$). § Difference from control significant ($P \leq .05$).

Table 2. Measurements of shell thickness and mercury residues among eggs of ring-necked pheasants maintained on feed containing ethyl mercury *p*-toluene sulfonanilide at 10 ppm (mercury equivalent, 4.2 ppm).

Parameter	Year	Control	Mercurial (10 ppm)
Number of eggs	1969	16	10
	1970	15	4
Mean shell thickness (mm)	1969	0.316	0.297
	1970	0.297	0.275
Range in shell thickness (mm)	1969	0.27 to 0.37	0.25 to 0.34
	1970	0.27 to 0.33	0.26 to 0.29
Mean mercury content (ppm, wet weight)	1969	<0.1*	1.64
	1970		1.15
Range in mercury content (ppm, wet weight)	1969		1.1 to 3.1
	1970		0.9 to 1.4

* Four eggs pooled, two from each year.

and 30 ppm, 70 days (range 57 to 102). Prior to death, birds showed signs of neurological disturbance similar to those described for chickens (*5*), including ataxia and torticollis.

Necropsies were performed on four pheasants fed the ethyl mercury compound at 270 ppm in the diet, on six fed 90 ppm, on six fed 30 ppm, and on four fed 10 ppm (killed after 350 days). Pheasants fed 270 ppm tended to be in good flesh and had substantial deposits of subcutaneous, abdominal, and coronary fat. The most striking lesion was subcutaneous edema, which was particularly evident along the sternal keel. In some birds there was also a marked intermandibular edema which gave a swollen appearance to the entire head. Birds on diets containing the compound at 90 or 30 ppm showed variable amounts of subcutaneous edema and decreasing amounts of subcutaneous and abdominal adipose tissue as survival time increased. Birds fed 10 ppm were normal in appearance.

The study of reproductive effects of dietary ethyl mercury *p*-toluene sulfonanilide during two breeding seasons began 5 February 1969 and continued through the 1970 breeding season. Facilities and procedures were similar to those of the lethality study. Six hens and one cock (first-year birds) were randomly assigned to each of eight pens. Birds in four pens received the compound at 25 ppm (10.4 parts of Hg per million of diet) until 27 February 1969, when it was reduced to 20 ppm (8.3 parts of Hg per million) because the lethality study indicated that birds could not survive 25 ppm indefinitely. The level was finally reduced to 10 ppm (4.2 parts of Hg per million) on 8 April 1969 because of mortality among hens. Birds in the other four pens received untreated diet.

Within the first 70 days of the experiment (22 days of 25 ppm followed by 40 days of 20 ppm and 8 days of 10 ppm of the mercurial), ten of 24 dosed hens died compared to one of 24 control hens. There were no further losses of dosed birds, and mortality among control hens was routine (five deaths dispersed throughout the remaining 14 months of study). There were no deaths among cocks.

Eggs were collected daily for 8 weeks each season, beginning 29 March 1969 and 11 April 1970. Eggs were counted and stored at 16°C and 55 percent relative humidity. Eggs were examined for cracked shells, and incubated in 2-week groups; they were candled at 2 and 3 weeks of incubation to determine embryonation and embryo viability. Hatchlings were identified with parental pen and were fed untreated feed for 14 days to measure survival.

Records for 2-week periods were combined to form two 4-week periods each season. Rates of egg production, cracking, embryonation, embryo viability, hatching success, and chick survival were evaluated by weighted analyses of variance by using numbers of eggs or birds per pen as weights (*9*). Angular transformation was applied to proportions.

Data on reproductive success are shown in Table 1. Egg production by treated birds was significantly ($P < .01$) reduced both years, ranging from 50 to 80 percent below the controls in 1969 and remaining 75 percent below controls in 1970. Although the percentage of eggs with cracked shells was numerically slightly higher among treated birds, and the rate of embryonation was slightly lower, neither difference was statistically significant ($P > .05$); however, embryo survival during the third week of incubation was significantly ($P < .01$) reduced. Survival among control embryos averaged about 95 percent both years, whereas among treatment embryos it averaged 75 percent the first year and 48 percent the second year. The other parameters investigated were not measurably affected.

Eggs were sampled both years for shell thickness measurements and mercury residue analysis. Contents were removed and shells were dried in forced air for 16 hours at 27°C. Thickness of shell plus membranes at the waist was measured with a Starrett dial gauge micrometer graduated in 0.01-mm units. Total mercury determinations were made by WARF Institute, Inc. (*10*). Shell thickness measurements and mercury residues are shown in Table 2.

The treatment difference in mean shell thickness was not statistically significant ($P > .05$) in 1969; data for 1970 were not analyzed statistically since only four treatment eggs were available.

Mercury in 14 treatment eggs averaged 1.5 ppm (range, 0.9 to 3.1 ppm) on a wet weight basis. It was less than 0.1 ppm in a pool of four control eggs. Comparable levels of mercury (1.3 to 2.0 ppm), accompanied by a severe reduction in hatchability, were reported in eggs of pheasants fed methyl mercury dicyandiamide in Sweden, where residues in eggs of free-living pheasants varied from 0.3 to 3.0 ppm (*3*).

In summary, as little as 30 parts of ethyl mercury *p*-toluene sulfonanilide per million (12.5 ppm of Hg) in the diet of adult pheasants was generally fatal within 2 to 3 months of feeding. Ten parts of the compound per million (4.2 ppm of Hg) in the diet reduced egg production 50 to 80 percent and increased embryo mortality in the few eggs laid. Mercury residues in 14 eggs were 0.9 to 3.1 ppm, comparable to those in eggs of pheasants fed methyl mercury dicyandiamide (*3*), in which hatchability was also reduced. Since similar residues of mercury have been found in eggs of wild pheasants (*3*) and several species of aquatic birds (*2*), we conclude that mercury pollution may be sufficiently high in some areas to affect avian reproduction.

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Gamma-Aminobutyric Acid Antagonism and Presynaptic Inhibition in the Frog Spinal Cord

Abstract. *The convulsant alkaloid bicuculline blocked presynaptic inhibition, dorsal root potentials, primary afferent depolarization, and depolarizing effects of gamma-aminobutyric acid on dorsal root terminals of the amphibian spinal cord, but did not block effects of other putative amino acid transmitters. These actions of bicuculline suggest that gamma-aminobutyric acid may be the transmitter involved in spinal presynaptic inhibition.*

There has been little agreement as to the identity of the transmitter compound that is released from synapses on primary afferent fiber terminals and is responsible for both the primary afferent depolarization recorded as the dorsal root potential and for the phenomenon of presynaptic inhibition. In 1963, Eccles and his colleagues (*1*) postulated that gamma-aminobutyric acid (GABA) might be this transmitter in view of the evidence that it depolarized dorsal root terminals in the cat. Subsequently, the finding that GABA depolarizes primary afferent terminals has been confirmed (*2, 3*) and has been denied (*4*), and the latter evidence used to deny the possible role of GABA in presynaptic inhibition.

Bicuculline, an alkaloid known to have similar convulsant properties in mammals and amphibians (*5*), has been shown specifically to block the action of GABA at a variety of synaptic sites in the cat central nervous system (*6, 7*), the cat autonomic ganglion (*8*), and the crayfish stretch receptor (*9*). Presynaptic inhibition is probably generated by similar mechanisms in the spinal cord of mammals and amphibians (*10*), because there is a great similarity in the dorsal root potentials and in their pharmacological properties (*2*). There are conflicting reports, however, about the effects of bicuculline on presynaptic inhibition. Levy and co-workers (*11*) showed that this alkaloid blocked primary afferent depolarization in the cat spinal cord, whereas Curtis and co-workers (*6*) were unable to demonstrate any effect on presynaptic inhibition of the spinal monosynaptic reflex in the same species. This study is concerned with the effects of bicuculline and GABA on presynaptic inhibition in the isolated frog spinal cord and adds direct evidence to the hypothesis that GABA is the transmitter involved in spinal presynaptic inhibition.

The isolated frog spinal cord was employed because potential changes at the dorsal root terminals produced by various drugs and transmitters can easily be measured. In addition, this preparation circumvents difficulties seen in intact vertebrates when drugs and transmitters are administered intravascularly.

By the use of conventional methods (*12*), spinal cords and roots were removed from 9-cm-long frogs (*Rana pipiens*), were hemisected sagittally, and were perfused with oxygenated amphibian Ringer solution (*13*) in a temperature-controlled Lucite bath. A flow rate of 2.0 to 4.0 ml/min was used in different experiments. Dorsal and ventral roots 8 and 9 were pulled through slits in thin plastic partitions sealed with Vaseline. For a-c recordings and stimulation, the roots were placed on pairs of Ag-AgCl electrodes (interelectrode distance 12 to 15 mm) and covered with mineral oil to prevent desiccation. For d-c recording, nonpolarizing Ag-AgCl electrodes were used. Contact was made with the roots by means of a cotton wick (*14*). Stable recordings could be obtained from a preparation

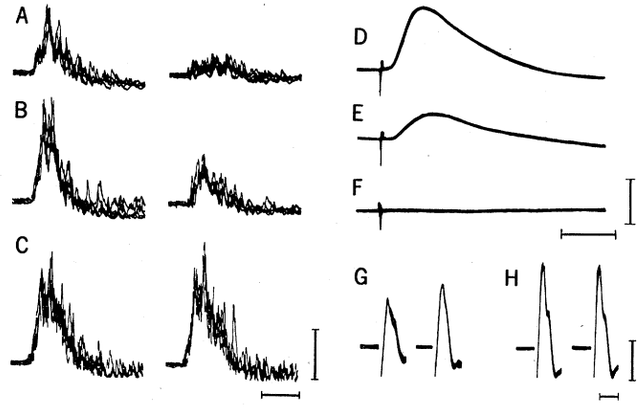

Fig. 1. (A–C) Effect of bicuculline on presynaptic inhibition. (Left) Polysynaptic ventral root reflexes produced by supramaximum stimuli applied to dorsal root. (Right) Inhibition of polysynaptic reflex when stimulation of adjacent ventral root preceded dorsal root stimulation by 50 msec. (A) Before, (B) 4 minutes, and (C) 11 minutes after addition of bicuculline (10 μg/ml) to Ringer solution. Records obtained by superimposing six traces. Bath temperature, 13°C. (D–F) Effects of bicuculline on dorsal root potential. (D) Dorsal root potential evoked by supramaximum stimulation of ventral root. (E) Ten minutes and (F) 20 minutes after bicuculline (10 μg/ml). Dorsal root potential completely abolished. Bath temperature, 15°C. (G–H) Effect of bicuculline on primary afferent depolarization. (G) (Left) Antidromic response recorded from dorsal root produced by constant-current, local stimulation in dorsal horn by a micropipette. (Right) Primary afferent depolarization as manifested by increased excitability of dorsal root terminals produced by stimulation of ventral root 50 msec before. (H) Sixteen minutes after bicuculline (10 μg/ml). Six superimposed traces in each figure. Bath temperature, 15°C. Vertical calibration: (A–H) 1 mv. Horizontal calibration: (A–C) 40 msec; (D–E) 100 msec; (G–H) 1 msec.