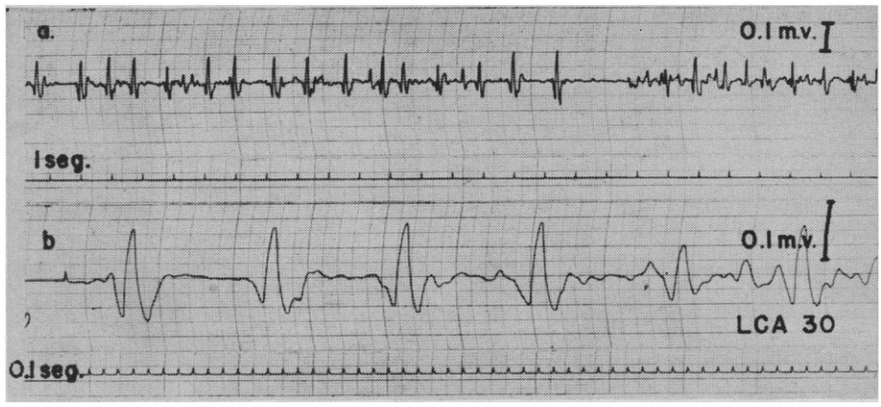

Fig. 1. Electroencephalogram of a dog 32 days after the injection of 100 μg of tetanus toxin into the left ectosylvian gyrus. The animal showed convulsions from the second day after injection. The "tetanic spike" is shown with different speed and amplification in records *a* and *b*. Grass polygraph.

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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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

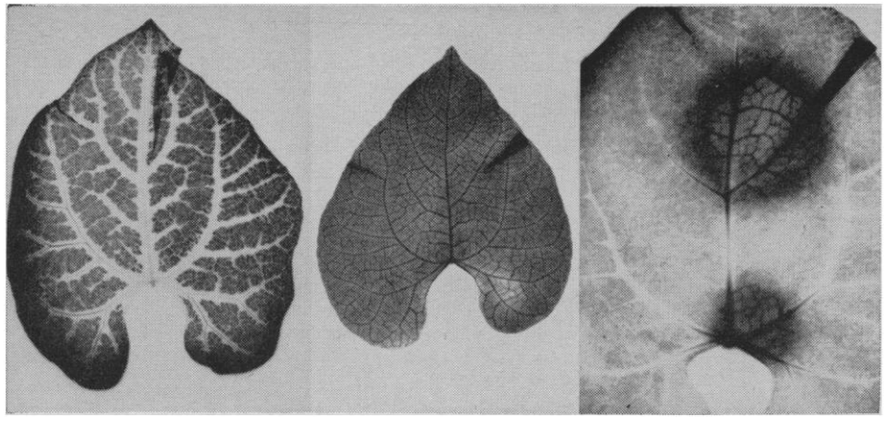

Fig. 1. Autographs of primary leaf of bean plant grown for 3 days in 750 ml of aerated complete Hoagland solution to which was added 1.0 μc of P^{32}. (Left) Oven-dried at 80°C. (Center) Freeze-dried. (Right) Oven-dried at 80°C between perforated plates.

(*Phaseolus vulgaris*) were autoradiographed after they had been dried in an oven at 80°C, prior to exposure to Kodak no-screen x-ray film. Plants were grown in complete Hoagland solution (*6*) under constant conditions of light (16 hours), air temperature (23°C), and humidity (55 percent). Irregular distribution in a haphazard fashion was noted in leaves treated with 1.0 μc of sodium phosphate in $10^{-3}M$ carrier applied as single or multiple drops of 0.01 ml each.

When distribution was even after leaf treatment, and in all cases of root treatment, the primary leaves showed veins depleted of tracer and the tracer seemed to be retained in what appeared to be some sort of precipitation in the interveinal areas (Fig. 1, left). The fact that the tracer had moved away from the veins, leaving broad clear areas, indicated that phosphorus is highly mobile in killed tissues. It appears as if phosphorus was concentrated in the stomatal chambers, from which water had evaporated and left the leaf, and was not fixed to any appreciable degree in the dead cells of the interveinal tissues. Figure 1 (left) shows a faint residue of tracer in the veins but practically none in the adjacent parenchyma; this mobility of phosphorus in killed tissues is unexpected, considering its known functions in living cells.

Autoradiographs of replicates which were frozen immediately after harvest and dried in that state under vacuum, by a method slightly modified from the one reported by Yamaguchi and Crafts (*7*), always showed even distribution of P^{32} in the whole vein network, in particular along the movement route to the petiole. No particular accumulation in the interveinal tissues was noted (Fig. 1, center).

In an attempt to explain these differences, plants were kept after harvest for periods ranging from 0 to 120 minutes at 25°C and 80°C prior to freeze-drying. No difference was noted in the redistribution of the P^{32} in the dried leaves. During these periods at 25°C, or as the leaves were heated to reach equilibrium with the preheated oven at 80°C, leaves were by no means dry, and cell metabolism should have continued normally or increased in rate. Since no difference could be found I concluded that metabolic activity of tissues following harvest and prior to complete drying was not responsible for this artifact. The possibility that this difference was masked by the dose used was investigated by increasing and decreasing the dose up to tenfold; there was no difference in the results. Neither the position nor size of the drops nor the concentration (activity) of solution was responsible for the pictures obtained. No difference was found in the pattern of distribution, whether plants were dried whole or sectioned, and whether oven temperatures varied from 40° to 120°C.

In normal oven-drying, more consistent results were obtained when harvested leaves were dried between blotters and kept flat during the process by sandwiching them between aluminum plates. To determine whether this artifact was due to a drying gradient arising in the leaves, some were placed in the oven between plates which had holes drilled into them. The regions where these holes occurred dried faster than the remainder of the leaves. Upon autoradiographing, it was found that, where drying had been faster, an accumulation of P^{32} occurred, whereas the rest of the leaf showed the appearance described above. When the rate of drying was adjusted, the distribution of P^{32} in the veins and parenchyma tissues became similar to that found after freeze-drying (Fig. 1, right). It thus appears that the artifact reported is of a purely physical nature. It is probable that cells rendered permeable by heating allow a rather free movement of solution from the veins, which dry slowly, to the veinlets and parenchymatous tissues of the leaves, which dry faster.

These results indicate that for accurate autoradiographic studies of the distribution of mobile isotopes, freeze-drying of plant material is essential even when treatment periods exceed 24 hours. They may help interpret some unexpected results obtained with autoradiographs which have been explained on a metabolic basis, such as accumulation of nutrients in leaf edges, erratic distribution of isotopes applied to leaves, and accumulation of tracers in cells simultaneously with what appears to be lack of movement of chemicals (*8*).

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