

thalamus following the parenteral administration of the NE precursor, 3,4-dihydroxyphenylalanine (DOPA), is very close to the distribution reported here (20). This suggests that exogenous NE and NE formed endogenously from DOPA finally localize in the same sites within the hypothalamus despite a widely divergent intermediate pathway. However, the structural and chemical limits of the specificity of localization require further definition.

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Intravenous Injection of Thalidomide in Pregnant Rabbits

Abstract. When administered orally to pregnant strain III rabbits, thalidomide is teratogenic to their fetuses. However, when the drug is injected intravenously to females of the same genetically controlled stock, it has no observable effect. This finding indicates that a metabolite of thalidomide formed in the intestinal tract may be the real teratogenic agent.

Thalidomide fed to pregnant rabbits is teratogenic to their fetuses (1-3). It is not known whether the teratogenic agent is thalidomide itself or one or more of its metabolites, nor is the mechanism by which thalidomide produces its effects known (4). Hydrolysis of thalidomide before oral or intraperitoneal administration markedly reduces its teratogenic effects in strain III (5) and other rabbit stocks (6).

We have studied the effects of intravenous injection of thalidomide into pregnant rabbits because this method facilitates a rapid and direct delivery of the drug to the fetus, and thereby controls the onset of exposure and reduces the amount of drug which must be given to the doe to obtain concentrations in the blood which will be teratogenic to the fetuses. The low solubility of thalidomide prohibits its intravenous injection in large dosages. However, with the inclusion of carboxymethyl cellulose (CMC) and glucose, a supersaturated solution (1 mg of thalidomide per milliliter of solution) which is stable at 40°C for a period long enough to allow intravenous injection at a rate of 5 ml/min can be made.

One hundred milliliters of the solution was prepared by placing 0.5 g CMC (type 120, high viscosity) in a 500-ml Erlenmeyer flask which contained a test tube with circulating cold water. The flask and test tube served as a reflux condenser. Then 100 ml of distilled water, plus 8 percent to com-

pensate for water loss during condensation, were added. The distilled water was boiled slowly for 10 minutes or until the solution became homogenous. The heat was then removed and the condenser was allowed to operate for an additional 10 minutes. Five grams of glucose and 100 mg of thalidomide (fine powder, sieve 200) were added, and the solution was heated to a slight boil until all thalidomide was dissolved (about 20 minutes). The clear solution was immediately filtered to remove any dust or fibers and was cooled to 50°C by placing it in a water bath (50°C). We injected the solution with a 30-ml syringe equipped with a two-way valve, 61 cm of polyethylene tubing (inside diameter, 0.12 cm; outside diameter, 0.16 cm), and a 22-gauge needle. The temperature of the liquid injected into the ear was about 40°C.

Satisfactory intravenous injections of 20, 50, and 150 mg of thalidomide per day to each doe were routinely made for periods varying from two to five consecutive days. Seven does of strain III produced treated litters; two of the seven produced litters at all three dosages. Each doe also produced control litters and was bred to the same male for both her control and treated litters. Controls were offspring of does which had received no intravenous injections during that pregnancy. Control and treated matings were made to avoid bias due to season or parity. All mothers were palpated for pregnancy 10 to 14

Table 1. The effects of intravenous injection of thalidomide on reproduction and on malformation of fetuses. Pregnant rabbits were injected with varying amounts of thalidomide each day for two to five consecutive days, beginning on different days of the gestation period.

Dose given		Pregnancies per matings	Classifiable fetuses (No.)				Weighted abnormal*	Unclassified resorbed fetuses (No.)
Amount (mg/day)	Days of gestation		Total	Viable	Dead	Abnormal		
0		9/12	60	48	12	38	7	3
20	7 and 8	4/9	19	18	1	6	0	0
50	6-9	0/3						
50	6-11	0/2(1R†)						
50	8 and 9	2/5	17	15	2	12	0	1
50	9 and 10	1/2	8	6	2	7	0	0
150	6-8	1/1	7‡	0	0	0	0	1
150	6-9	0/2(1R†)						
150	6-10	4/4	19	15	4	13	1	7

* Those animals having malformations only of skull, sternum, or tail were classified as normal since this is considered to be normal strain variation (1). † Of the two does bred, one was palpated pregnant but she resorbed her complete litter. ‡ Taken at 21 days gestation because the doe had a broken back.

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 ad-

ministered 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.