

DISCUSSION

M. A. Grayson:¹ During the past 10 years, particularly in 1961 and 1962, we have found 11 avian species in Panama naturally infected with Venezuelan encephalitis virus. The virus was detected in their tissues or blood, or through antibodies in the birds' blood serum. We have also subcutaneously injected the Panamanian 3880 strain of the virus into five native bird species. All the native birds were given approximately 100 weanling mouse intraperitoneal LD₅₀ of the 3880 strain and were then bled daily for one week, after which they were sampled periodically for antibodies. Very low titers of VE virus circulated in the gray-headed chachalaca and the black vulture for a few days, but the yellow-backed oriole, mealy parrot, and striated heron were considerably more viremic.

To my knowledge, the only naturally infected viremic bird so far reported has been *Butorides virescens*, or the green heron. In 1962 we captured an apparently sick green heron fledgling in the grassy edge of a fresh water swamp, bled it in the field, and sent it alive back to our laboratory where we observed it for 13 days. Virus was isolated from the blood serum obtained in the field, and we detected HI antibody titers of 1:20 and 1:80 in specimens taken six and 13 days, respectively, after the bird's capture. The bird thus proved viremic, and converted serologically within about two weeks.

As a group, green herons are distributed worldwide in temperate and tropical climates. The taxonomy of the group is a little confused: some authorities believe it is a superspecies comprising several species, while others say it is several subspecies composing one species, which they call *B. striatus*. At any rate, the many recognized subspecies or races may be divided into three major morphologic groups.

One group is found in Australia, Asia, Africa, and the islands of the Indian and Pacific oceans; another inhabits North and Central America and the Caribbean islands, and the third group is to be found in South America and Panama.

The last group consists of two types, one of which I will refer to as *B. striatus*, or the striated heron, which ranges in Panama from the Colombian border to the central part of the Isthmus. The other type in the last group, *B. virescens*, consists of two races. One, *B. virescens maculatus*, is a very common resident of the Almirante area of western Panama where we isolated virus from it. *B. virescens virescens*, the other race, is a common winter visitor to Panama, particularly the central and western parts, between October and April. It breeds from southern Ontario and Quebec through the Atlantic seaboard and into the southern part of Mexico. Since our virus isolation from the green heron fledgling was made in August, we feel it came from a *B. virescens maculatus* rather than one of the migratory variety.

Hérons may be found not only on sandy coastal beaches but also along coastal rivers and estuaries. They are common where the crab hole-breeding *Deinocerites pulex*, one of the epidemic vector mosquito species, lives.

In addition to finding natural VE virus infection in the green heron, we conducted some experiments with the striated heron. Nine wild-caught, serologically negative birds were intra-abdominally injected with approximately 100 mouse intraperitoneal LD₅₀ of the 3880 viral strain, and all developed viremia within the first four or five days after injection. Peak titers, which we express as weanling mouse intraperitoneal LD₅₀ per 0.02 of a milliliter of inoculum or of plasma, were recorded on the second day after injection. These titers were apparently high enough to infect at least some known vector species of mosquitoes.

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In additional experiments with the same species of heron, we were able to infect several birds with the virus. Once we fed two mosquito species on an infected heron and recorded titers of 3 to 4 logs of virus, and both species later transmitted the virus to uninfected golden hamsters in the laboratory. The mosquito species used in these transmission experiments were *D. psendes*, an apparent vector of epidemic VE in Texas and perhaps Costa Rica that has been found infected in endemic areas of Mexico, and *Culex aikenii*, a vector of endemic VE in Panama. In one instance, *C. aikenii* transmitted virus from an infected to an uninfected striated heron, which four weeks later developed HI antibodies.

There is a great deal to be learned about the role of birds in the natural history of endemic VE. Although they may further the maintenance or amplification of virus activity in enzootic cycles, I feel that their real importance—if it exists—lies in their migratory habits and consequent distribution of the virus from one geographic area to another. Although most migration studies have dealt with European and North American birds, tropical birds do migrate. W. H. Hudson, the author of *Green Mansions*, showed many years ago that some Argentine species migrate toward the equator at the start of the Argentine winter and return at the onset of the Argentine summer. More recently, Wetmore has found in intensive studies that tropical South American birds migrate considerable distances.