glucose-oxidase system (*11*). The occurrence of D-gluconic acid in *E. decipiens* is another example of a well-known substance that occurs in unusually high concentration in an arthropod defensive secretion.

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Clostridium botulinum Type F: Seasonal Inhibition by Bacillus licheniformis

Abstract. Clostridium botulinum *type F has been identified during the summer months in mud samples from a small stream. Its absence during the period from October to April in these mud samples is attributed to the presence of* Bacillus licheniformis.

During the summer, a survey of sewage lagoons and adjacent streams in eastern North Dakota produced two samples (from a sewage lagoon and a small stream) which yielded cultures producing *Clostridium botulinum* type F toxin. During the fall and winter months the organism could not be identified (as determined by toxicity assays) from mud samples obtained from the small stream. It was determined that *Bacillus licheniformis* was present during these periods and was at least partially responsible for the inhibition of growth and hence toxin production of *Clostridium botulinum* type F.

Previous isolations or identifications of *Cl. botulinum* type F have been few (*1*), and it is altogether possible that seasonal or other variations occur in areas other than reported here. Other workers have noted that certain organisms will inhibit *Cl. botulinum* growth and toxin production, such as *Cl. sporogenes (2), Bacillus sphaericus* (*3*), *Brevibacterium linens* (*4*), and *Escherichia coli* and *Streptococcus faecalis* (*3*).

Mud samples which yielded *Cl. botulinum* type F toxin (or no toxicity) were placed in 1-g amounts into 9 ml of sterile, previously boiled and cooled, brain heart infusion (BHI) broth (Difco). All tubes were incubated in a Brewer anaerobic jar at 30°C for 36 to 40 hours. The supernatant of the culture (4340*g* for 10 minutes) was injected into 6-week-old Swiss mice in 0.4-ml amounts by the intraperitoneal route. The animals were observed for periods up to 5 days; however, all deaths attributable to toxin occurred within 2 days.

The toxin (0.4 ml of the culture supernatant) was identified by injection into mice that had been protected with antitoxin to *Cl. botulinum* types A, B, C, D, E, or F or tetanus (see Table 1). Heated supernatant alone (100°C for 10 minutes) failed to kill mice.

Mud samples obtained in June, July, and August were positive for *Cl. botulinum* type F, whereas samples obtained any time between October and May were negative. These negative samples were inoculated with a toxin-producing strain of *Cl. botulinum* type A (American Type Culture Collection No. 7948) and subsequently cultured as described above. No deaths occurred when culture supernatant was injected into mice, while controls with pure cultures of *Cl. botulinum* type A killed mice and showed type-specific neutralization with antitoxin (Table 2).

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, centrifuged supernatant of cultured mud samples; HS, heated supernatant (100°C for 10 minutes).

Source of toxin	Antitoxin	Results
S	None	15/15
S	ABCDE	20/20
S	F	0/10
S	Tetanus	4/4
HS	None	0/8

Serially diluted cultures of these negative mud samples yielded pure cultures of a variety of organisms; of these only *B. licheniformis* inhibited the growth and toxin production of *Cl. botulinum* type A when the two organisms were cultured together in BHI broth. *Bacillus licheniformis* had no effect on the preformed toxin, on the basis of the fact that supernatants of 36-hour cultures of *Cl. botulinum* type A remained toxic after extended incubation in fresh medium with *B. licheniformis*. Mixed cultures of the above organisms (when *Cl. botulinum* type A was initially present in concentrations of 1.6×10^3 organisms or less per milliliter) resulted in nontoxic cultures, provided that *B. licheniformis* was initially present in concentrations above 7.3×10^3 organisms per milliliter (Table 3).

Bacillus licheniformis produces the antibiotic, bacitracin (*5*), which is probably responsible for the growth-inhibiting phenomenon observed here. It is not known why *B. licheniformis* is

Table 2. Inhibitory effect of mud on toxin production by *Cl. botulinum* type A. Results are given as the number of mice that died out of the number tested.

Media inoculated with	Antitoxin	Results
Cl. botulinum type A	None	6/6
Cl. botulinum type A	A	0/6
Cl. botulinum type A + mud	None	0/6
Mud alone	None	0/6

Table 3. Inhibition of toxin production by *B. licheniformis*. Results are given as the number of mice that died out of the number tested. The media was inoculated with 1.6×10^3 *Cl. botulinum* type A per milliliter and various amounts of *B. licheniformis*.

B. licheniformis (cells/ml)	Results
7.3×10^6	0/3
7.3×10^5	0/3
7.3×10^3	4/6
7.3×10^1	3/6
0	6/6

not present in sufficient numbers during the summer months to inhibit the growth of *Cl. botulinum* type F, but since bacitracin production is apparently related to spore-formation in the organism (*6*), it is suspected that certain factors enhancing sporulation are present during winter but not summer months.

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Erythrocyte Chimerism after Injection of Spleen Cells into Anemic Mice of the W-Series

Abstract. *Anemic mice* (W^vW^v) *when injected at birth with a mixture of isologous (genotype* W^vw) *and homologous spleen cells, showed an improvement in their peripheral blood picture when adult. Red blood counts, red cell size, and electrophoresis of the hemoglobins indicated that, in some cases, the homologous tissue had become implanted and, in others, the isologous tissue was functioning.*

After a lethal dose of irradiation to a mouse, death can be averted by the administration of spleen cells from a healthy donor (*1*). Subsequently, such animals become red-cell chimeras (*2*). Spleen is also commonly employed to create immunological tolerance, by administration at birth, which results in cellular chimerism. Donor cells have regularly been demonstrated in the host lymphoid system (*3*), and Wilson and Talmage (*4*) have provided immunological evidence that such animals are also red cell chimeras.

Mice of the genotype W^vW^v have a genetically determined lifelong macrocytic anemia. Hematopoietic tissue from normal animals will implant in these animals and transform the blood picture to normal. Bernstein and Russell (*5*) used isologous and Seller and Polani (*6*) used homologous fetal liver cells as the source of hematopoietic tissue. Seller (*7*) subsequently showed that when the donor's hemoglobin differs from that of the recipient, its presence can be detected in successfully treated animals a year or more after treatment.

In the work reported here anemic mice W^vW^v were treated with spleen cells as an alternative source of hematopoietic tissue. The W-series mice (*9*) were a mixed stock with CBA/Gr and C57BL/Gr as part of their background. The donor animals were a pure-line, CBA strain maintained at Guy's Hospital, London. In early experiments, it was found that skin grafts exchanged between CBA animals and mice of the W-series were rejected in 11 days.

Originally, a spleen cell suspension prepared from adult CBA mice was injected intravenously into the W^vW^v animals a few hours after birth. However, initial experiments showed that the recipients succumbed to runt disease at about 21 days of age. Only one animal survived to adulthood. Russell (*8*) showed how death from runt disease may be circumvented by the addition of isologous spleen cells to the homologous inoculum. Accordingly, spleen cells from animals of the genotype W^vw were also injected. This led to an increase in the survival rate, although there was some evidence of slight runting during the juvenile period in the survivors, with subsequent recovery in adulthood, although an occasional survivor did die.

The whole spleens were removed from one adult CBA and one adult W^vw mouse; they were cut into fragments, sieved through one layer of bolting silk (14N, St. Martins), and rinsed with Hanks balanced salt solution (BSS), *p*H 7.2. This treatment produced cell clumps, which were broken down to single cells by drawing them (approximately 20 times) through two layers of bolting silk contained in a filtering nozzle of a hypodermic syringe. The suspension was centrifuged at 1000 rev/min for 5 minutes and the cells were resuspended in 0.5 ml of BSS. The genotypically mixed cells (10 to 15 × 10^6) were injected into the anterior facial vein of newborn W^vW^v recipients.

At varying intervals during maturation the peripheral blood picture was examined. Blood was taken from the tail of the animal. Hematocrit values were estimated with the Hawksley microhematocrit centrifuge, but very small capillary tubes (3 cm by 1 mm) containing heparin were substituted for the usual tubes. Red blood counts were made by the conventional hemocytometer method with Hayem's fluid as the diluent.

Electrophoresis was performed on the hemoglobins when the mice were approximately 6 months old. Cellulose acetate paper was used as the supporting medium in a barbitone buffer (5,5-diethylbarbituric acid), *p*H 8.6, ionic strength 0.05, and the electrophoresis was conducted for 3 hours at 5 volt/cm. Afterward, the papers were stained with a 0.2 percent solution of Ponceau S in 3 percent trichloroacetic acid.

In all ten W^vW^v mice that survived to adulthood after treatment at birth with 10 to 15 × 10^6 CBA/W^vw spleen cells, the peripheral blood picture changed from the anemic type. The extent of the change was not the same in all the animals. While in some, the blood picture became that of a hematologically normal mouse—the criteria being red blood counts, hematocrit values, and mean corpuscular volumes—

Table 1. Red blood counts, hematocrit values, and mean corpuscular volumes of CBA, W^vW^v, and W^vw mice, and of W^vW^v mice treated at birth with a CBA/W^vw spleen cell mixture.

Animals (No.)	Red cells ($\times 10^6$/mm^3)		Hematocrit (%)		Mean corpuscular volume μ^3	
	Mean	Range	Mean	Range	Mean	Range
			W^vW^v			
15	7.20	5.48– 8.11	38.2	31.3–46.5	53.7	41.3–71.0
			CBA			
7	11.97	11.61–12.61	43.9	39.8–49.1	36.6	32.8–38.9
			W^vW^v *treated with* W^vw*/CBA spleen, with CBA hemoglobin*			
6	11.77	11.17–12.68	45.1	41.6–47.2	40.1	37.2–43.8
			W^vW^v *treated with* W^vw*/CBA spleen, with W hemoglobin*			
4	10.28	9.89–10.55	43.5	40.8–45.6	42.2	38.9–46.2
			W^vw			
11	9.89	9.16–10.80	42.5	34.1–47.1	43.1	35.6–50.9