

A POX VIRUS OF THE SLATE-COLORED JUNCO

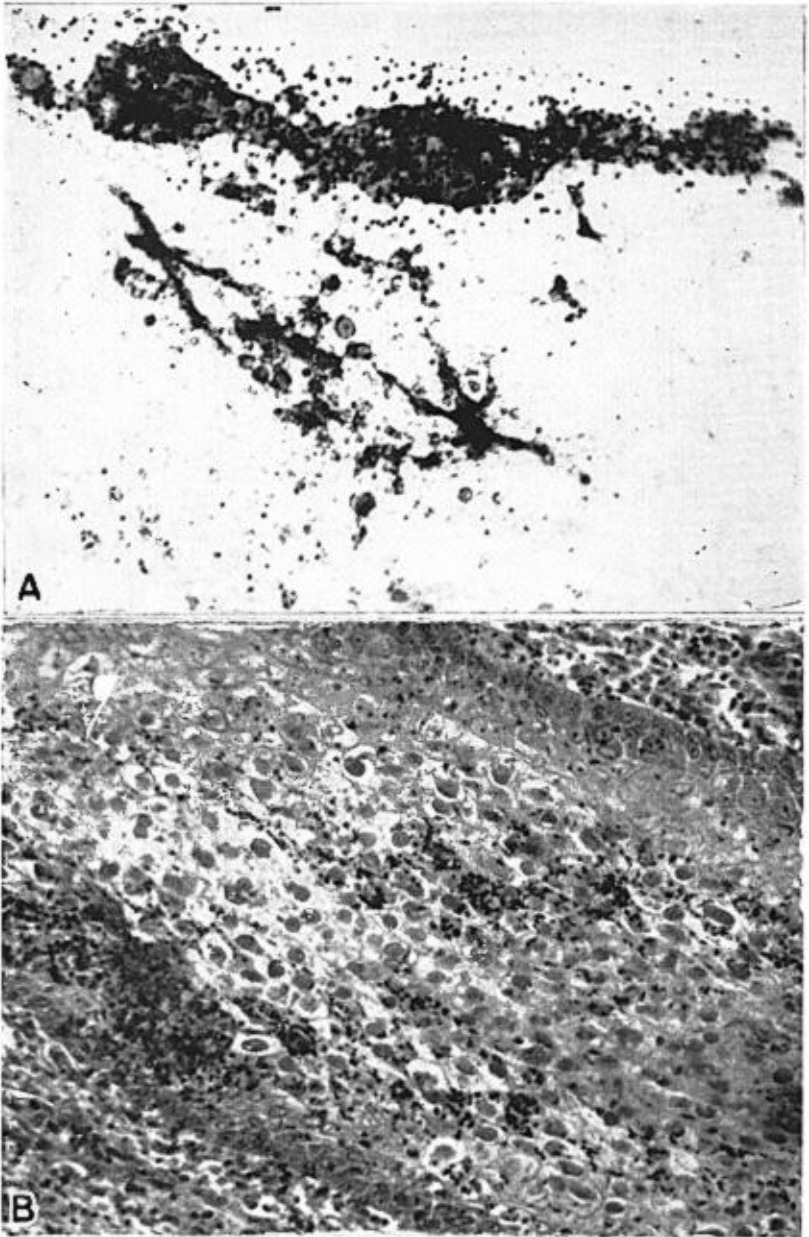
BY C. BROOKE WORTH

MUSSELMAN (1928) first reported foot-disease in Chipping Sparrows (*Spizella passerina*) and demonstrated that it could be transmitted by successively pricking the tumor-like lesion on the toe of a diseased bird and immediately pricking a healthy bird's toe with the contaminated needle. Although authorities have long suspected that the disease is caused by a virus, that fact has not heretofore been confirmed. This paper reports a demonstration of the viral etiology of "foot-disease" in a closely related species of bird and also characterizes some of the unique aspects of the virus. The work here reported was performed under the direction of Dr. Carl TenBroeck, while the author was Assistant in Animal Pathology at The Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, Princeton, New Jersey, on leave of absence from the Department of Biology, Swarthmore College, Swarthmore, Pennsylvania.

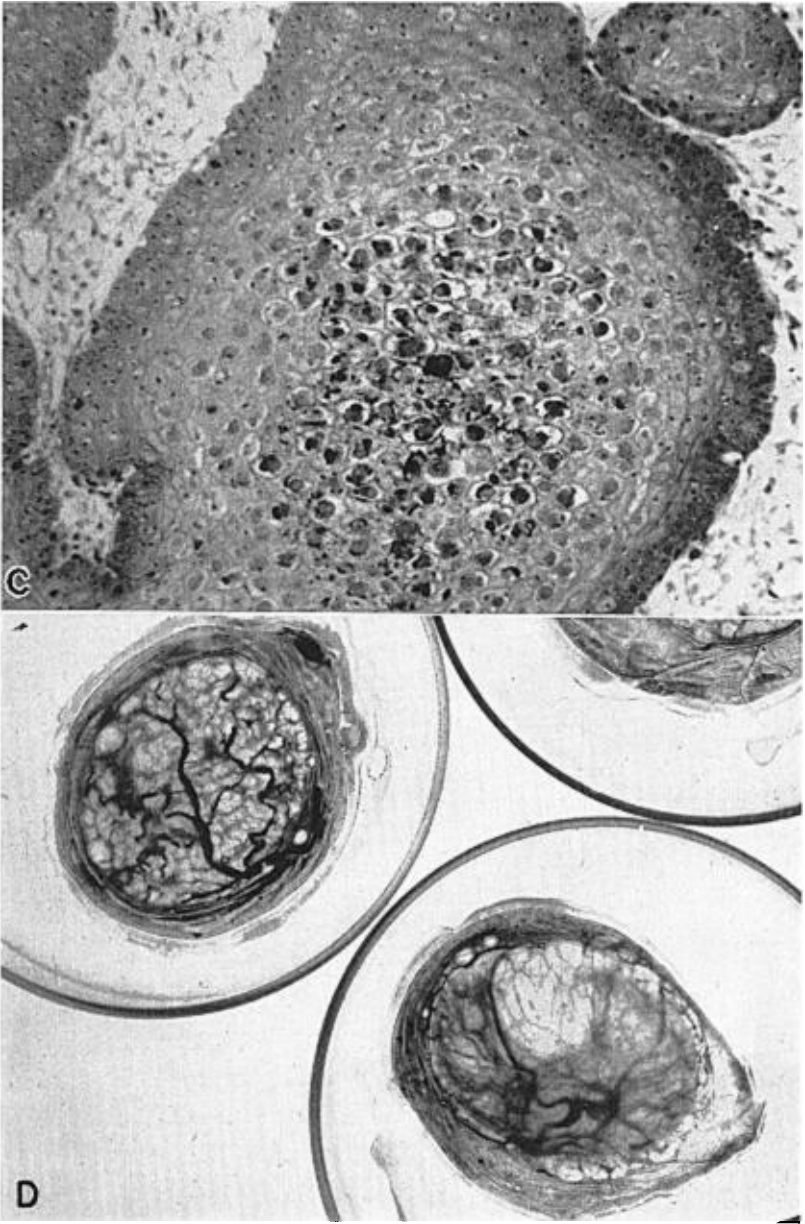
"Foot-disease."—Bird-banders are almost universally familiar with foot-disease. It may be seen in a wide variety of small, ground-feeding birds, especially those of the finch family, and I have also observed it naturally (but infrequently) in the Blue Jay (*Cyanocitta cristata*), and even in the Chimney Swift (*Chaetura pelagica*). Although lesions may occur at the angle of the beak and elsewhere, they are located most typically on one or more of the toes. A lesion consists of an indurated swelling, up to 5 mm. in diameter, that becomes more or less spherical. After a week or two it may regress, or else it softens and breaks open. In the latter case secondary infection may lead to the loss of the toe, the foot, or the entire tarsus.

Isolation of the virus.—Late in 1939, an opportunity to study foot-disease in the Slate-colored Junco (*Junco hyemalis*) presented itself. A bird-trapping and banding station was established at Princeton, New Jersey, and as cold weather set in, many Juncos entered the traps. The observed incidence of foot lesions in these birds was about one per cent.

The diseased toe of one randomly selected Junco was incised and material within the nodule was suspended in one ml. of sterile physiological salt solution. This suspension was inoculated into: the toe pad of an apparently healthy Junco; the toe pad of a chicken; and some freshly denuded and scarified feather follicles of both a Junco and a chicken. The chicken's toe failed to respond, but a typical lesion was reproduced in the Junco's toe. The cutaneous follicles



A POX VIRUS OF THE SLATE-COLORED JUNCO. A. Elementary bodies in the chorio-allantoic membrane of the hen's egg. $\times 1500$. B. Inclusion bodies in ectodermal cells of chicken skin. $\times 282$.



A POX VIRUS OF THE SLATE-COLORED JUNCO. C. Inclusion bodies in hyperplastic ectoderm of embryonated hen's egg. $\times 235$. D. Infected chorio-allantoic membranes of hens' eggs inoculated with Junco-virus. $\times 0.8$.

of both the Junco and the chicken developed nodules that were indistinguishable from lesions of fowl-pox as commonly seen by poultrymen.

On the seventh day of its infection the chicken was killed and material from a follicular nodule was obtained aseptically from the flesh side of its skin. This matter was triturated, suspended in sterile physiological salt solution and deposited on the surface of the chorio-allantoic membrane of ten-day-embryonated hens' eggs. Seven days after inoculation the membranes showed discrete nodular opacities up to 5 mm. in diameter. The membranes were harvested, triturated, suspended in sterile saline solution and passed successively through nine additional series of hens' eggs. By this time the inoculation of undiluted membrane suspension caused a massive, confluent lesion of the recipient chorio-allantoic membrane, while discrete lesions could always be produced by suspensions diluted to 10^{-5} and sometimes to 10^{-6} .

Films from infected chicken skin and egg membranes, when stained by Morosow's silver-impregnation technique (1926), showed abundant elementary bodies (Plate 12, A). Histologic sections of the corresponding tissues revealed many ectodermal cells occupied by large intracytoplasmic inclusion bodies (Plate 12, B; Plate 13, C).

Suspensions of heavily infected egg membranes, when inoculated into Juncos' toes, caused the appearance of nodules resembling the initial lesion of the wild-caught Junco. The inoculum, incubated in broth tubes, failed to reveal bacterial contamination, nor was there detectable bacterial growth in hens' eggs after seven days' incubation.

It was concluded that the agent isolated from the Junco's toe was a virus of the pox-producing type.

Cross-immunity tests.—A suspension of Junco-virus was rubbed into freshly denuded and abraded feather follicles of several chickens. Another group of chickens was similarly infected with a strain of typical fowl-pox virus. All birds developed typical and identical follicular nodules. After one to three months, when the lesions had regressed, all birds were reinoculated at fresh cutaneous sites with the virus to which they had originally been exposed but also with the opposite virus. A third group of chickens, never exposed to either virus, was inoculated with both viruses simultaneously. The latter birds (controls) showed typical reactions to both viruses.

Chickens originally inoculated with Junco-virus showed no reaction to a second exposure to this agent but gave full reaction to fowl-pox virus. Chickens originally inoculated with fowl-pox virus showed no

reaction to a second exposure to this agent but gave slight or moderate reactions to Junco-virus.

Cross-immunity tests with other avian pox viruses were not performed.

Host range.—Typical cutaneous follicular lesions were produced by egg-membrane suspensions of Junco-virus in: squabs (*Columba livia*), a Blue Jay (*Cyanocitta cristata*), White-throated Sparrows (*Zonotrichia albicollis*), Fox Sparrows (*Passerella iliaca*), and Song Sparrows (*Melospiza melodia*). Lesions failed to appear in: unconfined adult pigeons (*Columba livia*), a Mourning Dove (*Zenaidura macroura*), nestling and adult Screech Owls (*Otus asio*), House Sparrows (*Passer domesticus*), Tree Sparrows (*Spizella arborea*), and a Laughing Gull (*Larus atricilla*). Lesions failed to appear also in laboratory rabbits, white mice, and a single human volunteer following shaving and scarification of the skin with subsequent exposure to a rich virus suspension.

Egg-membrane reactions. Macroscopic.—The most striking feature of the heavily infected egg-membrane, inoculated on the tenth day of incubation and harvested at seventeen to twenty days, was edema. Some membranes weighed as much as one-third of the embryo's bulk. The superficial blood vessels were frequently markedly dilated (Plate 13, D). Dehydration of an embryo sometimes led to its death. However chicks were hatched successfully from some heavily infected eggs, and these birds were found to be susceptible to cutaneous infection with Junco-virus at four months of age.

Microscopic.—Histologic sections of seventeen-day egg membranes showed extreme thickening and hyperplasia of the chorionic ectodermal layer (Plate 13, C). The mesoderm was greatly thinned out by the edema. No evidence of cellular infiltration could be seen, but this may have been because of the rarefaction of such elements by the edema. The endoderm showed no reaction. The ectodermal proliferation seemed to be most active at the deepest, or germinal, level, since this tissue buckled inward at many points, causing an appearance as of long, finger-like extensions of ectoderm invading the mesoderm.

Discussion.—The strain of Junco-virus here described differs from other known bird-pox viruses in several respects. That it has distant relationship to fowl-pox virus has been demonstrated by total cross-immunity in one direction but only partial cross-immunity in the opposite direction. Its behavior in embryonated hens' eggs also is at variance with the fowl-pox reaction, for the latter is fully evident by the third day, when the Junco-pox reaction is still only partially developed. Moreover edema is less evident in the fowl-pox reaction,

and the endoderm in fowl-pox-infected eggs may show hyperplasia with a few cells containing inclusion bodies (Woodruff and Goodpasture, 1931).

References in the literature to other avian viruses throw additional light on the unique nature of Junco-virus. Pigeon-pox virus produces the same reaction in hens' eggs as fowl-pox virus (Beaudette and Hudson, 1938). However the susceptibility of squabs but not "wild" adult farm pigeons to Junco-virus suggests that the adults may have had a pox disease that protects them from Junco-virus, so that Junco-pox may be related to pigeon-pox. Canary-pox virus cannot be transmitted to chickens (Lahaye, 1930; Kikuth and Gollub, 1932; Eberbeck and Kayser, 1932; Burnet, 1933). Virus from quail (species?) imported from Mexico that developed "epithelioma contagiosum" caused pock lesions in chickens but not as severe as those of ordinary fowl-pox (Gallagher, 1916).

Failure of Tree Sparrows to respond to inoculation with Junco-virus may reflect true immunity through prior exposure, since "foot-disease" was first recognized in Chipping Sparrows belonging to the same avian genus. But species immunity in the Tree Sparrow would be astonishing in view of the ability of Junco-virus to infect birds far removed from the Family Fringillidae.

Acknowledgement.—The assistance of Dr. John B. Nelson, who supplied the fowl-pox virus used in this investigation and also made valuable technical suggestions, is gratefully acknowledged.

Summary and conclusions.—A virus was isolated from a nodule on the toe of a Slate-colored Junco afflicted with "foot-disease." The virus was easily grown in chicken skin and in embryonated hens' eggs.

Cross-immunity tests in chickens with Junco-virus and fowl-pox virus indicated that the viruses, although possibly related, differ widely from one another.

The most characteristic effects of Junco-virus on the chorio-allantoic membrane of embryonated hens' eggs are: proliferation of the deeply-lying ectodermal cells and extreme edema of the mesoderm.

The host-range of Junco-virus was partially investigated. Squabs, a Blue Jay, and White-throated, Fox, and Song sparrows were successfully infected.

The behavior of the virus does not conform to reports on other bird-pox viruses in the literature, including fowl-, pigeon- and canary-pox viruses. However the susceptibility of squabs but not feral adult pigeons to infection by Junco-virus may indicate a relationship of this virus to pigeon-pox virus

LITERATURE CITED

- BEAUDETTE, F. R., and C. B. HUDSON. 1938. Cultivation of pigeon-pox virus on the choriollantoic membrane. Journ. Amer. Vet. Med. Assoc., **43**: 146-150.
- BURNET, F. M. 1933. A virus of the Canary of the Fowl-pox Group. Journ. Path. and Bact., **37**: 107-122.
- EBERBECK, E., and W. KAYSER. 1932. Über das Vorkommen von Pockenerkrankungen bei Kanarienvögeln, Buchfinken und Sperlingen. Archiv für Tierheilkunde, **65**: 307.
- GALLAGHER, B. 1916. Epithelioma Contagiosum of Quail. Journ. Amer. Vet. Med. Assoc., **50**: 366.
- KIKUTH, W., and H. GOLLUB. 1932. Versuche mit einem filtrierbaren Virus bei einer übertragbaren Kanarienvogelkrankheit. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, **125**: 313-320.
- LAHAYE, J. 1930. Contribution à l'étude comparative de diverses varioles animales. Annales de Médecine Vétérinaire, **75**: 515.
- MOROSOW, M. A. 1926. Die Färbung der Paschenschen Körperschen durch Versilberung. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, **100**: 385.
- MUSSELMAN, T. E. 1928. Foot Disease of the Chipping Sparrow (*Spizella passerina*). Auk, **45**: 137-147.
- WOODRUFF, A. M., and E. W. GOODPASTURE. 1931. The susceptibility of the chorio-allantoic membrane of chick embryos to infection with the fowl-pox virus. Amer. Journ. Path., **7**: 209-222.

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