

with specific toxic effects on the spreading elements of the organisms. The purpose of this note is to describe the transformation of strains of *Proteus mirabilis* to the L form of growth under the influence of sterile broth filtrates of antagonistic strains.

The test organisms used in this study were selected by streaking opposite poles of nutrient agar plates with recent isolates of *Proteus mirabilis*. Antagonism between two strains was evidenced by a line of demarcation between the spreading elements of the organisms. The L type of growth was induced in nutrient agar pour plates containing high concentrations of filtrates from 10-day-old broth cultures of a selected strain in conjunction with an appropriate test organism. The L colonies were similar to the 3B L colonies obtained by Dienes (J. Bacteriol., **57**, 529, 1949) with the use of penicillin. In most cases L transformation was not complete insofar as smooth, nonswarming colonies of the test organism were also present. The L forms could be maintained in subculture only in the presence of the filtrate. Reversion to the normal bacillary state was easily accomplished by streaking the L forms on filtrate-free plates. The reverted organisms were identical with those of the parent strain and showed no increased resistance to the action of the filtrate. Repeated cultivation in the presence of the filtrate did not slow down the process of reversion or increase the resistance to the filtrate. The metabolic activity of the L

forms as evidenced by biochemical characteristics was found to be very similar to the parent organisms. Microscopic observations of the transformation of bacilli to large bodies and L forms in slide cultures showed processes similar to those reported in other investigations (Dienes, J. Bacteriol., **57**, 529, 1949; Stempen and Hutchinson, J. Bacteriol., **61**, 321, 1951).

Dienes (*personal communication*) indicated that it was doubtful that the action between the spreaders and the inhibitory action of old broth cultures depended on the same mechanisms, since the antagonism between the spreaders is specific and does not affect the small bacterial forms. Preliminary data indicate that several factors may be present in filtrates which induce L transformation. Furthermore, we have demonstrated that the filtrate also induces L transformation on its homologous source. However, the latter effect is not as marked as that which occurs against the heterologous strains.

Although many conditions that induce L transformation are artificial, it is felt that the observation that *Proteus* cells can give rise to a spontaneous product of growth capable of inducing L transformation would seem to indicate that such a process might occur in nature. The observation also lends support to the statement by Dienes and Weinberger (Bacteriol. Revs., **15**, 245, 1951) that L transformation may be the result of general injury to normal bacterial growth rather than a specific effect of a specific toxic substance.

ISOLATION OF VESICULAR STOMATITIS VIRUS FROM MOUSE MOTHERS AFTER INOCULATION OF SUCKLING MOUSE LITTERS

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In the course of titration of New Jersey and Indiana types of vesicular stomatitis virus (VSV) contained in infected bovine tongue epithelium or chorioallantoic membranes, 230 litters of suckling Swiss albino mice were inoculated (Fellowes *et al.*, Am. J. Vet. Research, **17**, 799, 1956). Of this number, 114 litters were inoculated with Indiana type VSV and 116 litters with New Jersey VSV. In the litters inoculated with the

latter virus, 9 of the uninoculated lactating mouse mothers died. No deaths of mouse mothers were observed in the Indiana type VSV groups of suckling mice inoculated. Inoculations were performed intra-abdominally (IA), each suckling mouse receiving 0.05 ml of various virus dilutions.

No attempts were made to determine the cause of death in the first 4 deaths of mouse

mothers, since these were considered unrelated to the inoculation. In later experiments, an additional 5 deaths of mouse mothers were observed. These deaths occurred 5 to 7 days after the litters were inoculated. No symptoms were observed in the others prior to death. Due to postmortem changes in 3 of the 5 cases, no examinations were made. In the other 2 instances, the bodies were examined; a brain and muscle pool was harvested from one and brain only was taken from the other. The tissues were prepared as 10 per cent suspensions in tryptose phosphate broth. Penicillin and streptomycin were added in amounts of 1,000 units and 1 mg, respectively, per ml of suspension.

A complement fixation test, using the combined brain and muscle suspension as antigen, revealed the presence of VSV in the sample. The brain suspension was inoculated via the allantoic cavity in 0.2 ml amounts into 8-day-old em-

bryonating chicken eggs. Suckling mice were also inoculated IA, using 0.05 ml each. The embryos of all inoculated eggs died within 48 hr, while all suckling mice died between 48 and 72 hr. Brains from the mice and chorioallantoic membranes from the eggs were prepared as 10 per cent suspensions. A complement fixation test was performed with these suspensions, which confirmed the presence of New Jersey type VSV in both eggs and mice.

At the present time, it is not definitely known how the mouse mothers became infected with VSV. It may be possible for infected suckling mice to mechanically injure the mammary gland of the mother while feeding and transmit the virus in this fashion. Since the mother licks the anal region of the young mice, it may be possible for her to ingest sufficient virus, if present in the excreta, to produce infection.

TOXIN PRODUCTION AND PROTEOLYTIC PROPERTIES OF *CLOSTRIDIUM NOVI*¹

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Clostridium novyi is usually considered as being unable to digest casein in milk media (*Bergey's manual of determinative bacteriology*, 6th edition) and most keys for the identification of the clostridia include milk as a differential medium to aid in separating this and similar organisms from the more proteolytic species. However, *C. novyi* has been described as digesting casein in milk (Reed, In *Bacterial and mycotic infections of man*, Lippincott, 1948) and we have encountered strains with this property in a collection of cultures that were identified as *C. novyi* on the basis of fermentative characteristics and the production of the species-specific alpha toxin.

Three casein-digesting strains and three strains that did not digest casein were investigated for

the production of α , β , γ , and ϵ toxins. They were also inoculated into gelatin, iron-brain, iron-milk, and coagulated serum media and were plated on milk agar made with a liver-infusion base. These cultures were incubated at 37 C for 10 days. All strains hydrolyzed gelatin; none of the strains digested coagulated serum; the digestion of casein in milk agar was equivocal. The results of the other tests are given in table 1.

It is apparent from the correlation between toxin

TABLE 1
Toxin production by proteolytic and nonproteolytic strains of Clostridium novyi

Strain	Digestion of Iron-Milk	Iron-Brain Blackening	Toxin Production			
			α	β	γ	ϵ
8	—	—	+	—	+	+
17	—	—	+	—	+	+
40	—	—	+	—	+	+
7	+	+	+	+	—	—
988	+	+	+	+	—	—
2,879	+	+	+	+	—	—

¹ Contribution from the Montana Veterinary Research Laboratory (Montana Experiment Station and Livestock Sanitary Board cooperating), Agricultural Experiment Station, Montana State College, Bozeman. Paper No. 394, journal series.