be considerably extended in one direction due to the use, by this population, of a migratory route to a winter roost. However, this certainly could not explain the successful return of this bat from the diversity of directions demonstrated in this study.

The failure of this individual to be recovered following her last release at Mammoth on 1 October was originally thought to be due to the fact that the bridge from which she was taken is used as a roost only during the warmer months of the year and was abandoned by all pallid bats some time between the period of 1 through 8 October. However, during repeated checks at this same bridge during the following summer we failed to recover this individual even though several other pallid bats, which had been used in the 1960 experiments, were in continuous residence throughout the summer.

From these results, it does not seem reasonable that chance alone would have permitted this bat to show such a consistency of return. Surely some abilities possessed by this bat, and not simply randomness, must have been in operation. The problem of low percentage of return, however, still remains and must be taken into account by any explanation of the mechanism of bat homing. Did this particular bat possess or develop abilities which are unique and which are absent in most other members of the same species? Individual variation is, of course, to be expected among the members of any species. Ordinarily, however, one would not expect variations in the presence of any aptitude or ability to range from extremely high values in certain individuals to near zero in others. Obviously, much additional evidence is needed to answer this and many other questions concerning homing in bats (*6*).

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5. Papers concerning these data (speed of flight and population home range) are in preparation. We hope that more direct evidence can be obtained with a subminiature radio transmitter being developed by Howard Baldwin of the University of Arizona.
6. This report is the result of activities supported in part by grants from the National Science Foundation (G-5209) and the National Institutes of Health (E-3147).

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Chronic Effect of Tetanus Toxin Applied Locally to the Cerebral Cortex of the Dog

Abstract. Tetanus toxin injected into the cortex produces convulsions, neurological disability, and a strychnine-like discharging focus, appearing after a minimal latency of 2 days and lasting more than a month. Such a focus, apparently caused by a blockade of inhibitory synapses, does not demonstrate any major damage in microscopic studies.

The present study was undertaken in order to find an effective method of producing a chronic "epileptogenic" focus in the gray substance without significant structural changes. The purpose of this preliminary communication is to show how this has been accomplished with tetanus toxin in the cerebral cortex of the dog.

The acute action of tetanus toxin upon the spinal cord was recently studied (*1*, *2*). Its chronic effect upon the iris (*3*) and the pathogenesis of "local" or peripheral tetanus is still a matter of controversy (*4*). To our knowledge, the chronic action of tetanus toxin applied locally to the cerebral cortex has heretofore never been studied.

Under sterile conditions, 50 to 150 μg of tetanus toxin was injected immediately under the pia mater in 66 dogs. The dry toxin ($LD_{50} = 1.8$ μg) was kept in a vacuum container and placed in a refrigerator. A 0.1-percent solution in saline was prepared immediately before its use.

Thirty-four animals (group I) showed major convulsions. These appeared between the second and seventh day after the operation and lasted until the animals were sacrificed, or died, usually as the result of recurrent convulsions. In three dogs which were allowed to survive for more than a month, the convulsions diminished and finally disappeared by the end of the second month. Between convulsions, the animals showed disturbances of motor performance and behavior. The most significant findings observed were diminished strength, awkwardness of movements, and a brisk withdrawal response of the limbs on superficial stimulation, apparently leading to "steppage" during gait. These effects were contralateral to the side of injection.

Five animals (group II) showed minor motor disturbances but convulsions were not recorded, although they could have occurred and escaped observation. Twenty dogs (group III) showed no convulsions and no motor disturbances. This group includes three controls injected with inactivated toxin and those who received smaller amounts of tetanus toxin (see below). Seven animals (group IV) died within the first 2 days of the operation; unobserved convulsions might possibly have been the cause of death in these cases.

The spontaneous electrical activity of the brain was recorded with a Grass polygraph in intact nonanesthetized and in curarized animals. Needle electrodes inserted through the skin and small (0.2 to 0.5 mm diameter) silver ball electrodes applied to the surface of the exposed cortex were employed. Electrodes implanted at the original operation were used in a few circumstances for recordings in unrestrained animals. The usual technique was bipolar recordings with a 2- to 3-mm interelectrode distance. Unipolar recording and bipolar recording with different sized silver balls, leading the smaller to grid one, were employed to ascertain the polarity of the electrical events.

The common finding was the recording of a triphasic spike with an average total duration of 250 to 300 msec and an average peak to peak amplitude of 0.2 mv when recorded from the skin and 1.5 mv when recorded from the cortical surface. The outstanding event was a negative component, of 100-msec duration, which was preceded by a sharp positive deflection and followed by a variable, usually smoother positive wave (Fig. 1). Such spikes would appear at random but more frequently they were observed at slightly irregular intervals, of about 1 second duration, with random short silent periods (Fig. 1) or in bursts of three to ten spikes. Any one of these three patterns of activity could be alternately observed in the same experiment. These "tetanic" spikes were similar to those observed after the application of strychnine to the otherwise normal cortex.

The records showed that these spikes occurred in a small area about 5 mm in diameter, centered at the site of the previous injection of tetanus toxin. Occasional spikes or slightly abnormal cortical activities were sometimes recorded in the adjacent area. The remaining ipsilateral cortical areas as well as the cortex of the opposite hemisphere were normal. Abnormal activity transiently spread to adjacent previously normal areas during convulsive discharges.

Such abnormal electrical activity was more remarkable in animals that demonstrated convulsions (group I). It was also seen in animals showing mild neurological abnormalities but no convul-

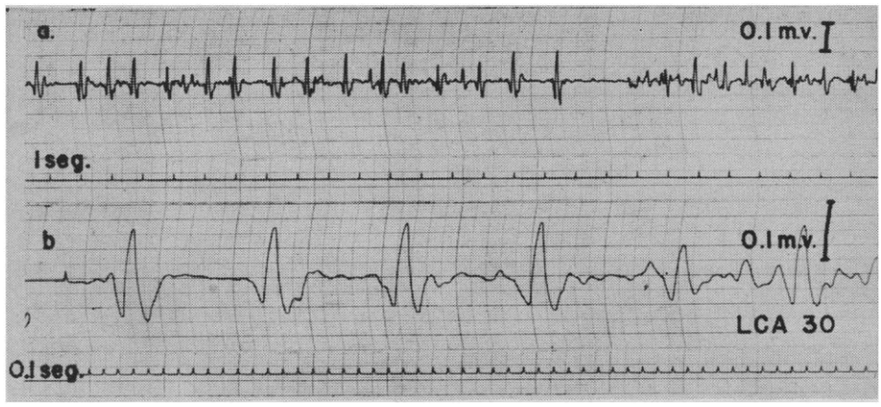

Fig. 1. Electroencephalogram of a dog 32 days after the injection of 100 μg of tetanus toxin into the left ectosylvian gyrus. The animal showed convulsions from the second day after injection. The "tetanic spike" is shown with different speed and amplification in records *a* and *b*. Grass polygraph.

sions (group II) and in some animals showing no fits and no neurological disturbances (group III). In such cases (groups II and III) the abnormal area was smaller and the discharges exhibited greater irregularity. These were the cases injected with a smaller quantity of tetanus toxin. Such electrical events were similarly observed between 2 and 37 days after the injection of tetanus toxin.

Topical application of a 1-percent solution of γ-aminobutyric acid to the discharging cortical focus completely reversed the polarity of the spike within 20 seconds; if the cortex was subsequently washed with warm Ringer's solution, the original spike reappeared. Topical application of a 1-percent solution of ϵ-aminocaproic acid to the discharging cortical focus increased the amplitude of the spike and sometimes initiated brief afterdischarges. The latter effects were immediately reversible. Topical application of 1-percent strychnine solution enhanced or minimally altered the tetanic spike. New "strychnine" spikes did not appear in the area of the "tetanic" focus. On the other hand "strychnine" spikes appeared under such circumstances in the previously normal cortex surrounding the discharging tetanic focus.

γ-Aminobutyric acid or ϵ-aminocaproic acid injected into the ipsilateral carotid artery, or intravenously, did not modify the tetanic spike. The spike, on the other hand, disappeared with anoxia, induced by interruption of artificial respiration. Intravenous injection of less than 10 mg of Nembutal per kilogram of body weight also eliminated "tetanic" spiking.

Neither macroscopic nor gross microscopic alterations were observed in the area of the "tetanic" focus. This, as well as the results of injection of γ-aminobutyric acid into the bloodstream, would indicate that the blood-brain barrier is unaltered. This seems to be one of the advantages of this method over ancillary techniques to produce a chronic cortical discharging focus. Microscopic alterations, which apparently occur at a cellular and perhaps only synaptic level, are under investigation.

It can be concluded from this study that the local injection of tetanus toxin in the cerebral cortex of the dog produces a chronic active lesion in a small, well localized area. Such a discharging focus appears after the second day post-injection, persists for more than a month, and is finally reversible. Present and previous evidence (*1*, *5*) indicates that the "tetanic focus" of discharge is probably the result of the focal selective blockade of the inhibitory synapses in the cerebral cortex. The data suggest that tetanus toxin may be a useful analytical tool in studying cortical synaptic organizations.

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29 January 1962

An Artifact in Plant Autoradiography

Abstract. An artifact in autoradiography of bean plants containing phosphorus-32 is reported. It was shown that the apparent accumulation of isotope in primary leaves that were oven-dried prior to exposure to x-ray films was not metabolic but due to a drying gradient occuring within the leaf. This artifact disappears when autoradiographs are made of leaves that have been freeze-dried under vacuum.

A number of reports (*1*, *2*) have pointed out the possibilities of autoradiography for plant physiological research and have described methods of preparation of plant material.

Possible artifacts have been reported which could lead to false interpretation of experimental results. Millikan (*3*) found that radioactive manganese had moved from interveinal tissues into veins of pea leaves when he studied successive autoradiographs of the same fresh plant material. He concluded that the movement was due to the enclosing of the plant parts between sheets of glass during exposure at room temperature, thus limiting evaporation and allowing movement of sap in veins. Rice and Rohrbaugh (*4*), who used 2,4-dichlorophenoxyacetic acid, reported movement of radioactive tracer in plants dried whole at 60°C. They found that this movement ceased when the plants were sectioned and explained the phenomenon on the basis of ease of movement of kerosene through intercellular spaces by capillarity. Crafts (*2*) and Pallas and Crafts (*5*), considering a critical preparation of plant material for autoradiography, discuss the importance of freeze-drying after treatment for short periods. They state, however, that with increasing time the difference between oven-drying at 80°C and freeze-drying is less evident. They reported that breaking frozen plants into sections and then drying them conventionally approximated freeze-drying. A number of reports have also appeared where autoradiographs, made after oven-drying or infrared-drying of plants, were used to interpret distribution of isotopes or radioactive compounds within the plants. In general, there seems so far to be agreement that for experiments lasting longer than 24 hours, drying of plant materials at about 80°C gives satisfactory results.

However, in a series of relatively long (3 to 7 days) experiments with P^{32}, it was found that an artifact opposite to that reported by Millikan occurred when primary leaves of bean plants