maximal destruction of red cells occurred at approximately 235 days in both guanacos. We do not know of any species with similar erythrocyte survival times. The elliptical erythrons of Camelidae may be unique in their longevity as compared to the circular, biconcave erythrons of other mammals (*9*).

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## Occurrence of a Porphyrin Pigment in Streptomycetes

*Abstract.* The formation of an intramycelial red pigment of coproporphyrin type in submerged cultures of *Streptomyces griseus* and *S. fradiae* was detected. Its production is more intensive in the variants of *S. griseus* Z38 that give a low yield of streptomycin, and in the absence of $Fe^{++}$ in fermentation media.

Despite the growing knowledge of the physiology of actinomycetes, relatively little is known about the chemical nature of their pigments that lack antibiotic activity. Many of the antibiotic pigments of actinomycetes are of the quinonic type (*1*), as are probably some of the nonantibiotic pigments which function as *p*H indicators. Some actinomycetal pigments that possess antibiotic properties, such as holomycin, thiolutin and aureothricin, are relatively simple derivatives of pyrrole (*1*); the orange antibiotic pigment found in a streptomycete related to *Streptomyces ruber* and *S. roseo-distaticus* (*2*) is prodigiosin-like in nature. However, the accumulation of true porphyrin pigments in actinomycetes has so far not been described.

During our study of the physiological relationships of streptomycin biogenesis in *S. griseus* Z38, we have observed the production of a mycelium-bound red pigment that was formed during the submerged cultivation of the organism on a reciprocal and rotary shaker, with a variety of complex and synthetic growth media. During the growth of the organism on Ferguson's (*3*) and other synthetic nutrient media, the production of the pigment was favored by the absence of iron salts. A pigment concentrate was obtained from the 5-day submerged culture by adjusting the *p*H value of the fermentation liquid to 2, separating the mycelium by filtration, and extracting it with an adequate volume of ethyl acetate. This crude ethyl acetate extract, which showed an intense reddish-violet fluorescence in ultraviolet light, was further purified by extraction of the red pigment from ethyl acetate to water at *p*H 6.0 and by repeated extraction to ethyl acetate, after acidification of the aqueous phase to *p*H 5.4. After this process had been repeated four times the pigment was transferred from the acidified aqueous solution to ether and then extracted with 0.1*N* hydrochloric acid. After adjustment of the *p*H of this extract to 2.0 the pigment was again extracted with ether. This final ether extract was evaporated to dryness, which left a purified concentrate of the pigment in the form of a dark violet amorphous residue. The amount of this concentrate was too small for further purification.

The solution of this material in ether showed absorption peaks at 598, 623.5, 569, 527, 499, and 397 m$\mu$ in order of increasing intensity, whereas its solution in 0.1*N* HCl showed absorption maxima at 590, 548, and 400.5 m$\mu$. The HCl number estimated by the method of Willstätter (cited in *4*) was 0.09. The pigment was not soluble in chloroform. These results clearly suggest that the pigment is a porphyrin. The analytical data obtained coincide with those given by Jope and O'Brien (*5*), Todd (*6*), and Lemberg (*4*) for coproporphyrin. The formation of this pigment was also markedly augmented during the degeneration, in respect to streptomycin production, of the strain of *S. griseus* Z38. A pigment of a similar type was also found in iron-deficient submerged cultures of *S. fradiae*.

These results show that the formation of porphyrin pigments, known so far in yeasts, fungi, and bacteria (*1*), occurs in actinomycetes as well.

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## Action of Tetanus Toxin in the Cerebral Cortex

*Abstract.* Injection of more than ten mouse lethal doses of tetanus toxin into cat's motor cortex produces seizures accompanied by cortical electrical convulsive discharges. During the hours preceding onset of large seizures, "antidromic" inhibition of evoked cortical activity is reduced. The similarity of these effects to those observed in spinal cord suggests operation of similar inhibitory transmitters in the two parts of the central nervous system.

Tetanus toxin has a specific pharmacological action in the spinal cord: it progressively reduces transmission at all inhibitory junctions. Since all types of inhibition are equally affected no matter what their central connections (*1–3*), one may infer that the transmitters at such junctions are very similar, or perhaps are the same substance. We have investigated the effects of tetanus toxin on the electrocorticogram and on inhibition in the cerebral cortex. It has been reported recently that cerebral injection of tetanus toxin may produce convulsive activity and foci of electrical discharge (*4*). The prerequisite for a study such as ours is knowledge about a form of synaptic inhibition that occurs in the cortex. This condition is met in the case of "antidromic" cortical inhibition: repetitive stimulation of the bulbar pyramidal tract causes inhibition of spontaneous activity of cortical units (*5*) and of responses to peripheral or cortical stimulation (*6, 7*).

Acute experiments were carried out with cats receiving artificial respiration while immobilized by intravenous injections of Flaxedil. After brain exposure under ether anesthesia the animals were maintained with a long-lasting anesthetic. Details of these methods have been described previously (*8*). Approximately 1 to $10^5$ mouse lethal doses of tetanus toxin suspended in $10^{-3}$ to $10^{-4}$

ml of Ringer-gelatin solution (for details, see *3*) were injected in different experiments into the motor area at a depth of 0.5 to 2 mm. Records were obtained from points on the cerebral surface, including the site of injection.

Injection of 100 to 1000 mouse lethal doses of toxin into the motor cortex led to development of small convulsive spikes and triggered seizure bursts within 2 and 6 hours, respectively. However, large convulsive discharges with intervening silent periods began only many hours later. In this later stage, surface responses could not be evoked by electrical stimulation of the foot or of the contralateral cortex. Observation of intact animals that had received injection of such doses of toxin under sterile conditions revealed progressive hyperreflexia of the limb into whose motor projection the injection was made, followed by a stage where relatively severe exertion, such as an animal righting itself from the supine position, resulted in a seizure consisting of clonic movements of the limb into whose cortical projection area toxin had been injected. This stage in turn gave way to regular convulsive episodes of that limb occurring regularly at rates of 30 to 60 per minute. The contralateral other limb, and both limbs ipsilateral to the injected brain side, became involved several hours later than the limb first affected. Hyperesthesia was never seen. Cortical injections of the same volume of solution containing only 10 to 100 mouse lethal doses produced the same sequence of symptoms; the onset of effects, however, was delayed by about 40 hours. Solutions containing but one mouse lethal dose produced no behavioral or electrical changes.

In order to study "antidromic" inhibition, test responses were recorded which were evoked in the forefoot area of the motor cortex by stimulation through two needles inserted into the skin of the contralateral forepaw. Amplitudes of evoked responses were measured from ten superimposed oscilloscope traces. Conditioning pyramidal stimulation consisted of 11 shocks spaced 5 msec apart, at strengths sufficient to produce half maximal antidromic cortical *a*-waves (*9*). The typical time course of "antidromic" inhibition of peripherally evoked responses is shown in Fig. 1 (open circles), recorded 4 hours after toxin injection from the point of injection, which was about 1 mm anterior to the cruciate sulcus. Test stimuli were adjusted to evoke three-quarter maximal responses. Inhibition is displayed as amplitude of conditioned responses expressed as percentage of control test responses (ordinates), plotted against the intervals *t*, in milliseconds, between the end of the conditioning train of pulses (zero time) and the onsets of the positive and negative deflections, which were 10 and 20 msec, respectively, after the testing stimulus (abscissas, see inset diagram in Fig. 1). The times labeled with negative numbers on the abscissae refer to the period of repetitive conditioning when response amplitudes usually fluctuated greatly. Percentage control amplitudes of surface-positive primary responses are plotted at the left, and those of subsequent surface-negative responses on the right, in Fig. 1. Peak latencies of these responses were 12 and 26 msec, respectively, and peak amplitude variation was about 10 percent. The inhibitory curves taken at 4 hours after toxin injection (open circles) resemble results obtained with normal animals in all respects (*7*). Reduction of the later phase of inhibition (after intervals of 40 msec) by toxin is reflected in the curves made at 11.5 and 13 hours after injection. In this experiment, very small spikes and waves appeared in the electrocorticogram between 3 and 4 hours after toxin injection, and equally small "bursts" between 6 and 7 hours. These bursts grew larger and became well-defined seizure discharges between 10 and 12 hours after injection. With the dose of toxin used, however, the amplitudes of even the largest discharges were only three times those of normal base-line fluctuations, or about 400 $\mu$v. The responsiveness of the cortex to peripheral or to antidromic stimuli remained constant throughout the experiment.

The type of result plotted in Fig. 1 was obtained in five experiments employing peripheral stimulation and in two where transcallosal excitation was used. Reduction of inhibition, which was followed satisfactorily in three experiments, always preceded onset of convulsive discharges of large amplitude. Test response depression during conditioning was highly variable before and after toxin injections. The early phase of recovery after conditioning stimulation remained unchanged by toxin or decreased only slightly; this period of depression may be mostly due to relative refractoriness or to inhibition generated in structures either insensitive to toxin or remote from the site of toxin injection. In contrast, inhibition after 40 msec gave way to facilitation in all experiments. Reversal from inhibition to facilitation has also been observed to follow action of tetanus toxin in spinal motor nuclei. For instance, reflexes of flexor digitorum longus are normally inhibited by conditioning stimulation of the quadriceps nerve. After intraspinal injection of toxin, this inhibition is replaced by facilitation (*1*).

Reduction of "antidromic" inhibition by tetanus toxin before the onset of

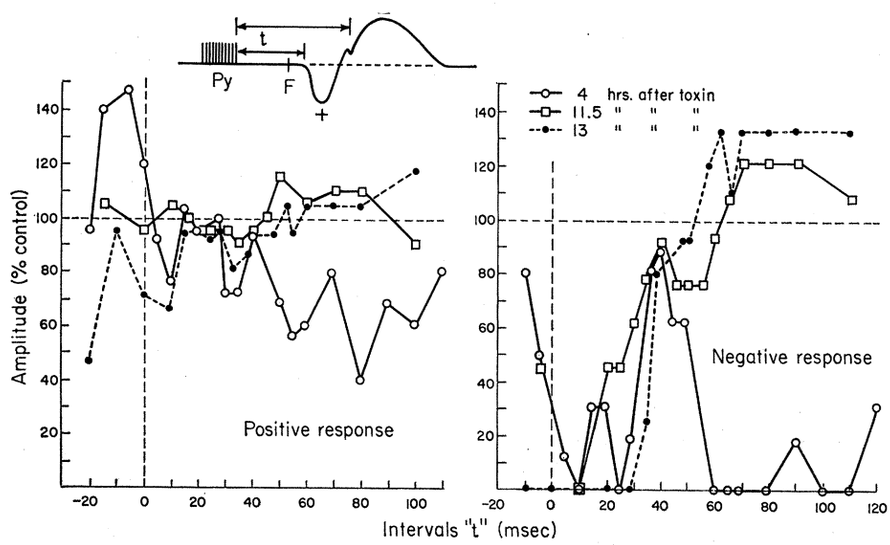


Fig. 1. Curves of antidromic inhibition of cortical-evoked surface responses. Ordinates of graphs on left side plot percent of control positive deflections, and those on right, percent of control negative deflections. Intervals between end (zero time) of repetitive antidromic conditioning (*Py*) and onset of respective potentials to foot stimulation (*F*) are plotted on the abscissa (*t*, see inset diagram). Base-line deflections due to pyramidal stimulation are not shown in the diagram. Key explains symbols; for details see text.

large cortical convulsions resembles the sequence of events in the spinal cord after injection of toxin into the cord or motor nerves. The similarities during treatment with the same pharmacological agent, tetanus toxin, suggest that the inhibitory transmitter operating at cortical synapses mediating the late phase of "antidromic" cortical inhibition is very similar to, if not identical with, the inhibitory transmitters active in the spinal cord (*10*).

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## Reappearance of Eulimnadia agassizii with Notes on Its Biology and Life History

*Abstract.* After being unreported for 83 years, *Eulimnadia agassizii* Packard was collected from a temporary pool in Woods Hole, Massachusetts, in 1956 and again in 1961. This conchostracan attains maturity in 5 days and may reappear several times during the summer months in the same location.

The conchostracan phyllopod *Eulimnadia agassizii* Packard 1874 has not appeared in the nets of collectors since its discovery more than 80 years ago on Penikese Island in Buzzards Bay, Massachusetts. It was first found in a small pool of fresh water by Walter Faxon on 27 August 1873, and immature stages were collected in the same pool in early August of the following year. This species has been rediscovered twice in recent years in a sand trap at the golf club at Woods Hole, Massachusetts. The animal was fittingly named in honor of Louis Agassiz by Alpheus S. Packard, Jr. (*1*). Packard, one of Agassiz's students and an instructor at the famous Anderson School of Natural History, was on Penikese Island and possibly was present at the time of the original collection.

Although several expeditions have sampled the small ponds on the island quite thoroughly (*2*) and various individuals have undoubtedly examined the fauna of these fresh waters, *E. agassizii* has not been reported since its original discovery (*1, 3*). Since its original discovery was not questioned, its absence was explained mainly on the basis of a theory that subsequent collections were not made sufficiently early in the year, when pond-water temperatures were low. However, current evidence indicates that *E. agassizii* is probably not a spring form but one that appears primarily in early fall in years of unusually heavy rainfall during large storms.

On 18 July 1956, 4 days after a very heavy rain, both the northernmost sand trap of the third hole of the Woods Hole Golf Course and a natural depression more than 50 feet away, separated from the sand trap by a low rise, contained 4.5 inches of water and many phyllopods bearing eggs. A representative sample of animals was collected, preserved, and sent to a specialist for verification of identification. The water disappeared the following day. The phyllopods decreased in number as the pools dried out.

From this time until the second rediscovery of these phyllopods, on 29 July 1961 after a heavy rain on 23 and 24 July, there had been insufficient rainfall during the warm months to keep water in the sand trap for much more than 24 hours. On 29 July the population was again large, and brown individuals were distributed throughout the pool. On 30 July they were carrying eggs, were starting to accumulate in small depressions, and had turned grass-green through ingestion of benthic diatoms that had appeared in large numbers. Sampling on both 29 and 30 July revealed no larval stages and no males. The only other macroscopic organisms were small brown diving beetles and waterstriders. The water temperature on 29 July at 11 A.M. was 32.5°C, and on 30 July at the same time, 32°C; both days were cloudless. By 5 P.M. on 31 July the trap was dry and no phyllopods were found.

On 23 August a rainfall of more than 1 inch filled the sand trap again. On 26 August larval forms appeared and doubled in size about every 12 hours until 28 August, when they became adults. On this day, just before the water disappeared from the trap, a small sample of *Eulimnadia* was collected (*4*) and placed in a battery jar with trap water. Here they remained alive for more than a week, eventually attaining maturity without developing eggs.

On 30 September, 5 days after severe rain accompanying hurricane "Esther" had deposited more than 2 inches of water, examination of the trap revealed nothing but a few aquatic beetles. The water disappeared within 24 hours.

The phenomenon of a species of phyllopod hatching more than once in a single location during the same season is probably better explained by the hypothesis that different groups of eggs of the same generation that had overwintered in soil, hatch after subsequent soakings than by the hypotheses that eggs of the second hatching need two soakings before developing, or that the second brood came from eggs of the first brood. The phenomenon resembles the situation in related branchiopods and in copepods, where repeated hatchings have been observed experimentally and in nature in dry depressions that have been occasionally filled by rain water.

Dexter (*5*) collected a series of three separate hatchings of the fairy shrimp *Eubranchipus vernalis* from a single pool in the seasons 1955–56 and 1958–59. The pool lost its water repeatedly by evaporation or by freezing solid. Larvae of the first two hatchings never reached maturity. It is obvious that all the eggs hatched that season were present from the beginning. Undoubtedly many others remained unhatched until a later time. It is not known why some eggs hatch at one soaking and others during a later soaking. Variability in hatching time may be related to racial differences in egg permeability, or to variations in their depth in the soil at the bottom of the pool.

These animals have an unusually short life cycle and pass through larval stages rapidly as compared with most conchostracans whose development is known. Certainly the relatively high temperature of the water acts as a stimulus to both growth and development; a somewhat analogous adaptation has been observed in desert Crustacea, with lapses of many rainless years between hatchings and unusually rapid passage through larval stages during the brief life of temporary pools.

The reappearance of *Eulimnadia agassizii* in this location appears all the more unusual in the light of informa-