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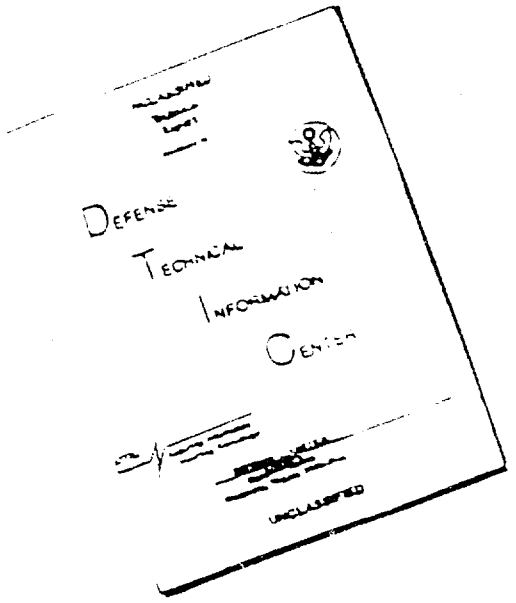

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METHODS OF DETECTING STAPHYLOCOCCAL ENTEROTOXIN

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METHODS OF DETECTING STAPHYLOCOCCAL ENTEROTOXIN

by

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Staphylococcal food poisoning is of great interest because in some countries it is more prevalent than that produced by Salmonella (Stolmakova, 1959).

Several biological methods to detect staphylococcal enterotoxin in bacterial cultures and food products have been described in literature. Opinions on the effectiveness of the various methods are conflicting. The Dolman test with kittens is now considered the most sensitive, but the seasonal availability of these laboratory animals makes it impossible to use them throughout the year. We, therefore, set out to investigate the possibility of using adult cats, frogs, and pigeons because the tests are easy to perform in the laboratory at any time of the year.

We used a total of 158 adult cats (1 to 3 kg in weight), 300 frogs of the species Rana ridibunda ridibunda (almost identical to Rana esculenta) and 26 young pigeons. Kittens served as the control. All told, 229 kittens up to two months of age (weighing 250 to 700 g) were used.

Eighteen strains of the staphylococci capable of producing enterotoxin were selected for the tests, 17 strains with proven pathogenic properties but not producing enterotoxin, and one nonpathogenic strain. Some of the enterotoxic strains were isolated in staphylococcal food poisoning and from the stools of nursing infants suffering from dyspepsia, while the remaining strains were obtained from the Czechoslovakian State Collection of Cultures and from the Hygienic Institute in Lubeck.

The strains had characteristic properties -- pathogenicity, high hemolytic titer, plasma coagulating activity, capacity to induce skin necrosis; they broke down mannite, etc.

The ability of the test strains to produce enterotoxin was established by several preliminary tests on kittens given the toxin orally, intravenously, and intraperitoneally.

The enterotoxin was obtained by culturing strains for six days in the air with 30% CO_2 at 37°. The nutrient medium was ordinary broth with 1% starch, 1% M solution of disodium phosphate, and 1% agar-agar with a pH of 6.2 to 6.4 The carbon dioxide was removed daily. The culture was centrifuged and passed through a Seitz filter. The filtrate was administered to the animals orally in a native state and parenterally after being heated for 30 minutes in a boiling water bath. The frogs were injected parenterally with heated and nonheated filtrates.

The initial experiments showed that the enterotoxin generally appeared between the third and sixth days of culturing (cf. Table 1). Other experiments revealed that the cats had to be fed one to two hours before injection if the tests were to be significant. Hungry cats frequently did not react to the enterotoxin.

TABLE 1

TIME OF APPEARANCE OF THE ENTEROTOXIN

No. of strain	Length of Cultivation in Days					
	1		2		3	
	special medium	ordinary broth	special medium	ordinary broth	special medium	ordinary broth
1	±	+	+	+	+	+
6	-	-	+	+	+	+
81	+	+	+	±	+	+
196	+	+	+	-	+	+
254	±	±	-	±	±	-

	4		6	
	special medium	ordinary broth	special medium	ordinary broth
1	+	+	+	+
6	+	+	+	+
81	+	+	+	+
196	+	+	+	+
254	+	-	+	+

Symbols: + = presence or traces of enterotoxin;

± = absence of the enterotoxin

Similar tests were performed simultaneously on kittens, adult cats, frogs, and pigeons. The animals were injected with the following doses of the same filtrate: kittens and cats -- 30 to 30 ml orally, 2 to 3 ml per kg intravenously and intraperitoneally; frogs - 0.5 to 1 ml orally, 2 ml into the dorsal lymph sac; pigeons - 1 ml intravenously. Serving as a control were the animals which did not receive the enterotoxin or were injected with sterile nutrient media ordinarily used to prepare the enterotoxin.

The reaction was judged to be positive if the following appeared: in the kittens -- diarrhea or vomiting 30 minutes to six hours after injection; in the cats - vomiting (not invariably) accompanied by diarrhea; in the frogs - the Robinton complex (change in color of skin, abnormal position of body, etc.).

The most reliable results were obtained from the kittens injected with the enterotoxin intravenously or intraperitoneally; the animals did not always react when it was administered orally (Table 2).

TABLE 2

RESULTS OF A COMPARATIVE STUDY OF THE STRAINS PRODUCING AND NOT PRODUCING ENTEROTOXIN

Culture	Kittens			Adult Cats		
	orally	intravenously	intraperitoneally	orally	intravenously	intraperitoneally
Producing enterotoxin	10/22	14/22	16/22	0/11	21/22	15/22
Not producing enterotoxin	0/19	0/19	0/19	-	0/19	3/19

	Frogs		Pigeons
	orally	dorsal lymph sac	intravenously
Producing enterotoxin	0/22	0/22	0.22
Not producing enterotoxin	0/19	0/19	-

NOTE: numerator - presence of the enterotoxin; denominator -- number of investigations

In the adult cats the greatest number of positive reactions resulted from intravenous injection of filtrates of the staphylococcal cultures; the results were less reliable after intraperitoneal injection. In some cases cats injected intravenously reacted to the enterotoxin and at times when the reaction in the kittens was indistinct (Table 3).

TABLE 3

RESULTS OF PARALLEL TESTS ON INTRAVENOUS INJECTION OF ENTEROTOXIN IN KITTENS AND ADULT CATS

No. of strains	Strain No.	Kittens	Adult Cats
12	1, 4a, 2-6, 81 (first time) 85, 87, 138, 147, 196, 254, 562, F 1	+	+
6	8 (repeated), 83, 86, 111, 113, 566	-, ±	+
1	239	-	-

Only adult cats injected intraperitoneally (3 cases) reacted positively to filtrates of staphylococci that were pathogenic but did not produce enterotoxin. This confirmed the view that nonspecific reactions also developed after use of this method.

To sum up, the tests on cats and kittens accurately reflected the amount of enterotoxin in filtrates of staphylococcal cultures. Thus, with oral administration 85.4% of the kittens (35 out of 41) accurately reflected its content; with intraperitoneal injection - 85.4% (70 out of 82 kittens and cats); with intravenous injection - 92.7% (76 out of 82 kittens and cats). Accordingly, with intravenous injection of kittens and cats, the percentage of reliable reactions indicative of the presence or absence of enterotoxin was higher than with the other methods of administration.

The frogs did not react in a characteristic fashion to the presence of enterotoxin, differing but little, as a rule, from the controls. It was only in rare cases that we could see, for a short period of time

Findings

1. The most reliable method of detecting enterotoxin in staphylococcal cultures is intravenous injection of cats with a filtrate of the cultures in doses of 2 to 3 ml per kg. The reaction does not vary with the age or weight of the animal. This confirms Stolnakova's finding that adult cats as well as kittens are suitable test animals to detect staphylococcal enterotoxin.

2. The enterotoxin appeared most clearly after three to six days of culturing the staphylococci.

3. The cats must be fed before being injected with staphylococcal cultures if positive reactions are to be obtained.

4. The large aquatic frog Rana ridibunda ridibunda, which closely resembles Rana esculenta, and young pigeons are not suitable for detecting staphylococcal enterotoxin.

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