days after coitus. Both the hormonal induction of parturition and the examination of newborn for malformations were in the manner of Sawin, Crary, Fox, and Wuest (*1*).

A significant increase in resorption of the fetuses ($P < .01$) was observed when 150 mg of thalidomide was administered 6 to 10 days after coitus, but no increase in the number of abnormal animals was shown by the data either before or after weighting it for normal strain variation (Table 1).

Abnormalities encountered were more varied in the control populations than in the treated ones. Thalidomide caused no significant increase in either type or incidence of anomalies in any of the treated groups ($P > .05$). Minor variations in the treated populations were, with one exception, in sternum and tail. These areas are subject to variations under normal conditions (*7*). The intravenous injection of placebos containing CMC and glucose into control animals to distinguish the possible teratogenic effects of CMC and glucose from those of thalidomide was deemed unnecessary since no real effect was observed in treated animals.

The high resorption rate observed in animals which received intravenous injections of thalidomide is similar to the 22-percent resorption observed in does given thalidomide orally (*1*). However, the high resorption rate may be due to the stress of injection on the doe before and during implantation of the egg, rather than to the presence of thalidomide. That the amount of injected fluid was about equal to the blood volume of the rabbit [about 50 ml/kg of body weight (*8*)] and no increase in malformation was observed supports this supposition. Runner (*9*) reports that mice injected with saline during early pregnancy have an increased rate of resorption.

The failure of thalidomide to cause teratism when it is injected intravenously could be explained if (i) the dosage were too low, (ii) thalidomide were rapidly eliminated from the blood stream so as not to produce malformations, or (iii) if thalidomide were metabolized in the intestinal tract to form a metabolite which is the teratogenic agent.

The first explanation (low dosage) seems unlikely since malformations have been induced in New Zealand White rabbit fetuses by oral doses of 50 mg of thalidomide per kilogram of body weight of the doe (*2*). Meier (*10*) reports, "When thalidomide is administered in an oral dose of 100 mg/kg in the rat, roughly 50 percent is absorbed and in the dog, about 30 percent; the nonabsorbed portion is excreted unchanged in the feces." Data for the rate of absorption in the rabbit are not yet available, but we believe the dosage was sufficient to have induced malformations comparable to those in our previous study (*1*) in which powdered thalidomide was given orally.

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Clostridium botulinum Type F: Isolation from Salmon from the Columbia River

Abstract. Clostridium botulinum *type F has been isolated from a salmon* (Oncorhynchus nerka) *taken from the Columbia River. Cultures of this type have been reported only twice before—once the toxin was identified and once the bacterium was isolated.*

During a survey of the natural distribution of *Clostridium botulinum* type E in the Pacific Northwest, one of the samples taken from a sockeye salmon (*Oncorhynchus nerka*) produced a mixed culture that contained *Cl. botulinum* type F toxin; a pure culture of the organism was isolated and its identity was verified. *Clostridium botulinum* type F has been reported only twice before —once the bacterium was isolated and once the toxin was identified. The organism was first reported and isolated from liver paste that was associated with human botulism on the Danish island of Langeland (*1*). One person died, and three others showed typical symptoms of the disease. The toxin of type F was demonstrated in cultures from two samples of marine sediments taken off the coasts of Oregon and California (*2*).

The salmon sample that yielded cultures of *Cl. botulinum* type F was collected approximately 32 km upstream from the mouth of the Columbia River. Gills and viscera were removed aseptically from the fish and placed in a plastic bag with 70 to 100 ml of Schmidt's trypticase, peptone, glucose (TPG) medium. As much air as possible was expelled from the bag, and it was closed and held with a rubber band. The sample was incubated for 5 days at 28°C. A portion of the fluid from the bag was centrifuged at 2500 rev/

Table 1. Neutralization pattern of cultures identified as *Clostridium botulinum* type F. Results are given as the number of mice that died out of the number tested. S is filtered supernatant; HS, heated supernatant (100° for 10 minutes).

Source of toxin	Antitoxin	Results
S	None	16/16
S	ABE and tetanus	6/6
S	ABE	4/4
S	Tetanus	2/2
S	A	4/4
S	B	4/4
S	C	4/4
S	D	4/4
S	E	12/12
S	F	0/4
HS	None	0/2
S*	ABCDE and tetanus	4/4

* Supernatant was mixed with the antitoxins in vitro and then injected into mice.

min for 30 minutes to remove the large particulate material, and 2 ml of the supernatant was filtered through a membrane filter that had a pore diameter of 0.2 μ. The sterile filtrate was activated with 1 percent trypsin according to the method of Duff *et al.* (*3*). One-half milliliter of 1 : 10 and 1 : 100 dilutions in gelatin phosphate buffer, *p*H 6.2, was injected intraperitoneally into each of two mice (Swiss Webster strain). The animals were observed closely for 48 hours for typical symptoms of botulism and for death; survivors were discarded after 4 days.

The toxin was identified by repeating the injections of diluted trypsin-activated filtrate into mice that had been individually protected with antitoxin to *Cl. botulinum*, types A, B, C, D, or E or with tetanus antitoxin. The toxic filtrate was also tested by mixing it with a combination of the five botulinum antitoxins in vitro, as well as with these five antitoxins plus tetanus antitoxin. Four mice were injected with the latter combination. The heat-labile character of the toxin was demonstrated by heating a portion of the culture filtrate to 100°C for 10 minutes and then injecting 0.5 ml of a 1 : 10 dilution of this filtrate intraperitoneally into mice. The results of tests for protection by antitoxins are shown in Table 1. Neutralization of the toxin was achieved only by type F antitoxin.

After typing the toxin, the original mixed culture was treated with alcohol as described by Johnson, Harmon, and Kautter (*4*) and then streaked on plates of liver-veal agar with egg yolk and on plates of agar containing 5 percent of blood. All plates were incubated in an anaerobic jar, and botulinum-type colonies were transferred to TPG medium; these were incubated for 5 days, and the toxin that was produced was typed by the method given above.

Animals protected with *Cl. botulinum* type F antitoxin were the only ones that survived. The titer of type F antitoxin was lower than that of the other types, which necessitated the use of 1:200 and 1:500 dilutions of filtrate, but these dilutions killed all mice except those protected by type F antitoxin. Identity of the culture has been confirmed by repetition of the typing procedure.

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Myotonic Response Induced by Inhibitors of Cholesterol Biosynthesis

Abstract. *Steroid inhibitors of cholesterogenesis containing nitrogen-substituted side chains induced electromyographic myotonia in rats. Cholesterol reduction or desmosterol accumulation, per se, did not cause myotonia, and cholestrol feeding prevented drug-induced myotonia. Desmosterol accumulation in combination with a specific drug effect may cause the observed myotonia.*

True myotonia is characterized by an abnormally delayed relaxation of skeletal muscle fibers which follows voluntary or induced contraction and is associated with repetitive depolarization of the muscle-cell membrane. Such a contraction, as recorded by an electromyograph (EMG), initiates a burst of action potentials of increased amplitude and high frequency which decreases over a period of seconds and is audible as a "dive-bomber" sound. The myotonic phenomenon has been observed in man (*1, 2*) and in the goat (*3*) after administration of 20,25-diazacholesterol, an inhibitor of cholesterol synthesis. Analyses of the sterol composition of plasma, erythrocyte stroma, and muscle showed a decrease in cholesterol and the appearance of its immediate precursor, desmosterol.

We now report the induction of EMG myotonia in rats by several cholesterol analogs and an androstene derivative and describe studies undertaken to determine the properties required for such agents to induce myotonia.

The agents (Figs. 1 and 2) were administered by daily subcutaneous injection to 20-day-old white, male Wistar rats, maintained on either a standard Purina rat diet or a 2 percent cholesterol diet. Electromyography (TECA electromyograph, Model B) was performed at weekly intervals with monopolar electrodes inserted into the thigh muscles of anesthetized rats. Animals were killed when definite myo-

Table 1. Electromyograph myotonia and mean concentrations of plasma sterol in rats. The number of animals treated is shown within the parentheses.

Agent	Treatment (wk)	Dose (mg kg^{-1} day^{-1})	EMG myotonia*	Plasma (mg/100 ml ± S.E.)	
				Cholesterol	Desmosterol
Control† (6)	6		0	64.6 ± 7.4	0.0
25-azacholesterol (4)	2	0.1	+	22.6 ± 4.9	28.2 ± 4.9
24-azacholesterol (2)	3	5	+		
20-azacholesterol (6)	6	10	0	59.0 ± 1.5	0.0
22, 25-diazacholestanol (5)	3	30	+	7.3 ± 2.1	43.9 ± 4.5
20, 25-diazacholesterol (5)	3	10	+	14.4 ± 2.4	40.7 ± 3.9
SC-13820 (3)	4	20	+	19.3 ± 1.6	24.3 ± 1.6
SC-12998 (3)	2	10	+	10.0 ± 2.8	37.8 ± 12.3
3β-(β-diethylaminoethoxy)-androst-5-en-17-one-methoxime (5)	4	15	+	3.7 ± 0.9	54.4 ± 11.3
Triparanol (6)	6	100	0	12.6 ± 2.8	29.5 ± 3.5
W-398 (3)	6	20	0	45.5 ± 2.5	0.0
W-398 + 20, 25-diazacholesterol (2)	6	20	+	13.6 ± 0.9	38.2 ± 2.2
2% cholesterol diet + 20, 25-diazacholesterol (6)	6	10	0	83.0 ± 2.8	6.7 ± 1.6
2% cholesterol diet + 20,25-diazacholesterol (2)	6	100	0	76.1 ± 6.3	2.8 ± 0.4

* Plus sign indicates presence of myotonia; zero indicates absence of myotonia. † Twenty percent methyl cellulose was used in place of the sterol.