between the location of the spots detected by iodine and the repellency as determined in the olfactometer. We have not yet identified the repellent substances. The significance of skin lipids in terms of their effect on the behavior of mosquitoes can only be conjectured. Perhaps the attraction of the host to the mosquito depends on a balance between naturally occurring repellents and attractants.

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Clostridium botulinum Type F from Marine Sediments

Abstract. Clostridium botulinum *Type F has been demonstrated in two samples of marine sediments. One sample was taken 83 kilometers off the coast of California; the other, 100 kilometers off the coast of Oregon. Cultures of this type have not been reported previously in the United States, and only once before in the whole world.*

In a survey on the incidence of *Clostridium botulinum* in the coastal areas of Alaska, Washington, Oregon, and California, two samples of marine sediment have yielded cultures of *Cl. botulinum* Type F. The first and only previous culture of *Cl. botulinum* Type F was isolated from a homemade liver paste connected with an outbreak of human botulism on the Danish island Langeland (*1*). One of the five persons who ate the liver paste suffered no harm, but three had severe attacks of botulism, and the fifth person died three days later (*2*). This culture has since been described as a prototype strain designated as *Cl. botulinum* Type F (*3*).

The marine sediments that yielded cultures of *Cl. botulinum* Type F in this laboratory were collected 83 kilometers from the coast of California (sample A) and 100 kilometers from the coast of Oregon (sample B). Sample A came from a depth of 1646 meters at 42°N latitude and sample B from a depth of 1326 meters at 43°N latitude.

Portions (approximately 5 g) of these mud samples were inoculated into 25 ml of broth containing glucose, peptone, trypticase, beef infusion, and ground meat, a modification of Dolman's medium (*4*). The inoculated tubes were incubated anaerobically (95 percent nitrogen and 5 percent carbon dioxide) at 25°C for 5 days. A portion of the broth was then centrifuged at 10,000 rev/min (12,000*g*). The supernatant was tested for toxicity by injecting two mice (Swiss Webster strain) intraperitoneally with 0.4 ml of a 1:2 dilution of the supernatant and gelatin-phosphate buffer. Characteristic symptoms of botulism, if present, occurred within 20 hours after injection.

The toxin was identified by injecting pairs of mice with 0.5 ml of mixtures composed of 0.4 ml of a 1:2 dilution of the supernatant and 0.1 ml of one of the following antitoxins: a polyvalent antitoxin (Types A, B, C, E, and F in equal concentrations); or an individual antitoxin (Types A, B, C, D, E, or F). Heat lability of the toxin was determined by injecting a pair of mice with 0.4 ml of a 1 : 2 dilution of the sample supernatant that had been heated for 10 minutes at 100°C and then cooled. All mice were observed for at least 6 days.

The data supporting the identification of the culture as *Cl. botulinum* Type F are summarized in Table 1. Neutralization of the toxin was achieved in both samples only by the Type F antitoxin and by a polyvalent antitoxin containing Type F. Heating the supernatant for 10 minutes at 100°C inactivated the toxin. The toxin of sample A was not neutralized by Types A, B, C, D, or E antitoxin, since the mice injected with this toxin died within 19 to 23 hours and had characteristic symptoms of botulism. The same results were obtained for sample B with the exception (not shown in Table 1) that Type E antitoxin showed some neutralization of the toxin, but only when the Type E antitoxin was in large excess. Cross neutralization between Type F and Type E was also reported from the Denmark isolate (*1, 3*). The titer of the toxin from both cultures was 6 to 20 minimum lethal doses (mouse) per milliliter of medium.

Table 1. Neutralization reaction pattern of cultures identified as *Clostridium botulinum* Type F. Results are given as number of mice dead out of number tested. S is supernatant; HS, heated supernatant.

Source of toxin	Anti-toxin	Result	
		Sediment sample A	Sediment sample B
S	None	6/6	6/6
S	ABCEF	0/2	0/2
S	A	4/4	4/4
S	B	4/4	4/4
S	C	4/4	4/4
S	D	2/2	2/2
S	E	4/4	4/4
S	F	0/6	0/6
HS	None	0/2	0/2

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Deformity of Forelimb in Rats: Association with High Doses of Acetazolamide

Abstract. *Deformities of the right forelimb occurred in a number of offspring of rats given high doses of the carbonic anhydrase inhibitor, acetazolamide, during pregnancy. In most cases this was the only deformity found. More than 20 times the usual therapeutic dose rate used for humans was required to produce this effect in rats.*

We have found a remarkably specific and reproducible malformation of the right front extremity in a number of offspring of rats given a diet containing large concentrations of acetazolamide during pregnancy. Acetazolamide (2-acetylamino-1,3,4,-thiadiazole-5-sulfonamide) is a potent carbonic anhydrase inhibitor which has been used as a diuretic in human patients since 1953.

Genetically heterogeneous albino rats of the Sherman strain from the Lederle colony were placed in cages as mating pairs and the females were checked daily for the presence of spermatozoa. When evidence of mating was found, the females were placed in individual cages and given a diet consisting of ground Purina Lab Chow containing acetazolamide at concentrations of 0.2 0.4, or 0.6 percent until parturition, when the offspring were examined and the mother returned to a diet of Purina Chow without acetazolamide. Control animals were given Purina Chow without acetazolamide. The young were kept with their mothers until the time of weaning (21 days) when, in most instances, they were killed and fixed, and cleared alizarin-stained preparations were made. Of 187 offspring of mothers which received diets containing either 0.4 or 0.6 percent acetazolamide, 45 showed a deformity characterized by a variable deficiency of elements of the postaxial border of the right forelimb. In the 225 offspring of animals which received a control diet or the diet containing 0.2 percent acetazolamide, no skeletal deformities were found (see Table 1).

The pattern of missing elements of the hand and forearm was quite constant. In its mildest form only digit 5 was missing (Fig. 1*a*). Most frequently there was an absence of digits 4 and 5 with the absence of corresponding metacarpals (Fig. 2). When two or more digits were missing the distal portion of the ulna was often absent, with an accompanying bowing of the radius and shortening of the forearm. The most severe deformity was a complete absence of the ulna and the presence of only digit 1 (Fig. 1*b*). The animal with this degree of deformity was the only one showing involvement of the humerus; the medial and lateral epicondyles were absent. Two weanlings with deformities of the right forelimb also showed a partial duplication of the 5th digit of the left hand. The weanling in which this duplication was most marked was that whose right arm is shown in Fig. 1*b*; its left arm is shown in Fig. 1*c*.

At birth these deformed rats behaved normally and, except for a slight disability, thrived as well as their normal littermates or control animals. In the rat, this deformity did not seem to invite maternal cannibalism to any degree. A few deformed rats were allowed to live for more than 18 months and showed no signs of other congenital anomalies either during life or at autopsy. Deformed rats of both sexes were fertile.

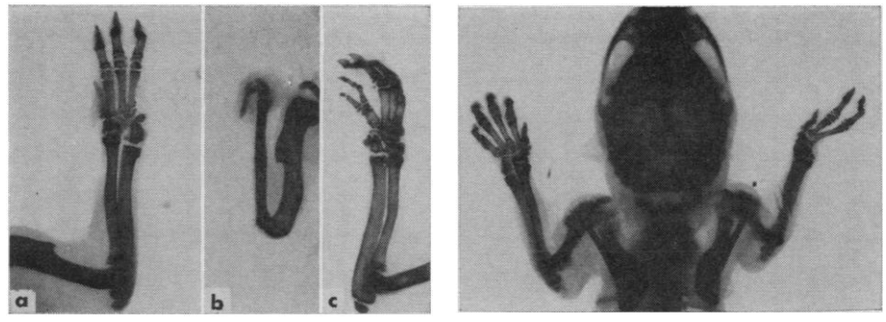

Fig. 1 (left). Offspring of rats given a diet containing acetazolamide (0.6 percent) during pregnancy. (*a*) Dorsal view, right forearm and hand; one digit missing. (*b*) Medial view, right arm; four digits and ulna missing. (*c*) Dorsal view, left hand of animal shown in (*b*); postaxial polydactylism. Fig. 2 (right). Offspring of a rat given a diet containing acetazolamide (0.6 percent) during pregnancy. Digits 4 and 5 missing from right hand.

In our study the deformity was restricted to the right side (except for the two cases of polydactyly). However, in a separate study of 232 Sherman rat fetuses from mothers given a diet containing 0.6 percent acetazolamide, several instances of bilateral forelimb deformity were found. Of the 76 fetuses showing the characteristic right forelimb deformity, nine showed, in addition, a deformity of the left forelimb. Bilateral involvement only occurred when there was a severe deformity of the right forelimb; the deformity on the right side was always more severe than that on the left.

Table 1. The incidence of deformities of the right forelimb in the offspring of rats given acetazolamide during pregnancy.

Acetazolamide		No. of litters	Offspring	
In diet (%)	Consumed* (mg kg^{-1} day^{-1})		Total	Deformed
0	0	26	275	0
0.2	115	5	50	0
0.4	208	7	66	6
0.6	284	6	64	16
0.6	†	8	57	23

* Milligrams of acetazolamide per kilogram of maternal body weight per day—average amount consumed during pregnancy. † Data not recorded.

Preliminary experiments have shown that this same right-sided deformity can be produced in Long-Evans rats or Manor Farms mice if large concentrations of acetazolamide are given to the mothers throughout pregnancy. The dosage required for the Long-Evans rat was the same as that for the Sherman rat (diet containing 0.6 percent of the drug). In the Manor Farms mouse (a genetically heterogeneous albino) a diet containing 0.2 percent acetazolamide (204 mg of drug per kilogram of maternal body weight per day) was needed. Here, because of maternal cannibalism, we have only been able to find the deformity in fetuses. In the mouse the most frequent deformity was the absence of the 5th digit. The similarity of the defect in the rat and mouse can be seen in Fig. 3. In this instance, digits 4 and 5 were missing in both species.

Attempts to produce this deformity in rabbits were fruitless because of the relatively high toxicity of acetazolamide for this species.

Table 2. Effect of administering a diet containing 0.1 percent acetazolamide continuously throughout two successive cycles of pregnancy and lactation.

Group	Matings		Number born			Average litter size	No. weaned (%)*
	Attempted	Successful	Alive	Dead	Deformed		
			First test cycle				
Control	20	17	175	2	0	10.4	94
Treated	18	14	139	0	0	9.9†	98
			Second test cycle				
Control	19	15	135	14	0	9.9	97
Treated	17	15	136	1	0	9.1†	99

* Animals surviving until weaning are expressed as a percentage of those remaining after litters containing more than nine members were reduced to nine 3 days after birth. † Not significantly different from control values at 95-percent confidence limits, as determined with the Rank-sum test (7).

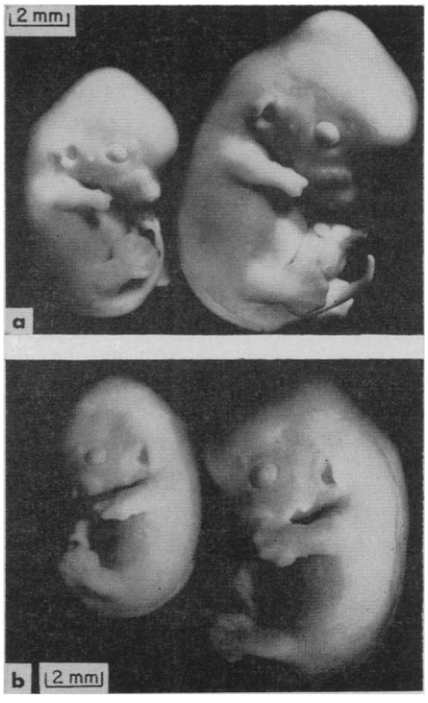

Fig. 3. Fetus of a mouse at 14 days (left) and a rat at 16 days (right) from mothers given acetazolamide during pregnancy. (*a*) Right side, digits 4 and 5 missing from hand. (*b*) Left side, no deformity.

The high dose required to produce teratogenesis in the rat is not well tolerated and results in a reduction in food intake (20 percent in the rats fed on the diet containing 0.6 percent concentrations of the drug), and the usual weight gain during pregnancy is greatly depressed.

In a study designed to evaluate the effect of a high and sustained but tolerated dose of acetazolamide on the reproductive process, male and female rats were fed a diet containing 0.1 percent of the compound. After 6 weeks of being fed on this diet, the rats were mated. Two or three weeks after the pups from the first litter were weaned, the rats were remated and the pups from the second breeding cycle were raised to weaning age, at which time the study was terminated. The diet containing the drug was administered throughout the entire test and the average drug intake varied from 40 to 60 mg per kilogram of body weight per day. Table 2 shows that acetazolamide had no demonstrable effect on male or female fertility or on lactation of pregnant females or on the offspring of treated parents.

Although acetazolamide has been widely used as a diuretic in pregnant humans (*1*), there are no reports of its proven or suspected teratogenicity in this species. This is reasonable if one compares the minimum teratogenic dose for the rat (more than 100 mg kg^{-1} day^{-1}) with the usual human therapeutic dose (5 mg kg^{-1} day^{-1}) and especially if one considers the very steep dose-response relationship (Table 1). This kind of postaxial arm defect is rare in man, and it is not the kind of deformity that can be easily overlooked. It is of interest that in man ulnar defects are reported to be predominantly right-sided (*2*).

The specificity of localization of this defect appears to be unique among either induced or genetically determined deformities. This specificity is manifest not only in a right-sided localization but also in an involvement of only the postaxial border of the forelimb without other skeletal or visceral abnormalities.

There are two similar deformities which should be mentioned. Searle (*3*) described a mutation of the mouse of the "luxate-luxoid" group, "postaxial hemimelia," which affects the postaxial side of the forelimb and sometimes also the hind limb. However, there is always a scapular defect and both sexes are sterile. The right forelimb and the left hindlimb tend to be more severely deformed.

Dagg (*4*) found that 5-fluorouracil given to pregnant mice could result in a number of malformations, including a reduction in the number of toes of the hind feet. The number of toes missing was a function of dose and when one toe was missing it was always the most postaxial (5th) toe. However, when two toes were missing these were digits 1 and 5.

The mechanism by which acetazolamide produces deformity is not clear. It has been postulated that spontaneous postaxial hemimelia is due to a defect in the induction of limb elements by the apical ectodermal ridge (*2*, *5*). Results of preliminary work we have done on rat embryos are consistent with this idea in that the defect is first visible at a stage of development during which the inductive action of the apical ectodermal ridge is taking place [Christie stage 22 (*6*), 12½ days after mating], but we have not made a detailed examination of the limb bud at this stage.

The only pharmacological activity ascribed to acetazolamide has been carbonic anhydrase inhibition, and we are not aware of any postulated role of carbonic anhydrase in limb development. However, it should be kept in mind that the exceedingly large amounts of acetazolamide required may produce other, as yet undiscovered, pharmacological effects.

Acetazolamide should be useful as a tool for the investigation of limb morphogenesis.

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27 May 1965

Thymine Photoproducts but not Thymine Dimers Found in Ultraviolet-Irradiated Bacterial Spores

Abstract. Bacillus megaterium *spores labeled with tritiated thymidine were irradiated with monochromatic ultraviolet light, and the DNA of the spores was analyzed for thymine-containing products. No thymine dimers were observed, but three other thymine photoproducts were found. The unknown products of radiation were produced in vitro by irradiation of DNA that had been dried in the presence of various salts.*

Ultraviolet irradiation of vegetative cells of various organisms and of DNA in solution forms dimers between adjacent thymine residues in DNA strands (*1*). Formation of dimers accounts for much of the failure of ultraviolet-irradiated DNA to prime DNA synthesis (*2*) and for the loss in transforming activity of irradiated *Haemophilus influenzae* DNA (*3*). The lethal effects of ultraviolet light on cells (*4*) has been attributed to the formation of dimers.

The resistance of bacterial spores to the deleterious effects of such agents as ultraviolet light, ionizing radiation, and