BACTERIAL POPULATION OF GROSSLY HEALTHLY FROGS

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ABSTRACT.—A study was undertaken to ascertain the bacterial flora existing in apparently healthy frogs (*Rana pipiens*) of both sexes and at all times of year. The frogs were obtained from several commercial sources in the North-Central and Eastern parts of the United States. After one week's isolation, the frogs selected were those without symptomatology. Cultures of the blood, peritoneum and viscera (excluding the stomach and intestines) were found to be bacteria-free. *Escherichia coli* especially and *Aerobacter aerogenes* less frequently were consistently isolated from the skin, mouth, intestines and cloaca. All cultural techniques were standard for the facultative aerobes. No attempt was made to identify fungi, Mycobacteria, sporulating anaerobes or animal parasites, although in a few instances non-sporulating anaerobes were isolated and identified generically only, e.g. *Bacteroides sp.*

Bacteria-free mammals have long been brought into prominence in the study of disease states and antigen/antibody interactions. Amphibians are just beginning to be subjects of interest in bacteria-free research because of the need to control the homogeneity of the stock to ensure the reliability of research data.

The frog is highly useful in animal experimentation, but before its status in experiments requiring homogeneity can be defined, it is necessary to ascertain the organisms that it may characteristically harbor without symptomatology. Much is known about its animal parasites, but very little about its bacterial populations, and it is reasonable to wonder whether bacteria are carried internally in symptom-free frogs. In this study the bacterial flora of grossly healthy frogs obtained from geographically diverse commercial sources were examined.

MATERIALS AND METHODS

Frogs were obtained from several supply houses in the North-Central and Eastern United States. They were inspected for superficial lesions and also for normal color, posture, reactivity and condition of the eyes as gross indices of health.

The frogs were kept individually in water at 4°C in repeatedly cleaned glass containers. These were kept in different rooms since the habitat may contribute to the species of bacteria isolated. The water used was considered to be bacteria-free, based upon bacterial checks performed here by standard techniques.

The animals were isolated for one week to observe signs of any latent disease, following which only grossly healthy frogs were selected for further tests. A total of 125 animals were selected without reference to sex or time of year.

Cultures were made of the skin, cloaca, mouth and nasopharynx. Then the abdominal skin of the chloroformed frog was swabbed with 2% Amphyl (Lehn and Fink) and an incision was made aseptically to sample the peritoneum, blood, spleen, liver, kidney, stomach and colon.

All specimens were placed in thioglycollate, and after 24 hours sub-cultured on blood agar, EMB agar, and SS agar. After another 24 hours, colonies were picked from EMB and SS agars and placed on Difco triple sugar iron agar (TSI); tryptose lactose iron agar (TLI); Simmons citrate agar; Christensen's urea agar; methyl red/Voges-Proskauer broth; and 1% tryptone for indole production. Colonies from the blood agar were picked and direct smears prepared, stained with Gram-stain and identified by morphologic characteristics and zones of hemolysis on the blood agar. Whenever *Staphylococcus* was identified, a coagulase test was performed to ascertain if the or-

ganisms were coagulase positive or negative. In every instance only coagulase negative forms were found.

Within 24 hours after the colonies had been placed on the differential media the data from the reactions were tabulated and the morphologic characteristics of the colony reexamined. Where positive identification could be made, the tests were concluded; otherwise, they were continued for another 72 hours, the biochemical reactions being noted daily. If at the end of 72 hours there was still some doubt, another 24 hours of incubation were instituted and the more definitive testing procedures of typing and/or agglutination were performed. Bacterial contamination was considered to exist in the host if bacteria could be consistently recovered from the same sampling sites.

Results

Bacteria were found to be common inhabitants of the skin and the alimentary canal. The species involved and their frequency of occurrence are listed in Table 1. *Escherichia coli* and *Aerobacter aerogenes* were the most frequent isolates.

No bacteria could be isolated from the blood, peritoneum, liver, lungs, and kidneys even after 96 hours of incubation in the thioglycollate gelatin media. Gross visual necropsy examination revealed no evidence of infection in any of these same bodily structures.

Table 1.—Bacterial Population of Grossly Healthy Frogs (*Rana pipiens*).

Number of Frogs Tested	Commercial Source	Bacteria Isolated	Site, Organ or Tissue Cultured and Number of Frogs Producing the Isolate[1]				
			Skin	Mouth	Stomach	Intestine	Cloaca
40	North-Central (Wisconsin)	Escherichia coli	40	40	None	40	40
		Aerobacter aerogenes	32	34	None	36	35
		Alpha-hemolytic Streptococcus	37	None	None	None	None
		Hem. Coag. (neg.) Staphylococcus	38	None	None	35	38
		Proteus mirabilis	5	5	5	5	5
		Bacteroides sp.	None	None	4	None	None
45	North-Central (Illinois)	Escherichia coli	40	42	None	42	42
		Aerobacter aerogenes	36	35	4	30	31
		Non-hem. Coag. (neg.) Staphylococcus	6	26	19	15	None
		Non-hem. Streptococcus	15	None	None	12	1
		Proteus mirabilis	10	None	None	8	8
		Streptococcus fecalis	None	None	None	5	None
20	North-Central (Illinois)	Escherichia coli	19	20	None	18	19
		Non-hemolytic Streptococcus	7	None	None	None	3
		Alpha-hem. Streptococcus	8	None	None	None	None
20	East (South Carolina)	Escherichia coli	20	15	None	20	20
		Aerobacter aerogenes	18	18	3	18	18
		Alpha-hem. Streptococcus	6	10	None	None	None
		Hem. Coag. (neg.) Staphylococcus	5	None	1	None	None
		Proteus mirabilis	8	None	None	8	7
		Pseudomonas aeruginosa	18	18	None	15	15

[1] No bacteria were ever isolated from the spleen, liver, kidney, peritoneum, or blood, so that no data from these areas are presented.

Discussion

It is apparent that bacteria-free frogs cannot be expected from commercial sources, but it also appears that if vigorous frogs are selected by the experimenter after a period of their isolation, they may be expected to remain free of disease. It is cautioned that there may be no firm relation between the locale of the vendor and the geographic source of the frogs. The animals tested here could have come from any region of the country.

It is of interest that bacterial sterility apparently exists in the blood and visceral organs of the grossly healthy frog and that such animals have bacteria only in the expected localities of the skin and alimentary tract, where they appear to reside as commensals. Several species of bacterial pathogens, described by Breed et al (1957), can potentially exist in the blood or tissues of frogs. These organisms, which include aeromonads, pseudomonads, *Brucella*, spirochetes, tubercle bacilli and others have been considered by Kaplan (1953), Reichenbach-Klinke and Elkan (1965), Gibbs et al, (1966), and many other investigators. In all cases where pathogenic bacteria have been isolated, however, there are associated characteristic signs of disease.

Due to the fact that frogs live in water into which they excrete their body wastes, freedom from bacteria has not been considered a practicable goal in the laboratory. A pathogen-free state, however, is readily attainable through careful control of the habitat, even though the skin, mouth, alimentary canal, and cloaca will always contain a normally harmless bacterial flora.

The evidence that reactive, healthy appearing frogs have bacteria-free blood and tissues except for areas exposed to the external environment supplements similar evidence obtained from dogs by Lutsky and Farmer (1966), although it is contrary to the questions raised by Flynn et al (1965) about the pathogen status of commercially produced mice.

The authors are aware of the restrictiveness of the techniques used. Thioglycollate broth, a single medium, might not be productive for all types of organisms that could be present.

No conclusions can be drawn from this study about the relationship between the presence of viruses and gross health. A widespread distribution of Western equine encephalitis virus was demonstrated in symptom-free frogs (*R. pipiens*) in Saskatchewan (Burton et al. 1966).

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Manuscript received Dec. 12, 1967.