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A TURTLE COLONY EPIZOOTIC APPARENTLY OF MICROBIAL ORIGIN

A septicemic, cutaneous ulcerative disease in laboratory colony turtles was reported by Kaplan (1957, Lab. Animal Care 7: 273-277). The disease was apparently introduced by several successive shipments of turtles (taxonomic identity not specified) from the north central region of the United States. Within 1 to 2 weeks, most of the turtles in the colony tank became lethargic and developed cutaneous ulcers on the limbs and fleshy areas of the body. Upon autopsy the animals were said to have multiple areas of necrosis in the viscera. Only one species, *Escherichia freundii* (now *Citrobacter*) was claimed to have been regularly isolated from cutaneous lesions, heart blood, and affected internal organs. Fresh saline suspensions of the bacterium grown on agar slants were reported to reproduce the disease when injected into apparently healthy turtles.

In January 1970, we received a single shipment of 6 males and 6 females of the painted turtle, *Chrysemys picta*, from the Minnesota-Wisconsin area. All specimens were sexually mature, uniformly large adults, especially selected for animal behavior studies. A few small lesions noted on three of the animals were assumed to be the result of mechanical abrasion during transport and not requiring treatment. The turtles were put into a large indoor tank which contained specimens representing the following taxa: *Pseudemys scripta elegans* (red-eared turtle), *P. s. scripta* (yellow-bellied turtle), *P. concinna suwanniensis* (Suwannee terrapin), *P. floridana peninsularis* (peninsula cooter), *P. nelsoni* (Florida red-bellied turtle), and *Malaclemys terrapin pileata* (Mississippi diamondback terrapin). Prior to the introduction, all of the resident turtles in the tank appeared healthy and were feeding normally. Some of the specimens had been captive for several years. Within a week, a number of individuals, which included all species present, developed whitish cutaneous patches (Figure 1) which agreed essentially with those observed by Kaplan (op. cit.). Feeding

began to decline as lethargy increased. On the 9th day following the introduction of the shipment into the tank, a male *Chrysemys picta* died.

Material collected on swabs from lesions was cultured for the isolation of gram negative aerobic rod forms. Material from each observable colony type was studied biochemically for identification. The methods of Edwards and Ewing (1962, *Identification of the Enterobacteriaceae*, Burgess Publ. Co., 201 pp.) were used for the culture, isolation, and identification of the bacteria. A rich and varied microflora was encountered. *Citrobacter* strains that fit the somewhat incomplete description available from Kaplan's (op. cit.) work were present in the lesions. The mixed microflora of the lesions also included *Serratia* in nonpigmented form, *Proteus*, and *Pseudomonas*. *Escherichia* was present in very limited numbers. Many isolates did not fit well into any recognized named group; many of these appear to be related to the *Serratia* organisms.

Within two months of the introduction, 10 of the 12 *Chrysemys picta* died, as well as 7 additional specimens which represented all of the previously mentioned taxa except *Malaclemys*. Gross examination of the viscera in the autopsied specimens revealed essentially the picture described by Kaplan (op. cit.) with the additional observation that the lungs of a few individuals appeared somewhat congested.

When dead turtles were dissected to observe internal pathological changes, the first operation conducted on the frozen carcass was to open the heart with aseptic precautions and collect a generous sample of the heart contents. Every one of the 15 animals thus examined yielded positive culture. Cultures from different animals were dissimilar. Some hearts yielded a single colony type apparently in pure culture, others yielded two to several colony types. Gram positive cultures were not studied but the gram negative bacilli were of various

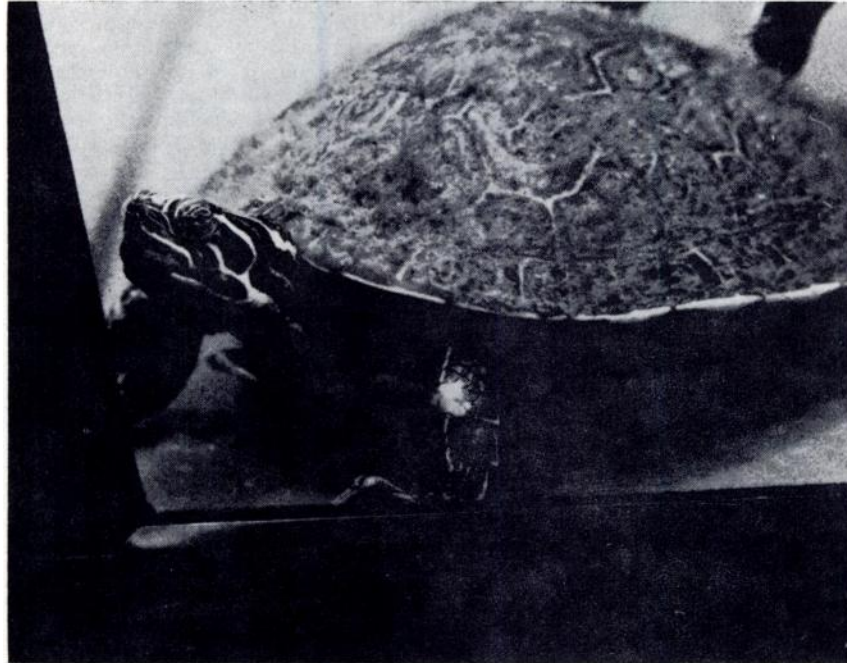


FIGURE 1. Cutaneous lesion on the left forelimb of *Pseudemys concinna suwanniensis*.

kinds. *Citrobacter* was recovered from 3 animals. From 5 animals *Aeromonas hydrophila* was secured, once as a pure culture. *Serratia* in nonpigmented form was encountered pure in one animal and mixed in 2 others. Organisms related to *Serratia* were numerous. *Proteus vulgaris* was found in 2 heart cultures and *Enterobacter hafniae* in 2.

Installation of an ultraviolet light source over the tank to provide several hours of UV radiation each day, and the addition of chloramphenicol to the water (approximately 250 mg/20 gallons) two or three times per week immediately prior to feeding, seem to be bringing the epizootic under control. The death rate has decreased sharply since these measures were instituted, but many individuals still possess the persistent whitish cutaneous patches. In our limited experience with this disease, we have noted juveniles and males to be somewhat less resistant. In the original description of

the epizootic, Kaplan (op. cit.) stated that his culture of *E. freundii* (*Citrobacter*) submitted to the Communicable Disease Center (CDC) in Atlanta for confirmation was diagnosed by the Center as mixed, containing *Serratia*. The *Serratia* he regarded as a contaminant, believing the causative organism of the disease to be solely *Citrobacter*, claiming *Serratia* was never isolated directly from the turtles.

On the basis of our experience, we suggest the possibility that a synergistic relationship exists between *Serratia* and *Citrobacter*. It seems possible that lipolytic, proteolytic action by *Serratia* on the exposed skin surfaces might facilitate subsequent entry into the body by *Citrobacter*, an organism capable of widespread systemic damage when it occurs internally outside the lumen of the gut. The potential pathological importance of colorless strains of *Serratia* has been mentioned by Fulton et al (1959, Can. J.

Microbiol. 5: 269-275), who also emphasized the ease with which the colorless forms could escape detection in routine examinations of enteric bacterial colonies. Duran-Reynals and Clausen (1937, J. Bact. 33: 369-380) described a new species, *Serratia anolium*, as a pathogen in a Cuban lizard. Clausen and Duran-Reynals (1937, Am. J. Path. 13: 441-451) reported that *Serratia anolium* when injected subcutaneously into the musk turtle (*Sternotherus odoratus*) produced lesions at the site of inoculation and was subsequently recoverable from the heart blood.

There is no clear way to evaluate the significance of our observations. The surface microflora of chelonians is rich in members of the family Enterobacteriaceae. Many forms seem to be present which have not been well studied, probably because they are infrequently encountered in the immediate microflora of man. In the present work, we observed *Citrobacter* which could well be the same organism studied by Kaplan (op. cit.). To the bacteriologist, the most impressive aspect would probably be the large assembly of forms related to *Serratia*.

These are forms which have received insufficient study and therefore methods for their analysis are still somewhat inadequate. The presence of *Aeromonas* among the microflora of turtles (Jackson et al, 1969, Assn. Southeastern Biologists Bull. 16 (2): 55; 1969, Bull. Wildlife Disease Assoc. 5: 328-329) has been repeatedly observed, and members of the genus can be pathogenic for fish and amphibians. While our observations do not contradict Kaplan's (op. cit.) conclusions, they seem to suggest that the problem may be broader than his work indicates.

Our experience in successfully maintaining laboratory turtle colonies covers fourteen years, and until the present epizootic occurred, we had never encountered any serious disease problem. While this pathogenic condition may be rare, we strongly recommend prompt and vigorous treatment be undertaken when cutaneous or behavioral symptoms appear. The highly fatal nature of this rapidly spreading disease poses a potentially grave problem where very rare or economically valuable species are being maintained.

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