

for diffusion and for the measurement of inhibition zone length.

Dilutions of antibiotics in nutrient broth are made up and poured into test tubes of such size as to accommodate two capillaries each. The volume of antibiotic solution is not important, about 2-4 ml being adequate. The test tubes containing antibiotic solution and capillaries are stoppered to prevent evaporation during incubation.

By a proper choice of test organisms and nutrient solution for each class of antibiotics, it is possible to find conditions which provide a sharp zone of intense aerobic growth of the test organism at the upper end of the inhibition column. Above this zone of growth almost no visible growth occurs, due to the absence of oxygen. The position of this sharp zone of aerobic growth depends upon concentration of the antibiotic. When the seeded agar is made with nutrient broth, the linear relation of log concentration versus zone length is approximated over the range from below 1 unit to above 1000 units per cc of streptomycin. If the seeded agar is made without nutrient, improved slope (zone length increase for a ten-fold increase in concentration) results, but with a radical deviation from linear of the log concentration versus zone length curve.

Automatic devices have been provided for rapid reading of zone lengths and for cleaning of capillaries and test tubes with steam after completion of the experiment.

The method has been applied successfully to the assay of streptomycin, dihydrostreptomycin, penicillin-G, and an alkyl thiomethylpenicillin using *B. subtilis* spores. Sharper zones of aerobic growth have been produced in assays of streptomycin than of penicillin, but the method appears to be adaptable to any diffusible antibiotic. The special conditions optimal for this test depend strikingly on the antibiotic being tested, upon the composition of the nutrient broth, and upon pH.

A standard curve is prepared from a series of known dilutions of the antibiotic, and the potencies of the unknowns are referred to this curve.

Test procedure and equipment for carrying it out, as well as factors determining slope, sharpness of zones, and other aspects of performances, will be described elsewhere.

Potentiality for Testicular Recrudescence during the Annual Refractory Period of the Golden-crowned Sparrow

Alden H. Miller

Museum of Vertebrate Zoology,
University of California, Berkeley

The inability of the pituitary-gonad mechanism of adult and immature golden-crowned sparrows (*Zonotrichia coronata*) to respond to light stimulation in the early autumn following arrival on the winter range has been demonstrated by experiments recently reported (3). This refractoriness was thought to be an attribute of the pituitary gland, but conceivably the gonad is refractory

instead, or in addition. In the autumn of 1948, during the refractory period of this species, gonadotropic hormone was administered to five males; two others served as controls. Pregnant mare serum was used (gonadin) from a preparation kindly furnished by the Cutter Laboratories of Berkeley. Daily dosage consisted of 1 cc of serum which contained approximately 50 international units. Controls received the same amount of physiological saline. Injections were made intramuscularly in the breast. Treatment began on October 19, some two weeks after completion of the fall migration, and all birds were autopsied by November 3 before the known conclusion of the light-refractory period.

The response of the testis was rapid and decisive in all experimental individuals. Increase in volume of the left testis from less than 1 mm³ to 4.2 mm³ occurred in 4 days; and one individual at the end of 14 days attained a testis volume of 20.9 mm³. Histologically this largest testis had reached stage 4 of Blanchard (1), with functional interstitial cells and primary spermatocytes in synapsis. The controls showed volumes of 0.26 mm³ and 0.78 mm³ at the end of the experiment on November 3.

The results indicate that the testis is able to respond to follicle-stimulating hormone during the period when this species of sparrow is refractory to increasing light dosages. The experiment, of course, does not eliminate the possibility that the testis at that time is unresponsive to the gonadotropic hormone of its own pituitary or that it may have a somewhat elevated threshold of response during that period. There seems no doubt, however, that the testis is capable of recrudescence if given a sufficient stimulus. Moreover, this is true equally of the immature testis that has never before enlarged and of the adult testis that previously has been functional, since both immature and adult birds were included in the experiment.

Kirschbaum *et al.* (2) have reported that the testis of young male English sparrows (*Passer domesticus*) can be stimulated at an early age by pregnant mare serum; adults also respond. However, in this species refractoriness to light stimulation occurs only in the adult in the autumn, immature birds showing recrudescence at that season, according to Riley (4). Nevertheless, the situation seems to be similar in the two species with respect to the capability of the testis to respond at all times to gonadotropic hormone and the apparent responsibility of the pituitary for refractoriness to light when and if this appears. Caution must be sounded against assuming that there are identical physiological mechanisms or systems of response in these species of sparrows, for the English sparrow is actually misnamed a sparrow and belongs to the weaver finch group, currently recognized as a family distinct from the Fringillidae to which the golden-crowned sparrow belongs. Moreover, the English sparrow is nonmigratory.

References

1. BLANCHARD, B. D. *Univ. Calif. Publ. Zool.*, 1941, **46**, 54.
2. KIRSCHBAUM, A., *et al.* *Anat. Rec.*, 1939, **75**, 257.
3. MILLER, A. H. *J. exp. Zool.*, 1948, **109**, 1.
4. RILEY, G. M. *Proc. Soc. exp. Biol. Med.*, 1936, **34**, 331.