trum. Since the wavelengths of light absorbed by the pigment are similar to those effective in phototropism, it is possible that the porphyrin is the photosensitive pigment; however, further investigation will be required to determine whether this interpretation is correct (*5*).

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Antigen Disappearance in Hibernating Ground Squirrels

Abstract. The rate of antigen disappearance was studied in hibernating ground squirrels (*Citellus tridecemlineatus*) injected with I^{131}-labeled bovine serum albumin. There was no detectable disappearance of antigen during 14 days of hibernation. The induction period, however, ended 5 days after arousal as compared to a 7-day induction period in nonhibernating ground squirrels.

The resistance of hibernating animals to infection has been studied by a number of workers (*1*). It would appear that resistance to infection is increased with entry into hibernation. There is no evidence to suggest that increased resistance to infection is other than a nonspecific effect associated with physiological changes accompanying this process. To our knowledge, the immune response of mammals in hibernation has not been studied. The present study is concerned with antigen disappearance in the hibernating ground squirrel, *Citellus tridecemlineatus* (*2*).

The ground squirrels were collected in northern Illinois during September 1960 and were maintained in a room at 23°C for 6 weeks before use. During this period they were individually caged and allowed free access to Rockland Guinea Pig Diet with supplements of carrots twice weekly. Hibernation was induced by placing the animals, individually caged in a deep bed of wood shavings without food or water, in a room at 5°C and 50-percent relative humidity. They were checked twice daily to determine their state of hibernation.

We followed the disappearance of I^{131}-labeled bovine serum albumin from the circulation of hibernating and nonhibernating ground squirrels. All squirrels received a single intraperitoneal injection of 10 mg of bovine serum albumin labeled with I^{131} by the method of Talmage *et al.* (*3*). Serum from ground squirrels was iodinated and injected by the same procedure.

Blood samples from the tail were collected on tared filter paper, weighed, and counted in a well-type scintillation counter. Counts were corrected for background, disintegration, and weight of sample, converted to percentage of the activity of the sample taken 1 day after antigen injection, and plotted on a semilogarithmic scale. The rate of antigen disappearance was in three phases, as indicated by marked changes in the slope of the plot. These three phases were, in the terminology of Dixon *et al.* (*4*), (i) the equilibration phase, (ii) the nonimmune elimination phase, and (iii) the immune disappearance phase which marks the appearance of antibody.

In Fig. 1 the mean slopes are plotted for each phase of antigen disappearance in four treatment groups. The mean slope for the period of equilibration is an average of the slopes of that phase for the individuals in the group. The mean slopes for the nonimmune elimination and the immune phases were obtained by means of the same method.

Group A was composed of 14 squirrels that remained at room temperature after antigen was injected. The first blood sample was taken 1 day after the injection, four during the next 6 days, and three in the course of the next 5 days. The period of equilibration was less than 1 day in ten animals and as long as 3.5 days in four animals where the mean half-disappearance time was 2.3 days. During the nonimmune elimination phase that followed, the rate of antigen disappearance slowed so that the antigen level decreased by half in 5.4 days. On the seventh day after antigen injection the half-disappearance time decreased to 1.5 days. This increase in rate of disappearance is considered to mark the end of the induction period and the beginning of the immune phase wherein the appearance of antibody in the circulation is followed by rapid removal of the circulating antigen-antibody complexes. This sequence is qualitatively similar to that observed by Dixon *et al.* (*4*) in rabbits.

Group B was composed of 11 animals that were placed in the cold room immediately after antigen injection. They all entered hibernation within a day. During the 2-week hibernation period only four samples were taken; after arousal the sampling schedule was the same as for group A. There was little or no disappearance of antigen during the period of hibernation. After arousal, the half-disappearance time became 4.4 days, which was not statistically different

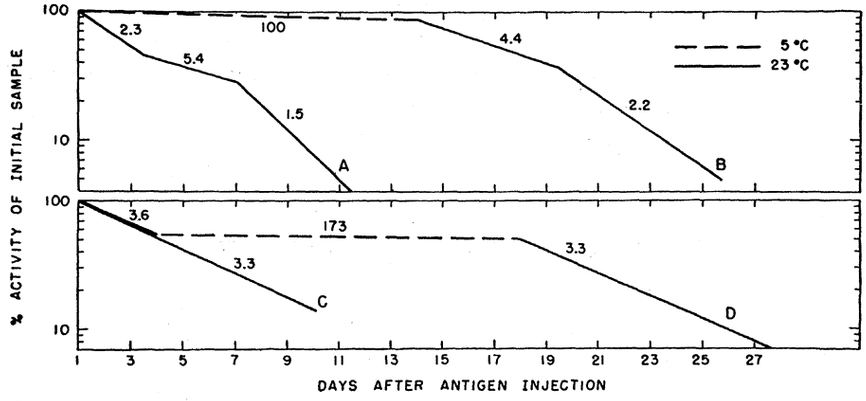

Fig. 1. A semilogarithmic plot of the mean disappearance curves of I^{131}-labeled bovine serum albumin in groups A and B, and of I^{131}-labeled ground squirrel serum in groups C and D. After injection of labeled material on day 0: (*A*) 14 animals maintained at 23°C; (*B*) 11 animals maintained at 5°C for 14 days in hibernation followed by a return to 23°C; (*C*) 4 animals maintained at 23°C; (*D*) 5 animals at 23°C for 4 days, then in hibernation at 5°C for 14 days followed by a return to 23°C. The numbers associated with each phase of the plot show the half-disappearance time in days.

from that of the nonimmune elimination phase in group A (5.4 days, $p > .20$). The induction period ended after 5 days when the half-disappearance time decreased to 2.2 days. The statistical significance of the difference between the immune disappearance times for groups A (1.5 days) and B (2.2 days) was equivocal ($p = .05$), but the difference between the induction period for group A (7 days) and the posthibernation induction period of group B (5 days) was significant ($p < .001$).

To determine whether the rate of disappearance of the antigen upon awakening from hibernation was comparable to the nonimmune disappearance rate or was actually the result of physiological processes peculiar to arousal, we studied the disappearance rate of labeled squirrel serum.

In four squirrels (group C) injected with labeled serum, the half-disappearance time was 3.3 days. In five squirrels treated similarly (group D) that entered hibernation 4 days after serum injection, the half-disappearance time upon arousal from 14 days of hibernation was 3.3 days. This was almost identical with the half-disappearance time of 3.6 days for the same animals during the 4 days before hibernation and for the group C animals that did not hibernate. In group D, as in group B, there was little or no disappearance of the labeled material during hibernation. Therefore, it is safe to assume that the disappearance rate of antigen in squirrels after arousal is the reflection of a physiological state (measured by antigen disappearance) comparable with that of the nonhibernating ground squirrels.

Although there is little or no disappearance of homologous or heterologous proteins from the circulation of hibernating ground squirrels, the induction period ends 5, not 7, days after arousal (group B, Fig. 1). Since the normal induction period is 7 days (group A, Fig. 1), it would appear that some of the events that occurred during the induction period transpired while the ground squirrels were hibernating. Work is in progress to determine whether the whole or only part of the induction period can be passed in hibernation.

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18 May 1961

Development of Needle Blight Symptoms on Rooted Cuttings from Diseased White Pine Trees

Abstract. A method of rooting cuttings from white pine trees is described. Approximately equal numbers of cuttings from diseased and healthy trees were rooted by this method. After the rooted cuttings were planted in the field, they were closely watched for root growth and the development of foliar injuries.

The disease, needle blight of eastern white pine (*Pinus strobus L.*), is characterized by an orange-red discoloration of the distal portions of current-year needles. For more than 50 years it was believed that the disease affected the tips of needles first, and that this was followed by a progressive dieback. To the contrary, it was recently discovered (*1*) that the injury began in semimature leaf tissues and then spread distally to more mature tissues. Based on data with respect to the nature and occurrence of the foliar symptoms, the hypothesis was set forth that needle blight is heritable (*1*).

Support for this theory would ensue if shoots removed from diseased or healthy trees and induced to root should produce phenotypic reactions similar to their parents when grown under similar environmental conditions. The question also arose whether cuttings from needle-blighted trees could be rooted, since diseased trees possibly lack food reserves because of reduced needle and shoot growth (*2*) and death of distal ends of needles.

Cuttings were taken from trees with histories either of disease recurrence or of freedom from blighting. The selected trees were 10 to 13 years old, which is considered fairly old by forest geneticists for obtaining high percentages of successfully rooted cuttings (*3*). Most conifer cutting experiments involve seedlings 3 to 6 years old. Trees this young, however, usually do not possess sufficient shoots to provide large enough clones for further experimentation.

Twenty-five cuttings were removed from each of four trees (two diseased and two healthy) in early September 1958, after shoot growth had ceased and buds had fully developed. Lateral shoots, 3 to 5 inches long, the full length of the current growth, were pulled from the trees. The basal fascicles were not removed. The "heels" were trimmed with a sharp knife, and the bases of the cuttings were dipped in 0.2 percent indolebutyric acid in talcum powder. The cuttings were placed immediately into pots 12 inches in diameter and 8 inches high. The medium used was Perlite (*4*), a chemically inert substance with good drainage and aeration properties. About one-third of the length of the cuttings was inserted into the firmed, moistened medium, and the cuttings were spaced about 1½ inches apart with 2 inches between rows. The 25 cuttings from each tree were placed in a separate pot. Wire hoops placed in the pots formed supports for thin polyethylene plastic covers. These covers were removed once a day, and the cuttings were sprayed with water until a fine film covered the needles. The covers helped maintain high humidities about the cuttings and also allowed an exchange of gases. The pots were kept at room temperature on a bench adjacent to

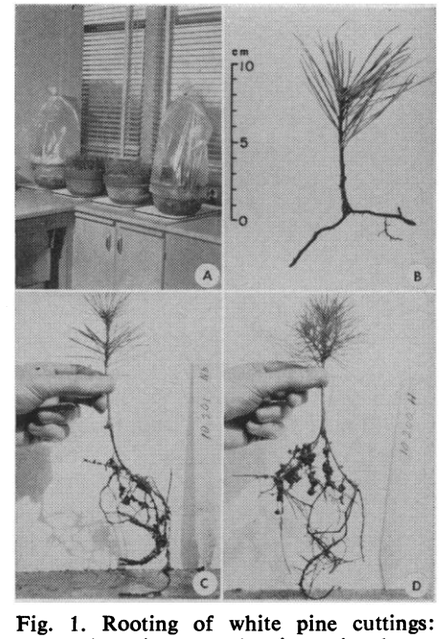

Fig. 1. Rooting of white pine cuttings: *A*, cuttings in pots showing wire hoops and polyethylene covers; *B*, cutting from a needle-blighted tree that rooted within 8 months; *C*, rooted cutting from a diseased tree 1 year after striking roots; *D*, rooted cutting from a healthy tree 1 year after striking roots.