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Intestinal Bacterial Flora and Transit Time of Three Neotropical Bat Species

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ABSTRACT

KLITE, P. D. (Middle America Research Unit, Balboa Heights, Canal Zone). Intestinal bacterial flora and transit time of three neotropical bat species. J. Bacteriol. **90**:375–379. 1965.—Quantitative studies on the intestinal bacterial flora of three neotropical bat species revealed the following average bacterial populations: *Molossus major*, $10^{4.8}$ bacteria per intestinal contents; *Carollia perspicillata*, $10^{3.3}$; *Chilonycteris rubiginosa*, $10^{3.9}$. In comparison, laboratory mice had an average of $10^{9.7}$ bacteria per intestinal contents. Of 236 bacterial isolates obtained from 60 bats, bacteria of the *Klebsiella-Aerobacter-Serratia* group were found most frequently, followed by enterococci and *Proteus* spp. Bacteria of eight other groups were less frequently recovered. A large intestine, cecum, or appendix was absent in all three bat species, and the intestinal length was one-third to one-fifth of that in a mouse of comparable weight. The transit time through the short bat intestine was 15 min. The possible relationship of these unusual anatomical and physiological phenomena to the ability of *Histoplasma capsulatum* to survive in bat feces is discussed.

Previous studies in this laboratory have demonstrated that neotropical bats on the Isthmus of Panama are naturally infected with *Histoplasma capsulatum* (Shacklette, Diercks, and Gale, 1962) and that some genera of chiropterans shed the fungus from intestinal lesions into their fecal contents (Klite and Diercks, 1965). These phenomena lead to the contamination of soil around bat harborages with *H. capsulatum* and the establishment of potential sites for human infection (Klite and Young, *in press*).

Although many pathogenic fungi have been found in feces-enriched soil (Ajello, 1956), the only other pathogenic, or potentially pathogenic, fungi that have previously been cultured directly from feces are *Candida albicans*, other *Candida* spp., *Geotrichum* spp., *Aspergillus* spp., and *Penicillium* spp. (Schnoor, 1939; Nachtigall and Von Riesen, 1959). The present investigation was undertaken to determine whether the intestinal environment of bats is, in some way, favorable for the survival of *H. capsulatum*. Data on the anatomy, bacterial flora, and transit time of the intestines of three bat species are presented.

MATERIALS AND METHODS

Test animals. Three species of Chiroptera, *Chilonycteris rubiginosa*, *Carollia perspicillata*, and *Molossus major*, previously shown to experience natural infection with *H. capsulatum* (Klite and Diercks, 1965), were chosen for study. The animals were captured in selected harborages, brought to the laboratory, and tested the same day.

Laboratory white mice, 4 to 6 weeks old, including ten mice inoculated intraperitoneally with approximately 10^6 *H. capsulatum* particles 1 month prior to testing, were also used in this study.

Bacterial studies. Twenty bats of each species and 20 mice, including the 10 previously inoculated with *H. capsulatum*, were killed with chloroform. Each animal was then weighed, immersed in antiseptic solution, and placed on a necropsy board. The skin over the abdomen was reflected; the peritoneum was incised, and the intestine was freed from its mesentery. The intestine was then cut below the pylorus and at the rectum, the length was measured, and, with a flat-edged forceps, the contents were milked into a sterile test tube. The fecal contents were suspended in 10 ml of saline. Serial 10-fold dilutions of the well-mixed stool suspension were then cultured on each of the following media: Eosin Methylene Blue (EMB) Agar, MacConkey Agar, Bismuth Sulfite Agar, Anaerobic Agar with blood, Thioglycollate Broth, LBS Agar, Blood Agar, and 5% blood-enriched Mycosel Agar (all media obtained from BBL). The bacterial cultures were incubated at 37 C for 48 hr; the fungus cultures, at 25 C for 30 days.

Total numbers of bacteria per intestinal contents in both bats and mice were estimated from

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the serial dilutions. From each of the above media, all morphologically different colonies were subcultured for identification according to the biochemical methods outlined by Edwards and Ewing (1962), with the use of TSI Agar, Urea Agar, Lactose Broth, Phenylalanine-deaminase Agar, Citrate Agar, Malonite Broth, Lysine Decarboxylase Broth, semisolid agar and the methyl red, indole, and acetylmethylcarbinol reactions (all media from BBL). Shigella, Alkalescens-Dispar, and Salmonella polyvalent antisera (Difco) were used to test organisms that gave biochemical reactions compatible with these groups.

Bacteria from the same bat recovered on different media, which gave identical biochemical reactions, were considered to be the same isolate. All organisms initially cultured anaerobically were subcultured both in Thioglycollate Broth and aerobically on EMB Agar to reaffirm their anaerobic growth. Facultative anaerobes, which, when subsequently cultured aerobically, gave biochemical reactions identical to an isolate obtained aerobically, were also considered to be the same isolate.

The *p*H of the intestinal contents of the bats and mice was determined from the undiluted saline suspension of intestinal contents on a Beckman Zeromatic *p*H meter. These measurements were made 2 to 3 hr after necropsy.

Transit time. Under light ether anesthesia, five bats of each species and five mice were intubated with a 21-gauge polyethylene catheter via the orogastric route, and 0.25 ml of indigo-carmine dye was introduced through the tube. The animals were killed at 5, 15, 30, 60, and 120 min, and the distance the dye had travelled was measured. India ink, similarly injected, was also used as a marker in three additional *Carollia*.

RESULTS

Anatomy of intestines. Several striking differences were evident in the gross anatomy of mouse and bat intestine. A large intestine, cecum, or appendix was not present in the bats, and their total intestinal length was shorter than that in a mouse of comparable weight. The mouse intestine was three times as long as that of *Chilonycteris* or *Carollia* and four to five times as long as that of the smaller *Molossus* (Table 1).

Measurements of pH. The *p*H of the intestinal contents of both bats and mice was slightly acid (Table 1). All animals gave *p*H values between 6.0 and 7.0. The *p*H of the mouse intestinal contents averaged 0.2 to 0.5 units higher than that of the bats. In a separate group of test bats and mice, *p*H measurements made at hourly intervals after necropsy revealed less than 0.2 unit change in *p*H within a 3-hr period.

Transit time. The transit rates of indigo-carmine were the same in the three bat species. Within 5 min, the dye had traversed approximately 30% of the intestinal length. In 15 min, dye was irregularly scattered through the gut but in all instances had reached the terminal portion of the intestine. Animals killed at 30 to 120 min still had dye within the intestine.

The actual rate of passage of indigo-carmine in mice was similar to that observed in the bats. Thus, in 15 min the marker had traversed 15 to 20 cm of intestine. This, however, represented only 30% of the total length. After 2 hr, the dye was in the terminal portion of the ileum but was not visible in the cecum.

India ink, when used as marker, gave comparable transit rates; within 30 min, two of three *Carollia* passed stools that contained the marker. Some ink, however, was present within the intestine of the third bat after 120 min.

Quantitative bacterial flora. Table 2 shows the total numbers of intestinal bacteria recovered from the animals tested. No more than 10^6 bacteria per intestinal contents were found in any of the bats. *Chilonycteris* had an average of $10^{3.9}$ bacteria, *Carollia* had $10^{3.3}$, and *Molossus*, $10^{4.8}$. The mice tested had an average of $10^{9.7}$ bacteria per fecal contents.

Qualitative bacterial flora. A total of 263 different bacterial isolates were obtained from the 60 bats. The average number of different bacterial isolates obtained from each *Chilonycteris* was 5.0 (range, 1 to 10); from *Carollia*, 3.4 (range, 0 to 7); from *Molossus*, 4.8 (range, 2 to 8).

Of the isolates obtained, 229 (84.7%) were identified as to bacterial group. These are listed in Table 3. The remaining 34 isolates did not conform to any of the biochemical patterns outlined by Edwards and Ewing (1962).

One hundred and four bacterial isolates of the *Klebsiella-Aerobacter-Serratia* group were recovered. Bacteria of this group were the predominant organisms in 43 bats. Enterococci were recovered 36 times from 36 bats, and were the predominant organisms in six instances. *Proteus* spp. were isolated 32 times from 31 bats, and were the most numerous group of bacteria in eight animals.

Bacteria of other groups were less frequently recovered. *Escherichia* spp. were found in 10 *Chilonycteris*, 1 *Carollia*, and 1 *Molossus*, and when present, were not the predominant organisms. *Alcaligenes* spp. were recovered only from *Carollia*; *Bacteroides* spp. were recovered only from *Chilonycteris* and *Molossus*. Seven bacterial isolates gave biochemical reactions compatible with those of *Shigella* spp. but failed to agglutinate in Shigella or Alkalescens-Dispar antisera and were unclassified.

There was no correlation between number or type of bacteria and *p*H.

TABLE 1. *Comparison of intestinal length and pH of intestinal contents among three bat species and laboratory mice*

Animal*	Avg wt	Avg intestinal length	Range of intestinal length	Avg pH	Range of pH
	g	*cm*	*cm*		
Chilonycteris	24.1	18.6	15.4–21.7	6.3	6.00–6.49
Carollia	17.3	20.0	13.6–21.7	6.2	6.02–6.35
Molossus	10.7	12.3	10.2–13.0	6.5	6.10–6.80
Mice	24.0	56.3	51.2–62.6	6.7	6.45–7.00

* For each species, 20 animals were tested.

TABLE 2. *Quantitative bacterial populations in three bat species and mice*

No. of bacteria per intestinal contents	No. of animals			
	Mice	*Chilonycteris*	*Carollia*	*Molossus*
10^{11}	1			
10^{10}	13			
10^{9}	5			
10^{8}	1			
10^{7}				
10^{6}		3	1	1
10^{5}		4	1	15
10^{4}		5	8	4
10^{3}		4	5	
10^{2}		4	3	
10^{1}			1	
10^{0}			1	

TABLE 3. *Frequency of occurrence of bacterial groups in intestinal contents of three bat species*

Bacterial group	No. of isolations		
	Chilonycteris	*Carollia*	*Molossus*
Escherichia	12	1	1
Shigella	0	0	0
Klebsiella-Aerobacter-Serratia	44	18	42
Salmonella-Arizona-Citrobacter	0	0	0
Proteus	12	10	10
Pseudomonas	0	1	1
Alcaligenes	0	8	0
Bacteroides	4	0	9
Bacillus	0	1	0
Clostridium	5	9	3
Enterococcus	12	8	16
Unidentified	11	10	13
Total bacterial isolates	100	68	95

H. capsulatum was recovered from two *Chilonycteris* and one *Carollia*; fungus concentrations were 8×10^2, 4×10^2, and 1×10^3 organisms per intestinal contents, respectively. The bacterial counts of these three bats, in the same order, were 10^2 *Clostridium* spp., 10^2 enterococci, and 10^3 *Klebsiella-Aerobacter* plus 10^2 enterococci.

H. capsulatum was not recovered from the feces of mice previously infected with the fungus. The livers and spleens of these mice, however, yielded *H. capsulatum* on culture.

DISCUSSION

Allen (1939) called attention to the absence of a cecum or appendix in most bat species. Others have confirmed this observation (Park and Hall, 1951). We have had the opportunity to examine a small number of preserved bat specimens from South Africa (*Epomophorus w. wahlbergi*, *Nycteris capensis*), Iran (*Rhinopoma hardwickei*), Malaya (*Megaderma spasma*), Thailand (*Rhinolophus affinis*), and Bechuanaland (*Hipposideros commersomi*). These bats were supplied by Charles O. Handley, Jr., Smithsonian Institution. In addition, Chester W. Emmons, National Institutes of Health, furnished two North American house bats (*Eptesicus fuscus*). With the exception of *E. wahlbergi*, a 90-g bat that had a 66-cm intestine, the gross configuration and length of the intestine of these bats were similar to those of *Chilonycteris*, *Carollia*, and *Molossus*.

The dye studies performed here demonstrated a 15-min transit time through the bat intestine. Whether this rapid passage of dye, and presumably foodstuffs, is a function of intestinal length and can be critically related to the numerical populations of intestinal bacteria has not been determined. It is certainly possible that the short intestinal length and the rapid transit time prevent the stasis necessary for colonization and multiplication of bacteria. Other factors, however, such as diet, environment, and intermicrobial antagonisms affect the qualitative and quantitative intestinal bacterial flora (Porter and Rettger, 1940; Donaldson, 1964), and must be evaluated.

Of the bats studied here, *Molossus* and *Chilonycteris* are insectivorous, and *Carollia* is a fru-

givorous species. Although the smaller *Molossus* had higher bacterial counts than the other species, and variations in the qualitative flora between species were noted, these differences were no more than were noted among bats of the same species. The intestinal bacteria of vampire, fish-eating, and carnivorous bats have not been studied, but food habits alone would not seem to account for the observed bacterial populations.

In addition to the general influence of diet on intestinal microbiology, specific microbial antagonists have been demonstrated in food. Recently, the poorly developed intestinal bacterial flora of some Antarctic birds (Sieburth, 1959) has been attributed to antibacterial substances in the algae that comprise the major portion of their diet (Sieburth, 1960). We have been unable to demonstrate a similar phenomenon in the bats; following filter-sterilization of bat stool suspensions, disc sensitivity testing revealed no antibacterial activity against mouse *E. coli* or *Klebsiella-Aerobacter* spp. These pilot experiments, however, do not begin to critically evaluate potential antimicrobial substances from ingested materials, the host, parasites, other bacteria, or fungi. Some information concerning the last of these is available. The presence of *H. capsulatum* does not appear to be responsible for the low bacterial counts, for the majority of bats had no cultural evidence of histoplasmosis.

The presence of *H. capsulatum* in bat intestinal contents is of interest because of the rarity with which pathogenic fungi have been cultured from feces (Schnoor, 1939; Nachtigall and Von Riesen, 1959). Studies designed to explain the overgrowth of *Candida* spp. after broad-spectrum antibiotic therapy have led to a realization that the normal intestinal environment is unfavorable for growth of fungi. Several hypotheses have been advanced to explain this phenomenon: (i) a competition for foodstuffs between bacteria and fungi that, because of a numerical advantage, favors bacterial growth and prevents extensive colonization by fungi (Gale and Sandoval, 1957); (ii) production, by bacteria, of oxidation-reduction potentials unfavorable for fungus growth (Paine, 1958); (iii) elaboration of inhibitory substances by enteric bacteria (Fisher, 1954; Fedors, 1959; Klite and Gale, 1961). It is apparent that each of these proposed inhibitory mechanisms would require the presence of a well-developed bacterial flora. The paucity of intestinal bacteria noted in the bats studied here would, therefore, minimize the influence of these types of inhibitory mechanisms on *H. capsulatum* shed from intestinal lesions. In addition, the rapid transit of *H. capsulatum* yeasts through the intestine would make it improbable that metabolic competitions would have time to exert their effects.

It is of interest to consider several other aspects of the intestinal microbiology and transit time in bats. These animals have been observed to eat up to one-half of their body weight per day (Allen, 1939) and defecate an average of 60 times per day (Klite, *unpublished data*). Although a normal spectrum of digestive enzymes has been demonstrated in *Myotis grisescens* (Buchholz, 1958), the intestinal physiology of chiropterans is otherwise unstudied. In view of the rapid transit time, the intestinal assimilatory processes of bats must be extremely efficient to support their high metabolic rate (Griffin, 1958). Also, the simple microbiology of the bat intestine would have application to the study of metabolic functions of bacteria, e. g., vitamin production and alteration of bile pigments.

Each of these investigations would require the use of captive animals. Unfortunately, bats have never been colonized in the laboratory. Even maintaining them in captivity in large numbers is difficult. Sparked by studies on their sonar apparatus (Griffin, 1958) and their ability to harbor the rabies virus (Enright, 1956), more interest has focused on this problem. The outstanding obstacle in maintaining captive bats is to satisfy their prodigious appetities; with the insectivorous species of North America, this can be a formidable task (Krutzsch and Sulkin, 1958). The fruit bats of the tropics, however, are easily maintained in the laboratory. We have kept *Carollia* for over 6 months on a simple diet of bananas and water without apparent ill effects. Utilization of these fruit bats as laboratory animals may provide a method for study of the many biological curiosities that have been noted in chiropterans.

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