THE PYROGENIC AND EMETIC EFFECTS OF STAPHYLOCOCCAL ENTEROTOXIN

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

A. Staphylococcal Enterotoxin

1. Historical Background

Soon after the discovery of staphylococci in pus by Pasteur in 1880, these organisms were among those isolated from foods implicated in a number of outbreaks of food poisoning. However, Barber in 1914 is considered the first definitely to establish staphylococci as causative agents in food poisoning. He described cases on a farm in the Philippine Islands which were traced to unrefrigerated milk or cream obtained from a cow whose milk contained a white staphylococcus. Since the early 1930's the role of staphylococci in food poisoning has been repeatedly substantiated. Staphylococci were responsible for approximately 80% of the outbreaks of food poisoning reported to the United States Public Health Service from 1945 to 1947 (Feig, 1950). This was four to five times more frequent than the next most common cause of food poisoning, namely, Salmonella species. Jordan and Burrows (1934) used the term "enterotoxic substance" to designate the toxic agent produced by staphylococci which caused food poisoning. Since then the term "enterotoxin" has been widely used. The literature concerned with staphylococcal food poisoning up to 1955 has been thoroughly reviewed by Dack (1956).

Staphylococcal food poisoning is characterized by a rapid onset, averaging $2\frac{1}{2}$ to 3 hours, with recovery usually complete within 1 to 3 days. The signs and symptoms in man include salivation, nausea, retching, vomiting, abdominal cramps, and diarrhea, although not all symptoms necessarily occur in a given individual. More severe attacks may be accompanied by headache, blood or mucus in the vomitus and stools, dehydration, marked prostration and perhaps shock.

2. Detection, Purification and Characterization of Enterotoxin

Since the 1930's numerous attempts have been made to develop suitable methods for detecting and measuring enterotoxin. Only man is sufficiently sensitive to enterotoxin to detect it in food samples. Other species can be used only to demonstrate whether or not a given strain of staphylococcus, when cultured under proper conditions, can produce enterotoxin. Vomiting in monkeys or cats has been the most useful endpoint for detection of enterotoxin. Other tests have included the production of diarrhea in rodents, antiperistalsis in decerebrate frogs (Robinton, 1950), decreased activity of nematodes* and even tests with fish (Raj and Liston, 1962). The tests with monkeys and cats provide only crude approximations of the amount of enterotoxin present in a sample when used as biological assays.

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*Mentioned by M. S. Bergdoll.

More recently, since the demonstration of the antigenicity of enterotoxin (Surgalla et al., 1954; Casman, 1958), the possibility of developing more sensitive and accurate tests for enterotoxin using immunological technics is being explored (Bergdoll et al., 1959b; Casman, 1960).

The interest in improving tests for enterotoxin led the staff at the Food Research Institute of the University of Chicago to develop methods for purifying enterotoxin to the extent that only a trace of one foreign substance, an apyrase (an enzyme with ATPase activity), remains. The purification process includes dialysis, acid precipation, alumina adsorption, alcohol precipation, Amberlite IRC-50 adsorption and starch electrophoresis. (See Bergdoll et al., 1959a for a detailed description of this procedure.) The only type of enterotoxin which has been purified by these methods at the present time is that isolated from staphylococcus strain S6. Purification of enterotoxin from strain 196E is presently being attempted (Bergdoll, personal communication). Strain S6 enterotoxin is a relatively heat-stable protein, containing no detectable carbohydrate or lipid, with a molecular weight of 24,000 $\pm$ 3,000 and an isoelectric point of 8.6 (Hibnick and Bergdoll, 1959). Its approximate amino acid content has been analyzed (Hibnick and Bergdoll, 1959). Evidence that the final material is essentially pure (except for the trace of apyrase) has been discussed by Bergdoll, Sugiyama and Dack (1959).

This evidence includes electrophoretic and ultracentrifuge studies, gel-diffusion tests with different concentrations of the purified material, and production of a single antibody by rabbits immunized with this material.

3. Immunological Studies

Recent immunological studies (Bergdoll et al., 1959b; Sugiyama et al., 1960a; Casman, 1960) indicate that at least two major types of enterotoxin are produced by staphylococcal organisms. Surgalla, Bergdoll and Dack (1953) reported that monkeys made tolerant to the emetic effects of enterotoxin from strain 196E still vomited in response to S6 enterotoxin. Bergdoll, Surgalla and Dack (1959) found that monkeys immunized against 100 minimally effective doses of partially purified S6 enterotoxin vomited in response to 10 to 12 minimally effective doses of 196E enterotoxin, indicating only partial cross-immunization. They also found that incubation of partially purified S6 antiserum with 196E enterotoxin before administration partially reduced the emetic effectiveness of the enterotoxin. In gel-diffusion tests, 196E enterotoxin did not form a precipitate with antiserum obtained from rabbits after four or five injections of highly purified S6 enterotoxin; and, conversely, this purified S6 enterotoxin did not react with antibodies produced by injection of partially purified 196E enterotoxin

into rabbits. These experiments suggested that S6 organisms may produce two types of enterotoxin, one of which is similar to 196E enterotoxin while the other is antigenically different and is the material present in the highly purified S6 enterotoxin preparations. Further evidence for the existence of at least two distinct enterotoxins produced by staphylococcal organisms has been provided by Casman (1960), who has divided enterotoxin-producing strains of staphylococci into two groups on the basis of serological tests involving interactions between their enterotoxins and antisera of strains S6, 196E and 243 (designated by Casman). One group produced only enterotoxin of the 196E type and had been isolated from foods implicated in outbreaks of food poisoning. The other group produced enterotoxin of the 243 type as well as that of the 196E type and had been isolated from patients with staphylococcal enteritis. Strain S6 produced both types of enterotoxin and can therefore be classified within the latter group. Strains which were non-enterotoxigenic by monkey-feeding tests did not produce either type of enterotoxin. On the basis of the above evidence, the purified S6 enterotoxin used for the pyrogenic and emetic studies described in this thesis is not of the 196E type but is presumably of the 243 type. A variant of strain S6 (S6R) produced enterotoxin which did not react with antiserum to purified S6 enterotoxin and which was apparently of the 196E type (Sugiyama et al., 1960a).

Similar evidence for different antigenic types of enterotoxin has been obtained by Sugiyama and associates (1960a). These relationships are summarized in Table 1.

TABLE 1

Strain(s) of Staphylococci	Type of Enterotoxin	
	196E	243
196E	+*	-
243	-	+
Food Poisoning	+	-
Enteritis	+	+
S6	+	+
S6R	+	-
Purified S6 Enterotoxin	-	+

* + = Produced
- = Not Produced

4. Fever Associated with Staphylococcal Organisms

Occasional mention has been made of fever accompanying staphylococcal food poisoning. Dack (1956) states that in severe cases "fever may occur, or the temperature may be subnormal." In a discussion following a report on staphylococcal food poisoning by

Slocum (1942), Bronfenbrenner cites an outbreak presumably due to staphylococcus in which approximately half of the people stricken with food poisoning at a banquet listed fever as one of their symptoms. In general, however, fever either seems to be absent in mild or moderate food poisoning or is minimal and overlooked among the more distressing symptoms. In a study of the effects of crude enterotoxin-containing filtrate in cats, Hammon (1941) reported pyrogenic responses, accompanied by chills, rising as high as 106°F from 2 to 4 hours after intravenous or intra-abdominal administration.

Recently there has been increased clinical interest in staphylococcal enteritis. Since staphylococci do not grow well in the presence of the normal intestinal flora, this disease occurs most often after abdominal surgery in patients being treated with an antibiotic to which the particular strain of staphylococcus is resistant. The symptoms in this disorder closely resemble those of staphylococcal food poisoning. In addition, fever is a common symptom (Dearing and Needham, 1960). Dearing and Needham were unable to detect any intestinal pathology associated with staphylococcal enteritis by post-mortem examination in patients who died of causes other than staphylococcal enteritis. This disorder is usually reversible with or without initiation of specific antistaphylococcic chemotherapy. Surgalla and Dack (1955) cultured staphylococcal strains isolated from 32 patients with staphylococcal enteritis. Thirty of these strains produced enterotoxin. These investigators

suggested the possibility that in vivo production of enterotoxin causes staphylococcal enteritis.

A more serious clinical syndrome which also strikingly resembles staphylococcal food poisoning, but which is associated with marked fever up to 107°F, is pseudomembranous enterocolitis (see review by Prohaska et al., 1961). This syndrome, which may be a severe form of staphylococcal enteritis, differs from enteritis in that it is accompanied by definite intestinal pathology including necrosis of the mucosa, hemorrhage, and a yellow-green pseudomembrane adhering loosely to the villi. The mortality rate is 60-75%. Prohaska and co-workers (1959) concluded that enterotoxin brings about the pathological changes in the gut in pseudomembranous enterocolitis for the following reasons: (1) Fatal pseudomembranous enterocolitis was produced in antibiotic-treated chinchillas by oral administration of cultures of enterotoxin-producing staphylococci obtained from patients with known pseudomembranous enterocolitis. (2) Fatal enterocolitis was produced in non-antibiotic-treated chinchillas by oral administration of enterotoxin-containing filtrates prepared from these same cultures and also from strain S6. (3) Non-fatal enteritis was produced in cats and kittens by oral administration of enterotoxin-containing filtrates. If enough enterotoxin is produced in the gut to produce lasting local damage, it is possible that other symptoms including fever are a result of systemic adsorption of enterotoxin.

The production of fever by intravenous administration of staphylococcal filtrates containing live or sterilized organisms has also been reported. Co Tui and Schrift (1939) sterilized horse serum incubated for 48 hours with Staphylococcus aureus by filtration through a Berkefeld filter. Injection of this filtrate into a dog caused a 2°F rise in temperature, leukopenia, emesis and shivering. These same authors (1942) report similar results from injection of a sterilized filtrate of Staph. aureus grown in broth. Atkins and Freedman (1960) produced fever in rabbits by injection of either live or autoclaved staphylococci. Passive serum transfer (see below) indicated that the fever was caused by an endogenous pyrogen in the serum. The entire organism rather than a bacterial product seemed to be required for pyrogenic activity. These authors postulated that a variety of unrelated agents may act on polymorphonuclear leukocytes to release endogenous pyrogen as the initial step in fever production. Other reports of fever after staphylococcal injections include those by Wylie and Todd (1948), Probey and Pittman (1945) and Braude and colleagues (1952). In these cases, it is unlikely that enterotoxin is involved since special conditions are required for its synthesis. The relationship of the above results to fever produced by endotoxins is likewise unestablished. Atkins and Freedman (1960) stated that staphylococci do not possess endotoxins. This view was supported by their inability to demonstrate cross-tolerance between the staphylococcal organisms

and endotoxin. Their observation that the whole organism was required for activity indicated that the agent responsible for inducing fever was not enterotoxin which is capable of inducing pyrogenic responses in a purified form completely free of any other cellular material (see RESULTS, below).

5. Studies of the Emetic Effect of Enterotoxin

Although the emetic effect of enterotoxin is well known, relatively few reported studies have been concerned with the mechanism or site of this action. A direct action on isolated strips of gut was not observed by Bayliss (1940) or Sugiyama, Bergdoll and Dack (1958). Increased tone in isolated rabbit intestine was reported by Richmond and associates (1942) and Anderson (1953) using high filtrate concentrations. Any relation of this action to emesis is doubtful because of species differences and also because emesis is produced primarily by actions of the respiratory and somatic musculature rather than by increased activity of the gut or stomach (Borison and Wang, 1953).

Bayliss (1940) studied the acute effects of a variety of surgical procedures on enterotoxin-induced emesis in cats. He found that vomiting was abolished by destruction of the vomiting center in the medulla or by brainstem transection anterior to the pons. Absence of the emetic activity of enterotoxin after decerebration has also been observed in chronic preparations (Schmidt and Borison, unpublished;

Clark et al., 1961). An emetic response to enterotoxin in one chronic decorticate cat (Borison et al., 1956), together with the above results, suggests a site of action in the region of the hypothalamus. However, this single observation requires confirmation.

Bayliss (1940) found that enterotoxin produced only retching in three cats with spinal cord transections at C7 but that vomiting still took place with cord sections at T2 or T5. After bilateral vagotomy in the neck only one cat in eight vomited, although some of the others did exhibit retching. Only mild retching could be elicited in cats in which the intestine or the entire gastrointestinal tract had been removed. From these experiments Bayliss concluded that a peripheral action of enterotoxin is more important than a central action in inducing emesis.

The effects of vagotomy at the diaphragmatic level and of ablation of the emetic chemoreceptor trigger zone (CTZ) in the medulla upon enterotoxin-induced emesis has been studied in chronic monkey preparations by Sugiyama, Chow and Dragstedt (1961). Vagotomy afforded some protection to all monkeys tested, and some animals did not vomit to as much as 100 minimal emetic doses of enterotoxin. Thus, monkeys are similar to cats in this respect. These authors also found that CTZ ablation was effective in protecting against the emetic effect of enterotoxin in monkeys.

Few emetic studies on the interaction between enterotoxin

and other drugs have been attempted. Bayliss (1940) reported that morphine and ergotoxine inhibited emesis while pentobarbital and atropine had no such effect. Sugiyama, Bergdoll and Dack (1958) found that sub-emetic doses of dihydroergotamine methanesulfonate given subcutaneously to monkeys increased their susceptibility to the emetic effect of oral enterotoxin. They suggested that this might be due to a summation of drug effects at a common site of action, perhaps the CTZ which is essential for ergot alkaloids to induce emesis (Wang and Glaviano, 1953). However, apomorphine, which also acts at the CTZ to induce emesis (Wang and Borison, 1952), in a small number of similar experiments did not seem to alter the incidence to vomiting after enterotoxin (Sugiyama et al., 1958). This may perhaps be due to the short duration of action of apomorphine. Reduced incidence of emesis has been shown in monkeys given intravenous perphenazine as late as 45 minutes after oral enterotoxin administration or given enterotoxin orally after pretreatment with reserpine (Sugiyama et al., 1960b). This protective action by a phenothiazine (perphenazine) again suggests the CTZ as the site of enterotoxin emetic action since in dogs, at least, chlorpromazine antagonizes the emetic effects of a variety of agents which act at the CTZ (Brand et al., 1954). Chlorpromazine itself, however, showed little if any protection against enterotoxin in monkeys (Sugiyama et al., 1960b). Similar results with perphenazine and chlorpromazine were obtained in dogs.

B. Endotoxin

1. Introductory Remarks

Endotoxins are high molecular weight lipopolysaccharides, derived primarily from gram-negative bacteria. They produce a wide variety of effects in animals and man including fever, alterations in blood leukocyte counts and glucose levels, the Shwartzman reaction, tumor necrotizing effects and cardiovascular effects. Endotoxins are responsible for various so-called "injection fevers" which have been of interest since the late 1800's and which are caused by contamination of solutions by gram-negative bacteria. The possibility of such contamination must be taken into account in any study of a pyrogenic effect supposedly induced by another agent. Recent reviews discussing various physiological effects of endotoxin include those by Bennett and Cluff (1957), Gilbert (1960) and Atkins (1960). The present survey will consider only those aspects of the pyrogenic effect and changes in leukocyte counts which are important for a comparison with enterotoxin and for an understanding of some of the problems involved in interpreting studies of pyrogenic agents.

2. Initial Pyrogenic Response to Intravenous Endotoxin

In the rabbit, the species most commonly used to study the pyrogenic effects of endotoxin, the first intravenous injection of a

sufficient dose of a purified endotoxin preparation or of a crude vaccine produces a biphasic fever with a latency of 15 to 30 minutes. The first phase of the fever usually reaches a relatively sharp peak at about 1½ hours; and the second phase or hump, with a more gradual rise and decline, reaches a maximum between 3 and 5 hours (Atkins, 1960). Minimally effective doses may cause only a brief monophasic pyrogenic response. Pyrogenic responses have also been reported in a number of other species including man, dogs, cats and horses. Sheth and Borison (1960) obtained a similar biphasic pattern after intravenous injections of 50 µg/kg of Salmonella typhosa endotoxin into cats. Ranson (1938) reported a regular triphasic response in cats to intravenous typhoid-paratyphoid vaccine with peaks at approximately three-quarters, 4½ and 15 hours.

Minimal pyrogenic responses of 0.6°C (1.08°F) have been obtained in rabbits after intravenous administration of as little as 0.0001 µg/kg of purified preparations of endotoxin. In a study of endotoxin from three bacterial strains (Salmonella enteritidis, S. typhosa and Serratia marcescens), Keene et al. (1961) determined the minimal pyrogenic doses as 0.0001, 0.0012 and 0.0003 µg/kg, respectively. However 1 µg/kg or more of these endotoxins was needed to produce a rise of 5°F. Cats and rabbits had similar thresholds for the pyrogenic effect of Serr. marcescens endotoxin in their study. Endotoxin preparations vary a great deal in their potency depending on the extent of purification and probably upon

the strain from which the endotoxin is derived. Therefore, dosage comparisons between studies using different preparations are usually not meaningful.

3. Tolerance to Intravenous Endotoxin

Tolerance to the pyrogenic effect of intravenous endotoxin that develops with repeated daily injections is characterized initially by a marked diminution or disappearance of the second phase, usually after two to four injections, while the first peak is still relatively unchanged. (See Bennett and Cluff, 1957; Atkins, 1960 for extensive discussions of endotoxin tolerance.) Following additional injections, the latency for the remaining peak increases and the peak itself decreases in amplitude,but usually it does not completely disappear, always giving a certain minimal response (Tennent and Ott, 1953; Beeson, 1947a; Bennett et al., 1957; Sheth and Borison, 1960). Morgan (1948), however, did observe complete tolerance in man after repeated endotoxin injections. When injections are stopped, complete recovery of the febrile response usually occurs within 3 weeks (Beeson, 1947a). Tolerance that develops to endotoxin from one bacterial species extends to endotoxin produced by other species as well. The shorter the interval between injections, the more rapidly tolerance develops (Beeson, 1947a). If sufficiently large doses of endotoxin are given repeatedly, tolerance to the pyrogenic effect may not be apparent. Tolerance to endotoxin is

not due to the development of immunity since the degree of tolerance cannot be correlated with the titer of circulating antibodies. Most of the present evidence indicates that an important factor in the development of tolerance to endotoxin is an increased rate of clearance of the endotoxin from the blood, presumably by the cells of the reticulo-endothelial system (RES), thereby effectively reducing the dose given. Beeson (1947b), using passive transfer of blood from non-tolerant and tolerant donor rabbits to non-tolerant recipient rabbits, showed that the tolerant rabbits cleared the injected endotoxin from the blood more rapidly than did the non-tolerant rabbits. Plasma taken from a non-tolerant donor 4 minutes after endotoxin injection caused a much greater rise in temperature when injected into a non-tolerant recipient than did plasma from a tolerant donor taken at the same time interval. He also showed that tolerance could be abolished completely by treatment of a tolerant rabbit with Thorotrast, a colloidal suspension of thorium dioxide which is taken up by the RES and thereby produces blockade, and that this loss of tolerance was accompanied by a decreased clearance of endotoxin. These observations have been confirmed by others and supported by studies in which labeled endotoxin and colloids other than Thorotrast were used.

4. Role of Endotoxin and Endogenous Pyrogen in Endotoxin-Induced Fever

The mechanism by which intravenous endotoxin produces fever has been the subject of much discussion and research. By passive

transfer of blood, plasma or serum from one animal to another, the presence of an endogenous pyrogen has been demonstrated. The technics used for these passive transfers are usually similar to that used for serum by Petersdorf and Bennett (1957). Blood obtained from the donor animal was allowed to clot at room temperature and then stored at 4°C overnight. It was then centrifuged and the serum stored at 4°C until used. Before injection into the recipient animal the serum was tested for sterility by culture in a broth. Sera from a number of donors could be pooled for injection. Atkins and Wood (1955a), using rabbits, studied the results of transfer of sera taken at different time intervals after endotoxin injection into different types of donors. Sera withdrawn at any time up to 2 hours from normal donors, i.e., donors previously untested with endotoxin, produced fever in the recipient. Sera from endotoxin-tolerant donors did not produce fever in the recipients. Sera from "sensitized" donors, i.e., rabbits which had received one or two previous endotoxin injections but at sufficiently long intervals so that they were not tolerant, produced fever if withdrawn within 30 minutes after injection or if withdrawn between 1 and 2 hours after injection but not if withdrawn between 30 and 60 minutes. They also found that sera taken at 5 minutes after endotoxin injection did not produce fever in endotoxin-tolerant animals indicating that the pyrogenic agent in these sera was uncleared

endotoxin (Atkins and Wood, 1955b). Sera taken at 2 hours produced monophasic fevers with short latencies in tolerant as well as non-tolerant recipients. The pyrogenic material in the sera taken at 2 hours, so-called "endogenous pyrogen" (EP), is indistinguishable from leukocytic pyrogen which can be obtained from polymorphonuclear leukocytes (Beeson, 1948). Both EP and leukocytic pyrogen cause fever with a short latency in normal or endotoxin-tolerant animals, are relatively unstable to heat as compared with endotoxin, do not cause a sustained or marked leukopenia or a local Shwartzman reaction, and do not induce tolerance when injected in a dose causing a monophasic pyrogenic response (Atkins, 1960). A preliminary investigation of the chemical properties of leukocytic pyrogen shows that it is a non-dialyzable protein with no lipid and little or no carbohydrate and that its pyrogenicity is destroyed by proteolysis with trypsin or pepsin (Rafter et al., 1960).

While it is generally accepted that the second phase of the pyrogenic response to endotoxin is an indirect effect involving release of EP, it is uncertain whether the first phase is also caused by EP or whether it represents a direct action of endotoxin. The evidence favoring these two possibilities has been reviewed by Atkins (1960) who favors the indirect mechanism. The direct mechanism is supported by Bennett and his associates. Sheth and Borison (1960) after a study of intraventricular and intravenous administration of S. typhosa endotoxin to cats concluded that the first phase is due to

a central action of endotoxin. Herion and associates (1961), however, have provided evidence which supports the hypothesis that leucocytic pyrogen (or EP) is necessary for both phases of endotoxin-induced fever. They found that no significant fever was produced by the injection of endotoxin into severely granulocytopenic rabbits. Since these animals exhibited a normal pyrogenic response to the injection of serum EP, the physiological systems involved were not impaired by the nitrogen mustard used to render them granulocytopenic. Endogenous pyrogen has been detected in the sera of leukopenic rabbits (white cell count less than $1000/mm^3$) (Gillman et al., 1961). The pyrogenic responses obtained in the recipients increased as the amount of serum transferred was increased. The responses obtained with sera from leukopenic donors were less than those obtained with equal amounts of sera from normal donors. These results indicate that the magnitude of the pyrogenic responses in the recipients was directly related to the amounts of EP transferred.

Some evidence is available that EP has a central site of action. King and Wood (1958a) showed that slow infusion of EP, as serum taken from a donor 2 hours after injection of endotoxin or as leukocytic pyrogen, obtained from peritoneal exudates, produced a higher fever with a shorter latency by intracarotid (5-15 minutes) than by intravenous administration (15-30 minutes). Serum containing EP was found to be 40 times more effective in producing fever after intrathecal administration than when given intravenously

(Bennett et al., 1957).

Evidence has also been obtained that endotoxin itself can produce fever when injected into cerebrospinal fluid at various sites. Bennett, Petersdorf and Keene (1957) were able to produce fever by injection of 1/1000 to 1/4000 of an effective intravenous dose of endotoxin into the basilar cistern of rabbits and dogs by direct puncture or via chronically implanted catheters. The onset of fever was shorter by this route of administration than after intravenous injection. Tolerance could not be induced by repeated injection into either normal animals or animals tolerant to intravenous endotoxin, and no EP was detectable in serum. Petersdorf and Bennett (1959) were able to produce a higher fever with 0.05 µg of Shigella endotoxin given intrathecally than with 5.0 µg given intravenously. Pyrogenic responses have also been demonstrated to endotoxin administered through a tube inserted through the fourth ventricle to the level of the corpora quadragemina in dogs (Penner and Bernheim, 1960). However, these responses were often accompanied by leukopenia which may indicate the passage of the endotoxin into the peripheral circulation as well as into the ventricular system (see below). Perry (1954) and Sheth and Borison (1960) have demonstrated fever production after endotoxin injection into the lateral cerebral ventricles of cats using the Feldberg and Sherwood technic. The latter authors found that 0.6 µg of S. typhosa endotoxin in 0.25 ml of saline solution

produced a maximal fever - a monophasic response with a latency of approximately 1 hour , peak at 4 hours and recovery to baseline usually within 24 hours. Increasing the dose increased the duration but not the peak of the fever. Tolerance to endotoxin did not develop with repeated injections. Cats tolerant to intravenous endotoxin, i.e., not showing a second phase, showed an undiminished response to lateral ventricular endotoxin.

Thus, both EP and endotoxin can apparently cause fever via central actions; but the relative roles of these actions in the response to intravenous endotoxins have yet to be definitely established.

5. Possible Role of Endogenous Pyrogen in the Pathogenesis of Fever

Atkins (1960) in his review of the pathogenesis of fever suggests that the release of pyrogenic materials from cells in the host may be the cause of fevers of various etiologies. Such endogenous pyrogens have been detected by passive transfer bioassay after injection of Newcastle disease virus (Atkins and Huang, 1958) and Old Tuberculin (Hall and Atkins, 1959; Moses and Atkins, 1961) as well as after endotoxins. The detection of an endogenous pyrogen after intravenous injections of either live or autoclaved cultures of staphylococci by Atkins and Freedman (1960) was mentioned earlier. Endogenous pyrogen has also been reported in the circulation of rabbits with peritoneal infections (King and Wood, 1958b).

6. Alterations in Leukocyte Counts Induced by Endotoxin

Another well-known property of endotoxin is the ability to cause leukopenia when given intravenously in adequate doses. This is due primarily to a decrease in the number of circulating granulocytes (Herion et al., 1960), although a lymphopenia also contributes. The disappearance of the leukocytes from the circulation is thought to be caused by their adhesion to capillary walls, particularily in the lungs, liver and spleen. Leukopenia is followed by leukocytosis, probably as the result of release of granulocytes from bone marrow and also of release of sequestered leukocytes. Leukopenia has not been observed after intrathecal endotoxin injection (Bennett et al., 1957).

C. Temperature Regulation and Measurement

1. General Considerations of Temperature Regulation

The thermoregulatory system in warm-blooded animals consists of three major divisions: (1) the afferent portion which includes peripheral and central thermodetectors and their central connections, (2) the efferent portion which comprises the mechanisms for production, conservation and loss of heat, and (3) the central mechanisms which integrate the activities of these afferent and efferent components to maintain a relatively constant internal body temperature. Recent reviews of the physiology of temperature regulation include those by Hardy (1961) and von Euler (1961).

Peripheral thermodetectors for both warmth and cold have been found in skin and in the tongue of cats and dogs. These receptors exhibit both a static discharge, which is proportional within certain ranges to the degree of warmth or cold applied, and a rapidly adapting phasic discharge, which is proportional to the rate of change of temperature. For example, if skin is heated slightly a transient feeling of warmth develops, presumably due to the phasic response of the warmth receptors. Raising skin temperature further increases the static discharge until eventually a continuous feeling of warmth is maintained without any further rise in temperature.

The presence of temperature sensitive elements in the

region of the anterior hypothalamus has been demonstrated by local heating or cooling of this region with various technics including the circulation of water through thermodes and heating with electrodes. Thus, heating the preoptic area causes a lowering of rectal temperature, while cooling the same area results in a rise in rectal temperature. Andersen, Hammel and Hardy (1961), measuring rectal temperatures in unanesthetized dogs, found that pyrogenic responses to endotoxin could be modified by altering anterior hypothalamic temperature. If heating was begun when the pyrogen was injected, the pyrogenic response was prevented as long as the heating was maintained. If heating was stopped within the period during which the pyrogen was still effective, a rise in temperature followed. The response could be interrupted by heating during fever development. When the anterior hypothalamus was cooled after pyrogen administration a higher fever than usual developed.

Whether receptors sensitive to both warmth and cold exist in the hypothalamus is a subject still under examination. Hardy, Hammel and Nakayama (1962) recorded electrical activity of single hypothalamic cells during local heating or cooling of the hypothalamus in cats anesthetized with urethane. They found cells in the preoptic region which showed increased activity with heating (Q_{10} = 5-10) but none which showed increased discharge rate with decreased temperature. They were unable to find temperature-sensitive

cells in either the supra-optic nucleus or the posterior hypothalamus.

The relative contributions of the peripheral and central thermodetectors to the regulation of body temperature have not been definitely established, but the consensus is that the central detectors are of primary importance in a warm or hot environment and that peripheral receptors are of greater importance in a cool environment.

The locations and functions of the integrating centers have been studied by ablation or stimulation of various regions and by the local heating or cooling technics previously mentioned. An important region for control of heat loss mechanisms is located in the dorsal part of the area ventral to the anterior commissure and dorsal to the optic chiasm. Lesions in this area in cats greatly impair ability to protect against heat loads (Frazier et al., 1936) while stimulation of the same region in goats evokes panting, peripheral vasodilatation and inhibition of shivering, responses which are effective in lowering body temperature (Andersson et al., 1956). Bilateral lesions in the lateral hypothalamus also markedly impair heat loss mechanisms. Bilateral lesions in the caudal part of the lateral hypothalamus greatly decrease the ability to protect against cold as well.

Andersson (1957) reported that stimulation of the septal area in unanesthetized goats produced shivering, peripheral vasoconstriction and occasionally piloerection at ordinary room temperatures. A cold environment facilitated shivering. Stimulation also inhibited on-going

panting. Stuart, Kawamura and Hemingway (1961) also reported that septal stimulation could produce shivering in cats. However, they found that stimulation of the dorsomedial posterior hypothalamus was more consistent in producing shivering and was effective at lower stimulus intensities. Septal lesions did not alter the ability of cats to maintain body temperature or to respond to a cold stress by increasing oxygen consumption (Stuart et al., 1962). Dorsomedial posterior hypothalamic lesions resulted in impaired shivering and decreased rectal temperature although other responses to cold, such as piloerection and huddling, were still present. These authors concluded that the dorsomedial region of the posterior hypothalamus is important in the efferent mechanism for shivering with some modulation probably arising from the septal region.

Body temperature in larger mammals is ordinarily maintained within narrow limits primarily by the interaction of mechanisms for heat production (increased metabolic rate due to shivering, exercise, etc.), heat conservation (peripheral vasoconstriction, piloerection) and heat loss (peripheral vasodilatation, sweating and panting). In a so-called "neutral" environment, alterations in peripheral vasomotor tone with associated changes in tissue heat conductance are sufficient to maintain heat balance. In a cold environment increased heat production is necessary to balance increased heat losses which cannot be prevented by further peripheral vasoconstriction. In addition to vasodilatation, sweating and panting are elicited to

maintain net heat loss in a warm or hot environment. Many other factors such as food consumption, clothing or fur, fat deposition, posture and hormonal activity will influence the balance between heat production and heat loss and the extent to which the other physiological mechanisms are activated.

2. Possible Effects of Pyrogens on Temperature Regulating Mechanisms

A pyrogenic agent can conceivably produce fever by any of a number of mechanisms. A commonly proposed mechanism for fever production by bacterial pyrogens and for other fevers is that of resetting the "thermostat", or thermoregulatory integrating mechanisms, in the central nervous system (primarily in the hypothalamus) to a higher level, thereby initiating events in the effector systems resulting in an increase in body temperature (Atkins, 1960). The pyrogen might also alter the sensitivity or threshold of peripheral or central thermodetectors, or alter the balance between the discharges from the warmth and cold detectors. These actions would have the same effect as resetting the thermostat and might actually be the mechanism of altering the set point of this integrating system. These effects could be caused by direct actions on the systems involved or possibly by indirect mechanisms such as changing the regional blood supply. Whether due to action on the integrating mechanisms per se or on the thermodetectors, the response should be independent of environmental temperature (Fox and Macpherson, 1959) except at

extremes. However, the relative contributions of the various efferent systems will vary with environmental temperature (Hardy, 1961). In a warm environment that causes sweating and/or panting, fever would be produced primarily by vasoconstriction and inhibition of sweating and panting, with little or no contribution by shivering. In a neutral environment not causing initial sweating or panting, shivering would be primarily responsible for the pyrogenic response. In a very cold environment in which heat conservation and production mechanisms were initially operating maximally, no fever could be produced.

On the other hand, the actions of pyrogens on the efferent components of the thermoregulatory system would be expected to vary with environmental conditions and with on-going activity in the system. In addition, the other efferent systems involved in temperature regulation should act as buffers. For instance, small doses of atropine will produce fever in a hot environment but not at a neutral temperature at which sweating does not occur (von Euler, 1961). Likewise, a peripheral vasoconstrictor drug may cause a slight increase in temperature in the neutral zone, but not in a cold environment where cutaneous vasoconstriction is already maximally effective in reducing tissue conductance, or in a warm environment where sweating or panting may occur.

Other possible means of increasing temperature include altering hormonal functions or metabolic rate more or less directly as with dinitrophenol.

Since the efferent mechanisms which mediate changes in body temperature vary with environmental temperature, it is essential that environmental temperature be kept relatively constant in any study of drugs which act through these mechanisms. It is also usually worthwhile to study the response at various ambient temperatures. However, in a study of the general characteristics of a response to an agent which acts on the afferent side of the reflex arc or on the integrating centers, a constant temperature environment is not necessary. If extremes of external temperature are avoided, the response should be very nearly the same even though the various efferent systems will be involved to different degrees.

3. Temperature Measurements at Various Sites in the Body

Since skin temperature varies between regions, weighting systems which take into account the proportion of total body surface contributed by each area as well as the temperature of that area have been developed to approximate "average skin temperature". Although a number of different internal body temperatures, including esophageal, stomach, liver, heart and bone marrow, have been measured, rectal temperature is still commonly used as a measure of internal or "core" temperature. Approximations of average total-body temperature, as are needed for studies of energy exchange, have been made by utilizing various proportions of rectal and average skin temperature such as $T_{ave} = 0.8\ T_r + 0.2\ T_s$.

For studies of the effects of temperature changes in the region of the central thermodetectors and integrating centers on temperature regulation, a closer approximation than rectal temperature is necessary. In local heating or cooling of these regions, direct temperature measurements are often made. Benzinger (1960) has used tympanic membrane temperature as a less direct measure of internal cranial temperature. Oral temperature is also considered by some to be a better measure of central temperature than is rectal temperature.

The important parameter in studies of the gross effects of pyrogenic agents is the change in temperature produced rather than the absolute temperature. Rectal temperature has generally been used in such studies. Sheth and Borison (1960) measured core temperature in the retroperitoneal space. No comparison was made between retroperitoneal and rectal temperatures. However, similar fever patterns are obtained with measurements from both sites, and factors such as peripheral blood flow or changes in position can exert little if any influence. The advantages of measuring retroperitoneal over rectal temperature are: (1) the thermocouple can be anchored and left in place continuously, (2) the animal is undisturbed by the physical presence of the thermocouple, and (3) the recording tip of the thermocouple is fixed with respect to the location of nearby blood vessels.

4. Measurement of Rectal Temperature in Rabbits

The setup used for measuring temperature in rabbits is generally the following or some modification thereof (Braude et al., 1960; Herion et al., 1960; Keene et al., 1961; Gillman et al., 1961; Martin and Marcus, personal communication): The animal is placed in a closely fitting stall or stock-like enclosure with some means of restraint around its neck. Temperature is measured by a rectal thermometer, thermister or thermocouple and is recorded at intervals by an observer. Time is usually allowed for the animal's temperature to stabilize before the injection is made, directly through an ear vein. This setup is used both to determine if a solution is pyrogenic by the criteria stated in the United States Pharmacopeia XVI and to study more carefully the characteristics of a pyrogenic response. As a bioassay for pyrogenicity, this procedure has a number of drawbacks. Martin and Marcus (personal communication), for instance, on the basis of responses to non-pyrogenic saline injection found that rabbits could be separated into groups ranging from stable animals which repeatedly showed no pyrogenic response (less than 0.6°C rise in temperature within 3 hours after injection of 10 ml/kg) to those which consistently gave a positive pyrogenic response (greater than 0.6°C rise). Therefore, it is important that the animals be conditioned to the stocks and to injections and that only stable animals be used. Martin and Marcus also found that occasionally even a usually

stable rabbit began to give erratic results after a large number (10-12) of injections made at 48 hour intervals. Another disadvantage of this procedure is the need to maintain the animal in a cramped, uncomfortable position for a long period of time.

D. Objectives

The primary objectives of this investigation were (1) to characterize the pyrogenic responses in cats to intravenous and lateral cerebral ventricular administration of S6 enterotoxin, (2) to compare the pyrogenic responses of enterotoxin with those of endotoxin since the possibility of endotoxin contamination must be considered in any study of this sort, (3) to elucidate the mechanism of emetic action of enterotoxin, and finally, (4) to analyze the importance of these results in relation to such things as food poisoning and the other physiological disturbances presumably caused by enterotoxin.

II. METHODS AND MATERIALS

A. Temperature Recording

The experimental arrangement for recording temperature is shown in Figure 1. Core temperature in an essentially unrestrained cat was recorded automatically by means of an iron-constantan thermocouple chronically implanted in the retroperitoneal space. Thermocouples were protected from body fluids by enclosing them in either vinyl (Temflex) or polyethylene tubing. Vinyl tubing was easier to seal properly and was more flexible than polyethylene tubing. However, diffusion of fluids through the vinyl tubing over a period of time caused the thermocouple leads to rust and eventually break. Polyethylene tubing was essentially impermeable but tended to crack at the knot around the rib or to open at the tip. With either type of protective sheath around the thermocouple a satisfactory recording period of 2 to 3 weeks could be expected, although an occasional thermocouple remained intact for longer periods. Thermocouples were replaced as often as necessary. The external leads of the thermocouple were fixed to a leather harness which was fastened around the shoulders and forelegs of the cat. From the harness, connecting wires led to the top of the cage and then to an input plug on a Leeds and Northrup Speedomax six-point recorder calibrated to record temperatures from 90 to 108°F. The input plugs were covered by a plastic box to prevent fluctuations in recorded

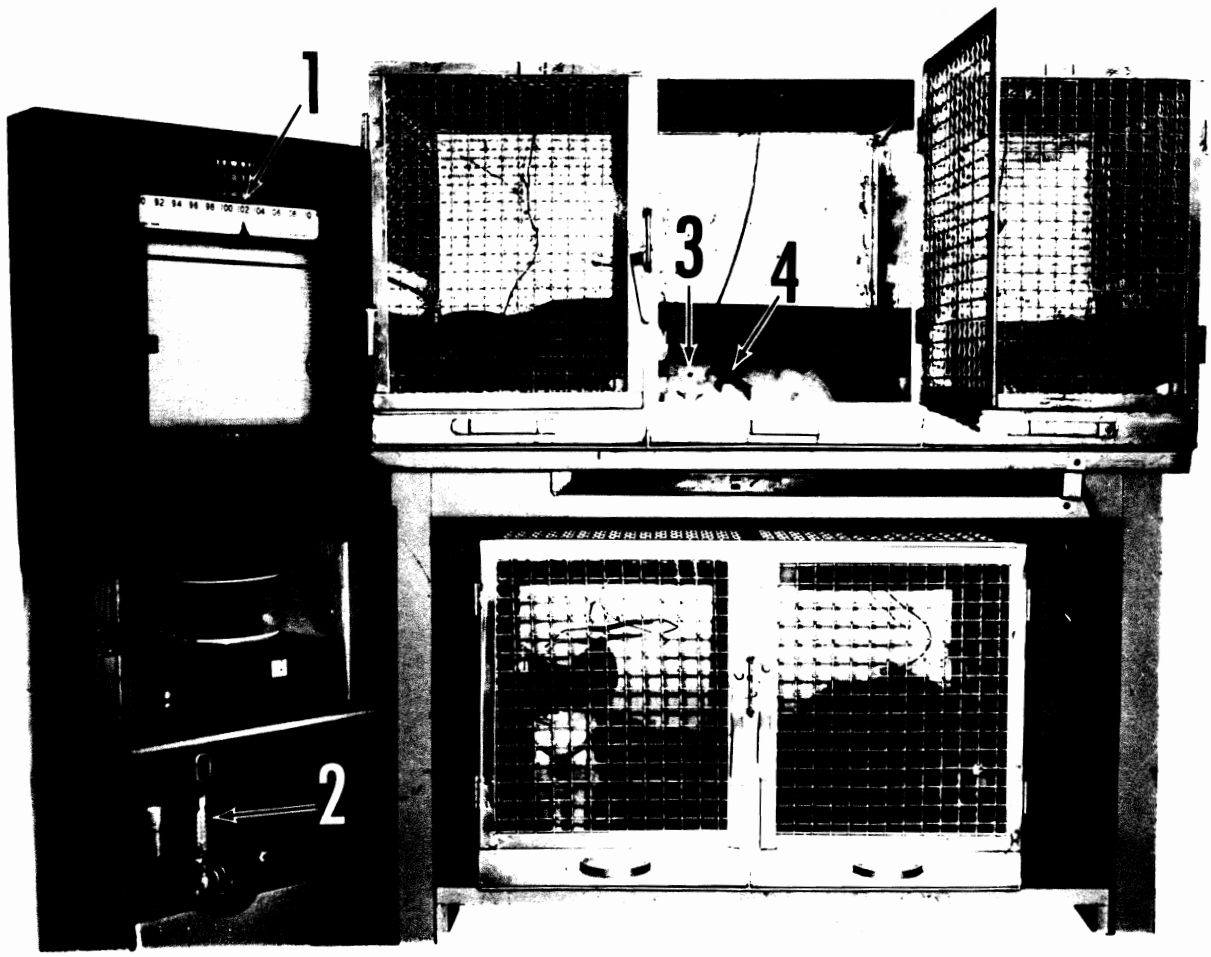


Figure 1. Arrangement for chronic recording of body temperature in cats. The numbers indicate respectively; (1) six-point recorder; (2) constant temperature bath; (3) lateral ventricular cannula; (4) harness.

temperature due to air currents around the plugs. Five channels were used to record body temperature in different cats. The sixth channel recorded the temperature of a Haake Type F Thermostat (constant temperature bath) set at 107°F and was used to monitor drift in the recorder. The unit was equipped with a cycle-timer and, although usually recording 6 of every 20 minutes, could be set to record continuously to follow relatively rapid responses. A complete cycle of all six channels was recorded approximately every 3 minutes.

The record was removed from the recorder daily, and the temperature curve for each cat was transposed to a condensed chart on which only hourly temperatures were generally noted. When rapid changes in temperature were recorded, however, the temperatures at shorter intervals were also transposed. Zero or baseline temperature for each day was taken as the average of the animal's body temperature at 8, 9 and 10 A.M. (This is a relatively constant temperature for an individual cat from day to day.) The temperature throughout the day is noted in all figures of temperature curves as the difference between the recorded temperature and this baseline temperature.

A rise in temperature of over 2°F from the baseline was considered to be a definite pyrogenic response. This value is based upon data of Sheth and Borison (1960) who found that, in 25 observations on eight untreated cats, the maximum diurnal rise was 0.4°F with a standard deviation of $\pm$0.8°F. A increase in temperature of over 2.0°F therefore falls outside of two standard deviations from the

mean and differs significantly from normal at the 5% probability level. No significant difference was observed when intraventricular saline injections were made. Similar technics and the same apparatus were used in the present experiments as used by Sheth and Borison, and the total daily range seen by the present author in untreated or saline-treated animals was usually less than 2°F. Therefore, the choice of 2°F as a dividing line, above which fever is definitely present and below which the presence or absence of fever must be determined by other criteria, was considered valid.

Animals were given milk and their cages cleaned between 9 and 10 A.M. Injections were made at 10 A.M. unless otherwise indicated. Usually, control injections and drug administrations were alternated on succeeding days. The entire setup was housed in a small, isolated room. Animals were disturbed as little as possible after 10 A.M. although some interruptions were unavoidable.

B. Surgical Procedures

All surgical procedures were carried out under sterile conditions. Animals were anesthetized with pentobarbital sodium (36 mg/kg) injected intraperitoneally and supplemented by intravenous injections when necessary. Procaine penicillin G (600,000 units, intramuscularly) was given postoperatively as a prophylactic measure against infection.

Implantation of the retroperitoneal thermocouple was carried out, after stripping the muscle from a short length of the last rib, by guiding the thermocouple posteriorly beneath the adjacent muscle layers until the tip rested between the kidney and the spinal column. The thermocouple was then looped and knotted once around the rib to hold it firmly in place. The thermocouple was led from the animal through a puncture made in the skin 1 to 2 inches from the incision. Cats were routinely intubated in case artificial respiration was necessary since pneumothorax was occasionally produced by this procedure.

The method of Feldberg and Sherwood (1953) was used for lateral cerebral ventricular cannulation. Proper placement of the Collison cannula was indicated by vomiting to the injection of 250 µg or less of apomorphine in 0.25 ml of saline solution (Borison, 1959) and, in appropriate instances, by post-mortem examination following the intraventricular injection of 0.25 ml of methylene blue dye after perfusing the head with formalin. The volume of 0.25 ml is sufficient to fill the ventricular system and to reach the subarachnoid

surfaces of the lower brain stem (Borison, 1959). This total volume was usually used for saline and drug injections.

Intravenous injections and withdrawals of blood samples were made through a permanently indwelling jugular catheter so as not to disturb an animal whose temperature was being recorded. The catheter was passed through a puncture in the skin at the back of the neck and fitted with a puncture-cap which was fastened to the harness.

Spinal cord transections were made between the levels of T1 and T3 after exposure of the cord by removing the overlying spinous processes and performing a wide laminectomy. The dura was opened with a longitudinal slit and the cord sectioned with a scapel blade. Gelfoam sponge was placed between the severed ends of the cord.

Transthoracic vagotomy was performed in artificially respired cats after opening the chest between the ninth and tenth or eighth and ninth ribs. After locating the two or three strands of the vagus in the loose connective tissue surrounding the esophagus, a section of each strand was tied off and removed. No intact branches could be found on post-mortem examination.

Ablations of the emetic chemoreceptor trigger zone (Borison and Wang, 1953) in the area postrema of the medulla oblongata were made by gentle thermal cauterization. Completeness of the lesions was indicated by lack of an emetic response to 0.15 mg/kg lanatoside C intravenously (Borison, 1952) or to 250 µg apomorphine in 0.25 ml saline intraventricularly (Borison, et al., 1960).

C. Miscellaneous

To prevent the bacterial contamination of the solutions, particularily by endotoxin, the following procedures were used: Bottles were thoroughly washed in soap and Alconox, carefully rinsed with distilled water and sterilized in an autoclave at 110°C for 30 minutes. In some cases sterilization was done at 170°C for 2 or more hours. Solutions were made using commercial, sterile, non-pyrogenic saline and were kept refrigerated at 4°C. All withdrawals from solutions were made with sterile, non-pyrogenic, disposable syringes. As a further check against pyrogen contamination, the same batch of saline which had been used to make the enterotoxin solution was employed for control injections.

Leukocytes were counted by the procedures described by Cartwright (1958).

After tests with large intraventricular doses of enterotoxin in 0.25 ml, fever occurred with the following saline injection although earlier injections had not produced fever. This pyrogenic response was presumably due to residual enterotoxin in the cannula and could be avoided by flushing with 0.05 ml of saline. For this reason all intraventricular toxin injections were made in a volume of 0.20 ml followed with a flush of 0.05 ml of saline solution. Intravenous toxin injections were flushed in with twice the volume of saline solution.

Materials were weighed on a semi-micro Sartorius-Werke double-beam balance with an accuracy of ± 50 µg. For more accurate weighing of small samples of enterotoxin, a double-beam balance with an accuracy of ± 10 µg was used.

D. Materials

All animals used were healthy, mongrel cats of either sex ranging in weight from 2.5-4.5 kg.

Purified enterotoxin prepared from staphylococcus strain S6 (Bergdoll et al., 1959) was kindly supplied by Dr. M. S. Bergdoll of the Food Research Institute of the University of Chicago. Unless otherwise noted, a preparation of greater than 90% purity was used. This preparation contains two or three impurities, detectable by gel-diffusion technics, in concentrations of 1% or less. No carbohydrate or lipid was detected in preparations of even less than 90% purity (Bergdoll, personal communication). In a few experiments, preparations of enterotoxin of approximately 70% and 20% purity were used. Estimates of purity were based on emetic tests in monkeys.

Crude enterotoxin filtrates were prepared as follows: The desired staphylococcal strain was plated on plain agar and cultured for 24 hours at 37°C. Brain-heart infusion was inoculated with this culture and incubated in a shaker for 48 hours at 35°C. This material

was then centrifuged at 3000g for 30 minutes at approximately 10°C and the supernatant frozen overnight. The supernatant was then filtered through a sterile Seitz filter. The filtrate was placed in boiling water for 30 minutes. This procedure yielded 140 to 150 ml of crude filtrate for 200 ml of brain-heart infusion broth inoculated.

S. typhosa lipopolysaccharide from Difco Laboratories was used as the standard endotoxin preparation. This same batch was used by Sheth and Borison (1960).

Other drugs used were apomorphine hydrochloride (Mallinckrodt), lanatoside C (Sandoz), pentobarbital sodium (Abbott), procaine penicillin G (Squibb, Wyeth) and heparin sodium (Organon Inc.).

III. RESULTS

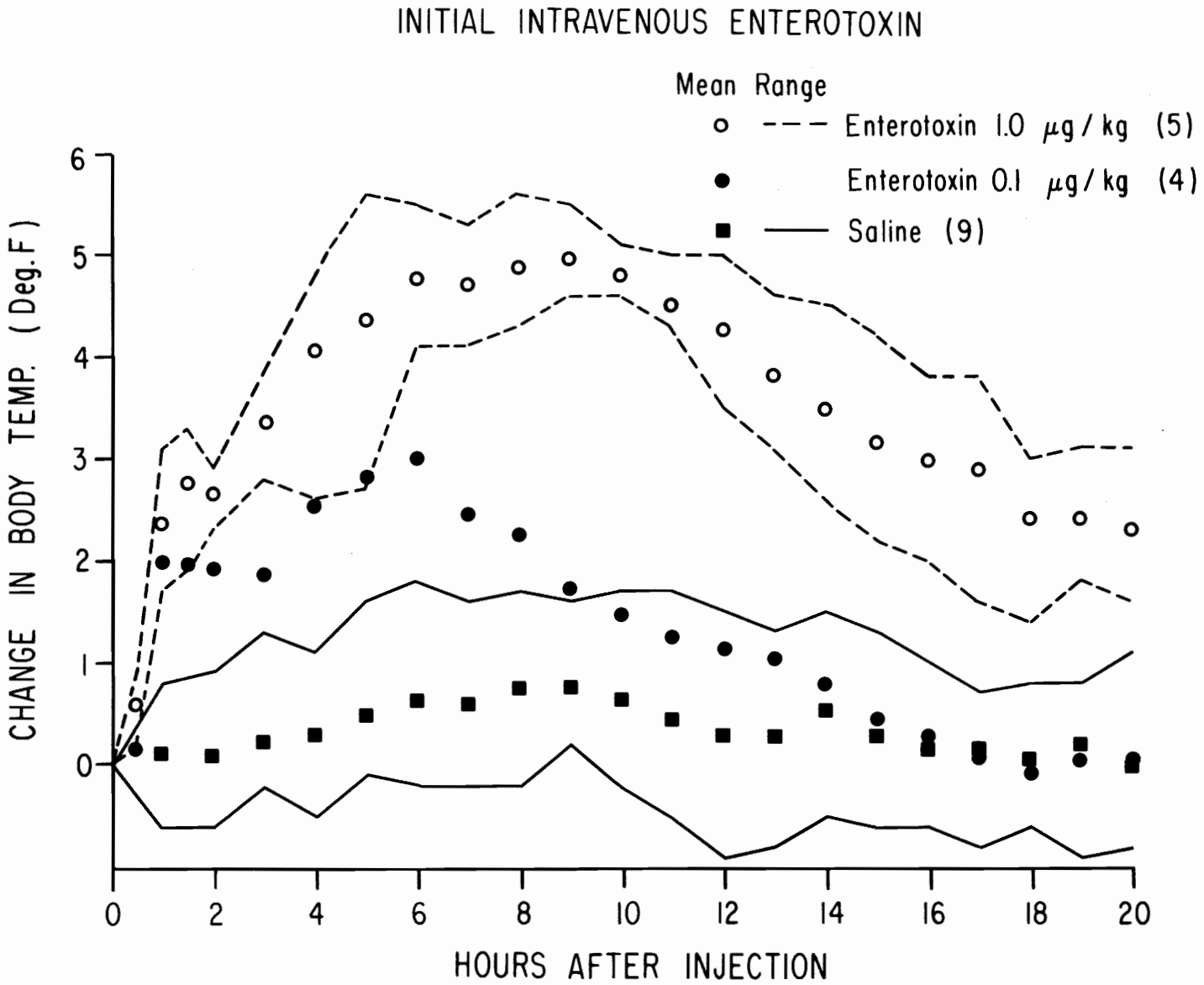
A. Pyrogenic Responses

1. Enterotoxin

a. Intravenous administration of enterotoxin

Initial response. - The mean and range of responses to initial injection of 1.0 µg/kg of enterotoxin in five cats are shown in Figure 2. After a latent period of 35 to 45 minutes, during which the temperature may have risen slightly, body temperature began to increase abruptly and maintained a rapid, linear rise for approximately 20 minutes. Shivering was usually prominent during this rising phase of the response. The temperature then continued to increase at a slower rate, or leveled out to a plateau or even fell slightly. This constituted the first phase of the pyrogenic response. The second phase of the response began with a gradual increase in temperature, approximately 2 hours after injection. The maximum level of the second phase was approached within 4 to 6 hours, with slight additional rises and plateauing up to 10 hours after injection. The average peak with this dose was 5.1°F. Recovery occurred within 24 to 36 hours. Injection of larger doses increased the duration of fever but not the maximum rise. Injection of smaller doses of enterotoxin also produced biphasic responses. The mean initial response to 0.1 µg/kg of enterotoxin in four cats is indicated in Figure 2. Two cats

Figure 2. Comparison of pyrogenic responses to initial intravenous injection of two doses of enterotoxin with responses to intravenous saline solution in the same animals. The numbers in parentheses in the key indicate the number of animals in each test. The solid and dashed lines indicate the range of variation for saline and for 1.0 μg/kg of enterotoxin, respectively. Note the biphasic nature of the responses to enterotoxin.


INITIAL INTRAVENOUS ENTEROTOXIN
Mean Range
Enterotoxin 1.0 μg / kg (5)
Enterotoxin 0.1 μg / kg (4)
Saline (9)
CHANGE IN BODY TEMP. (Deg.F)
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
HOURS AFTER INJECTION

tested initially with 0.01 µg/kg of enterotoxin gave responses which were within the range produced by injection of saline solution. Since 1.0 µg/kg of enterotoxin produced a maximal rise in temperature, this was chosen as the standard dose for use in subsequent intravenous tests. The mean and range of responses to injections of equal total volumes of saline in these same cats, 1 or 2 days prior to the enterotoxin injections, are also shown in Figure 2. None of these animals responded to saline injection with an increase in temperature greater than 1.8°F. The pyrogenic responses obtained after injections of enterotoxin-containing solutions could therefore not be attributed to the injection procedure itself.

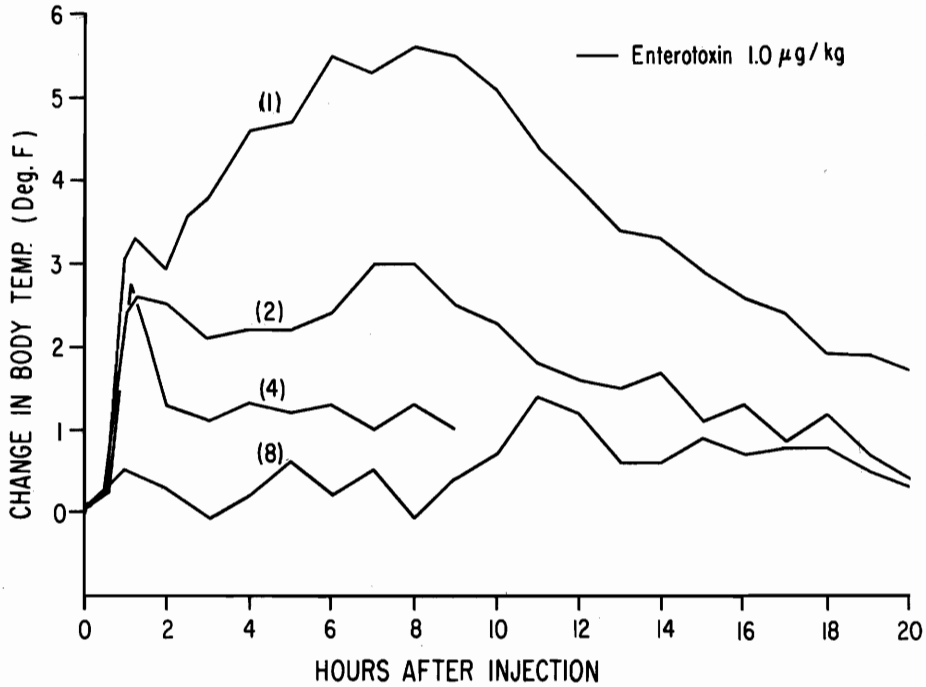
Pattern of tolerance development to intravenous enterotoxin. - Selected responses from a series of injections of 1.0 µg/kg of enterotoxin in one cat (eight injections over 16 days) are shown in Figure 3. The numbers in parentheses indicate the number of the response in the series. The second phase diminished considerably, as early as the second test, and was essentially absent by the fourth injection. Tolerance to the first phase developed more slowly, but was complete by the eighth test. Similar examples of the development of tolerance to enterotoxin are shown in Figure 4.

Intravenous injections of crude enterotoxin preparations. - In a few instances, crude culture filtrates from various strains of staphylococci, kindly prepared by members of the staff of the University

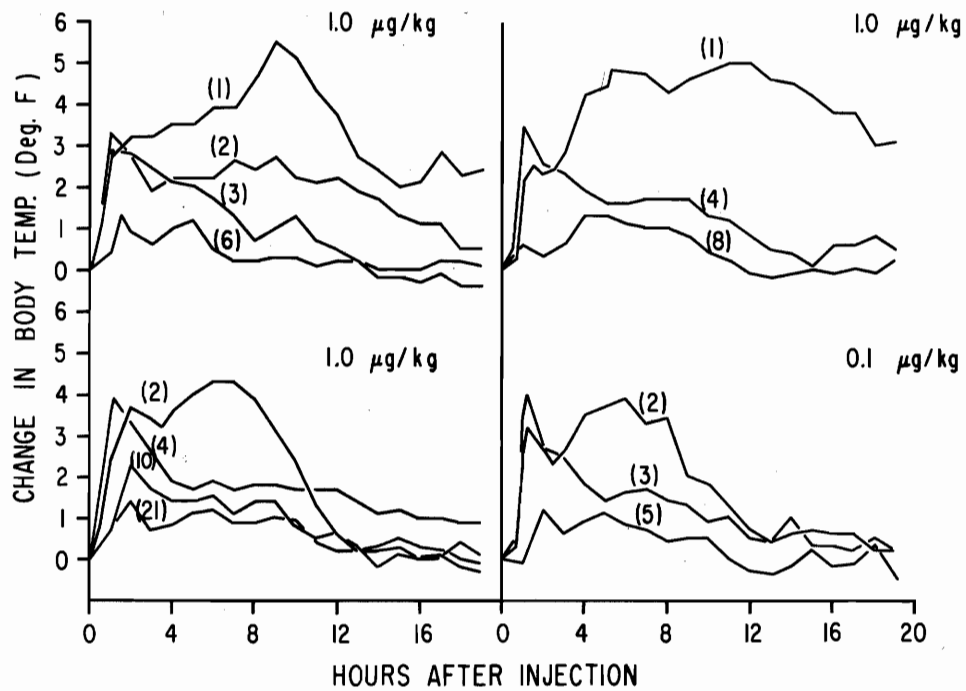
Figure 3. Representative responses to a series of intravenous injections of enterotoxin in one cat. The numbers in parentheses indicate the position of each response in the series. Note the early disappearance of the second phase while the first phase was still prominent.

Figure 4. Representative responses in four cats to repeated intravenous injections of enterotoxin at the doses shown. The numbers in parentheses indicate the position of each response in the series. The consistent pattern of tolerance development evident from these four examples deserves emphasis.

DEVELOPMENT OF TOLERANCE TO INTRAVENOUS ENTEROTOXIN



DEVELOPMENT OF TOLERANCE TO INTRAVENOUS ENTEROTOXIN



of Utah Department of Microbiology, were tested for pyrogenicity. Filtrates from strains S6, 196E, Fritchie* and LD2† all induced rises in temperature of at least 3°F, whether tested in novice or in partially tolerant animals. Doses of 1.0 or 2.0 ml/kg were injected. A dose of 2.0 ml/kg of filtrate from strain N4* produced a rise in temperature of 2.0°F in a partially tolerant animal. None of the filtrates, except S6, was tested more than once. Control responses produced by injections of growth medium into two novice cats were within the diurnal variations of the animals. The order of potency of three filtrates prepared simultaneously and tested in the same cat was S6 > LD2 > N4.

b. Lateral cerebral ventricular administration of enterotoxin

In early experiments of this series many of the animals responded with fevers as high as 5°F after lateral ventricular injection of saline. This was not due to incorrect placement of the cannulae since these animals vomited to intraventricular apomorphine. This difficulty was not noted by Sheth and Borison (1960) using similar technics. Upon repeated intraventricular injection of saline this pyrogenic response diminished and finally disappeared. This fever was not due to endotoxin contamination

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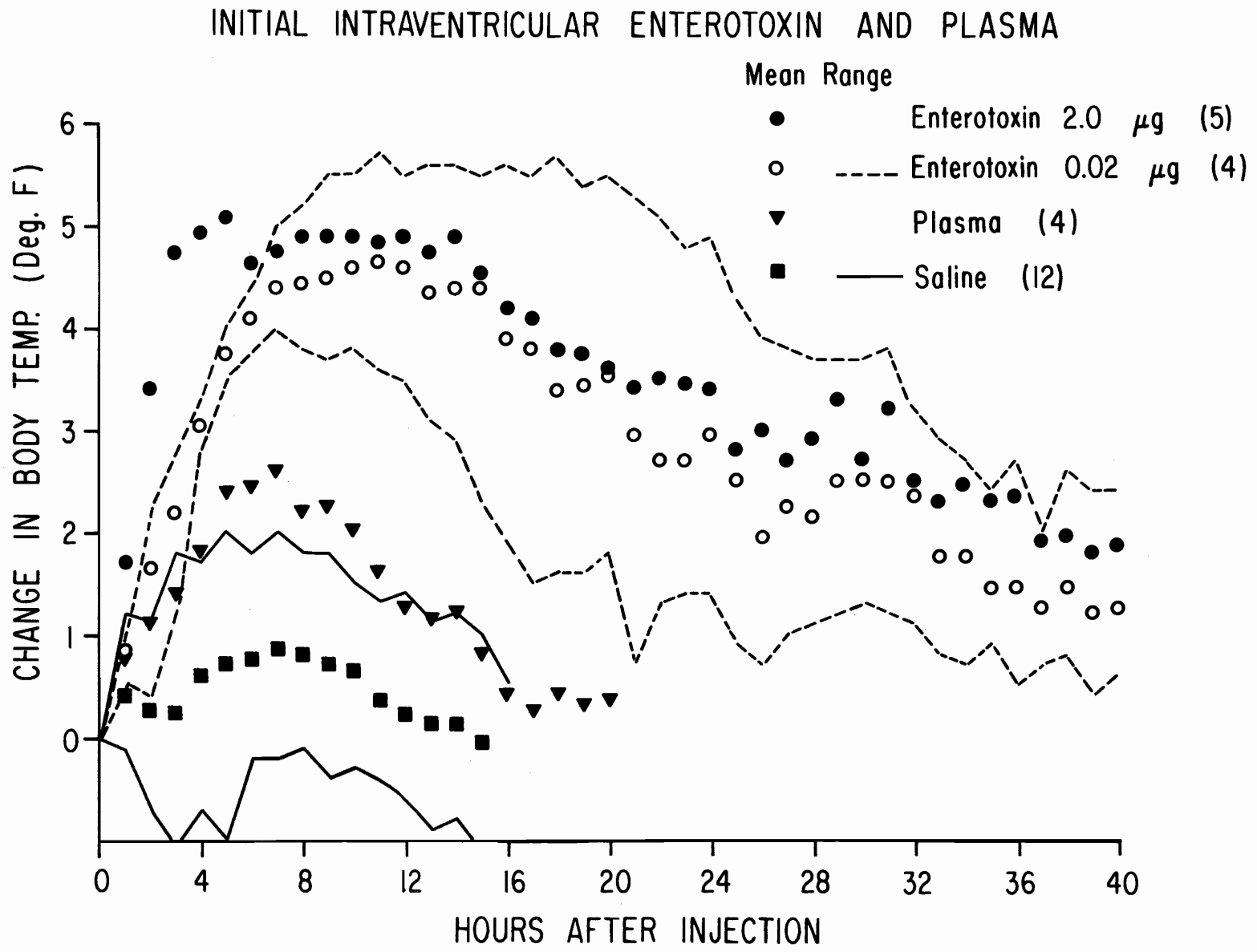
*Obtained from the Department of Microbiology, University of Texas Medical Branch, Galveston.

†Obtained from the Latter Day Saints Hospital, Salt Lake City, Utah.

since (1) tolerance to the pyrogenic effect of minimal doses of intraventricular endotoxin develops slowly if at all (Sheth and Borison, 1960; see below), (2) given samples of saline did not produce fever in all cats, and (3) normal responses to endotoxin could be obtained after this saline-induced fever had disappeared. It is felt that the disappearance of this saline-induced fever represents a stabilization and/or recovery of the animal after the trauma due to cannulation or a clearing of damaged tissue or other products from the area at the cannula tip. Subsequently, five to ten spaced saline injections were made in cannulated cats before thermocouple implantation. This procedure decreased the time lost before testing with enterotoxin or endotoxin could begin. No cat was used for tests with enterotoxin or endotoxin while its temperature rise after intraventricular saline was more than $2.0^{o}F$.

Initial responses to intraventricular enterotoxin. - Mean responses to lateral ventricular injections of 2.0 µg and 0.02 µg of enterotoxin into cats previously untested with enterotoxin are shown in Figure 5. The numbers in parentheses in the key indicate the number of animals tested. The range of responses to 0.02 µg of enterotoxin is also indicated. Except for the first few hours, essentially the same response was caused by 0.02 µg of enterotoxin as by 2.0 µg. Since 0.02 µg produced a pyrogenic response so nearly like that produced by 2.0 µg, and since 0.002 µg tested in one cat

Figure 5. Comparison of pyrogenic responses to initial intraventricular injection of two doses of enterotoxin and of normal plasma with the responses to intraventricular saline solution in the same animals. The numbers in the parentheses in the key indicate the number of animals in each test. The solid and dashed lines indicate the range of variation for saline and for 0.02 μg of enterotoxin respectively. Note the similarity between the responses to 0.02 and 2.0 μg of enterotoxin. Note also the small increase in temperature after injection of plasma as compared with the responses to enterotoxin.


INITIAL INTRAVENTRICULAR ENTEROTOXIN AND PLASMA
Mean Range
Enterotoxin 2.0 μg (5)
Enterotoxin 0.02 μg (4)
Plasma (4)
Saline (12)
CHANGE IN BODY TEMP. (Deg. F)
6
5
4
3
2
1
0
0
4
8
12
16
20
24
28
32
36
40
HOURS AFTER INJECTION

produced a response with a peak rise of only 3.5°F, 0.02 µg was chosen as the standard dose for most subsequent intraventricular injections. The fever produced by 0.02 µg of enterotoxin was a monophasic response with a latency of 1 to 2 hours; its peak, averaging 4.9°F, occurred approximately 8 hours after injection. About 48 hours were required for complete recovery.

Responses to 2.0 µg of enterotoxin differed from those obtained after 0.02 µg primarily in that the larger dose elicited a significantly more rapid rise ($p < .05$ by analysis of variance). The mean values at any hour were slightly higher but there was almost complete overlapping of the ranges for the two doses. The average peak after 2.0 µg of enterotoxin was 5.5°F.

Cats were also tested with injections of plasma (two cats) or plasma diluted 1:1000 (two cats). The mean response in these animals is indicated in Figure 5. The maximum rise was 3.3°F. The responses were essentially the same whether the plasma was obtained from the same animal or from another animal. The amount of protein in 0.2 ml of 1:1000 plasma is approximately 8 µg. These results show that the magnitude of fever produced by extremely small doses of intraventricular enterotoxin was greater than that produced by much larger doses of plasma proteins. The fever produced by intraventricular administration of enterotoxin must, therefore, be a specific effect of the toxin per se. Bennett and co-workers (1957) found that no fever was produced by intrathecal (intracisternal)

injections of serum, heparin or hemoglobin.

The mean and range of responses in these cats to injection of saline prior to tests with enterotoxin or plasma are also shown in Figure 5. These animals were adequately stabilized before the tests as indicated by the fact that none responded to intraventricular saline with a rise in temperature over 2.0°F. Injections of saline after enterotoxin or plasma produced responses that were not different from those obtained at the outset.

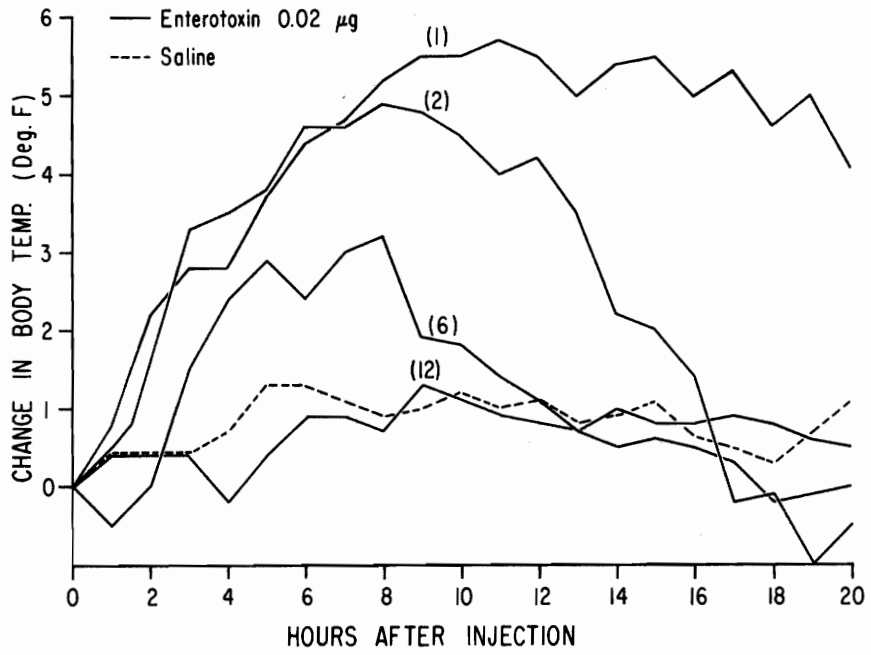
One other point of interest is that there appeared to be a ceiling above which the temperature would not rise. Thus even though the dose of enterotoxin was increased 100 fold, the increase in peak temperature was very slight and probably not significant. This ceiling seemed to be at approximately 107-108°F in cats. This phenomenon has also been observed by others (Keene et al., 1961).

Pattern of tolerance development to intraventricular enterotoxin. - Tolerance to the pyrogenic effect of intraventricular enterotoxin, as indicated by a progressive decrease in the response to a given dose, was evident at doses from 0.02 to 2.0 µg. Figure 6 shows representative responses to tests from a series of 0.02-µg doses given to one cat (12 tests over 19 days). The number of each injection in the series is indicated in parenthesis. The similarity between the responses to the twelfth enterotoxin injection and to saline administered later indicated that complete tolerance to this dose had developed. Similar tolerance patterns have been seen in four other cats with 0.02 µg, one

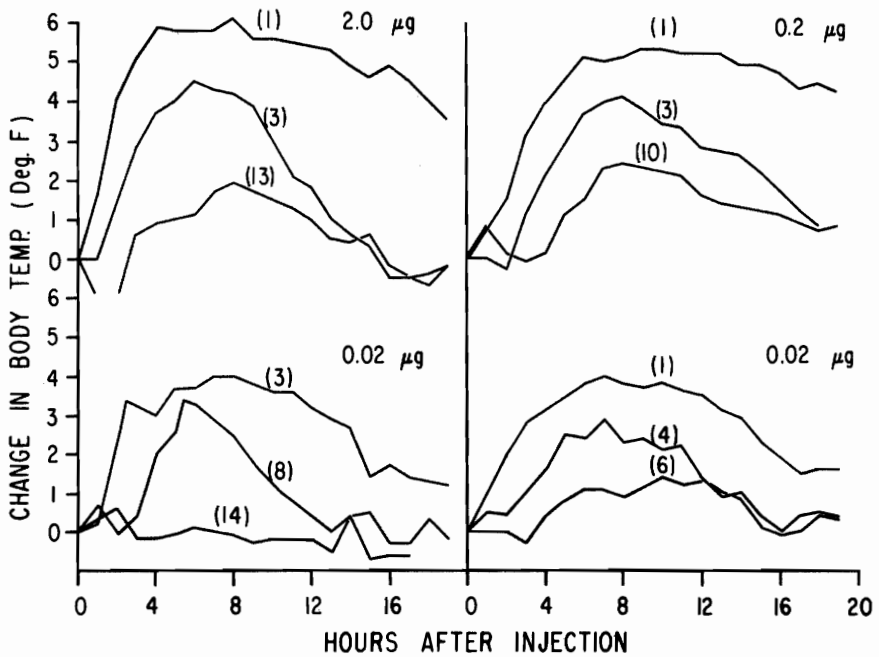
Figure 6. Representative responses to a series of intraventricular injections of enterotoxin in one cat. The numbers in parentheses indicate the position of each response in the series. Since the final response (12) to enterotoxin was indistinguishable from that obtained after injection of saline, complete tolerance to this dose had developed.

Figure 7. Representative responses in four cats to repeated intraventricular injections of enterotoxin at the doses shown. The numbers in parentheses indicate the position of each response in the series.

DEVELOPMENT OF TOLERANCE TO INTRAVENTRICULAR ENTEROTOXIN



DEVELOPMENT OF TOLERANCE TO INTRAVENTRICULAR ENTEROTOXIN



cat with 0.2 µg and three cats with 2.0 µg of enterotoxin. Examples from some of these cats are shown in Figure 7. Tolerance appeared to develop more rapidly when larger doses were interspersed in the series or when the intervals between injections were decreased. The greatest changes between successive responses were seen early in the series with the change from one injection to the next decreasing as the responses themselves diminished. The first manifestation of tolerance, especially with the higher doses, appeared to be a marked decrease in the duration of the response with little or no change in the peak. Tolerance to the larger doses developed more slowly and was less complete than that to smaller doses.

Intraventricular injections of less purified preparations of enterotoxin. - Samples of 20% pure and 70% pure enterotoxin were obtained from M. S. Bergdoll to determine whether pyrogenic activity parallels emetic activity. If such were the case it would indicate that the pyrogenic material is purified and concentrated concurrently with the emetic material or that, in fact, the same material, i.e., enterotoxin is responsible for both biological actions. The estimates of purity of the samples as 20%, 70% and 90%, based on the ability of these samples to produce emesis in monkeys, are at best only crude approximations. Tests for relative pyrogenic potency of the three samples were carried out by alternately injecting solutions of 90% enterotoxin and supposedly equivalent amounts of 70% or 20% enterotoxin during the development of tolerance. Four cats were used. In four

of five comparisons, 70% enterotoxin caused a smaller pyrogenic response than 90% enterotoxin given the following day. In the other comparison the responses were about equal. This indicates that 70% enterotoxin is less potent than estimated (since if the amounts of enterotoxin in both solutions were equal, 70% enterotoxin should have given the greater response because of tolerance development). In six of seven comparisons, 20% enterotoxin caused a greater response than 90% enterotoxin given the previous day. Twenty percent enterotoxin caused a smaller response in the other comparison. These results indicate that 20% enterotoxin is more potent than estimated on the basis of the emetic tests. Unfortunately, it is not possible to make any useful approximations of the relative pyrogenic potencies of the three samples of enterotoxin from the above data. Because of this and the uncertainty in the approximations of the emetic potencies, no valid conclusions can be drawn concerning the possibilities that the emetic and pyrogenic activities were concentrated concurrently or that enterotoxin exerts both biological actions.

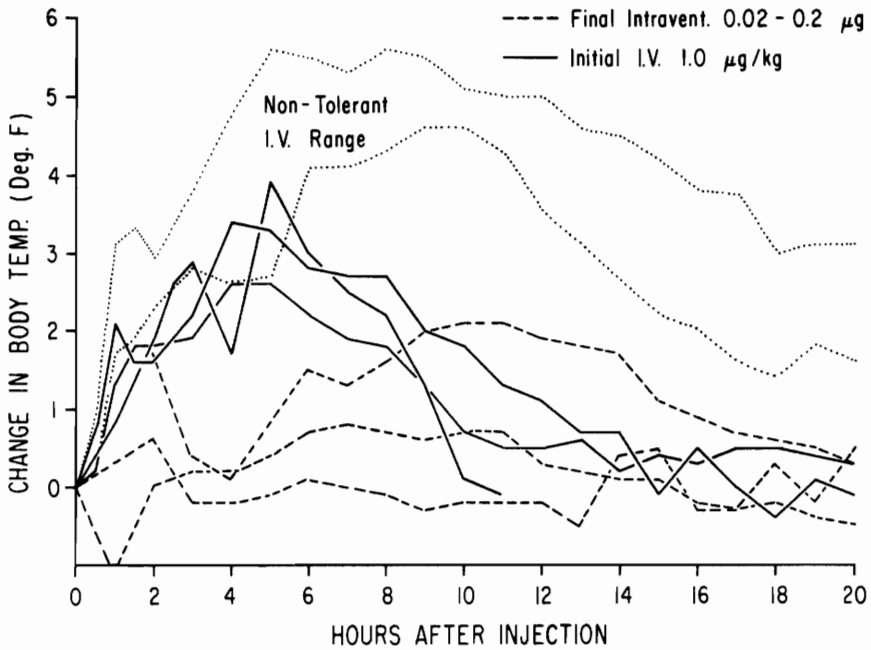
c. Cross-tolerance between intravenous and intraventricular routes of enterotoxin administration.

Initial response to intravenous enterotoxin after the development of tolerance to intraventricular enterotoxin. - Three cats were made tolerant to intraventricular enterotoxin, two cats to 0.02 µg and one cat to 0.2 µg. Their final, or tolerant, responses to intraventricular injection are shown in Figure 8 as dashed lines. Tolerance

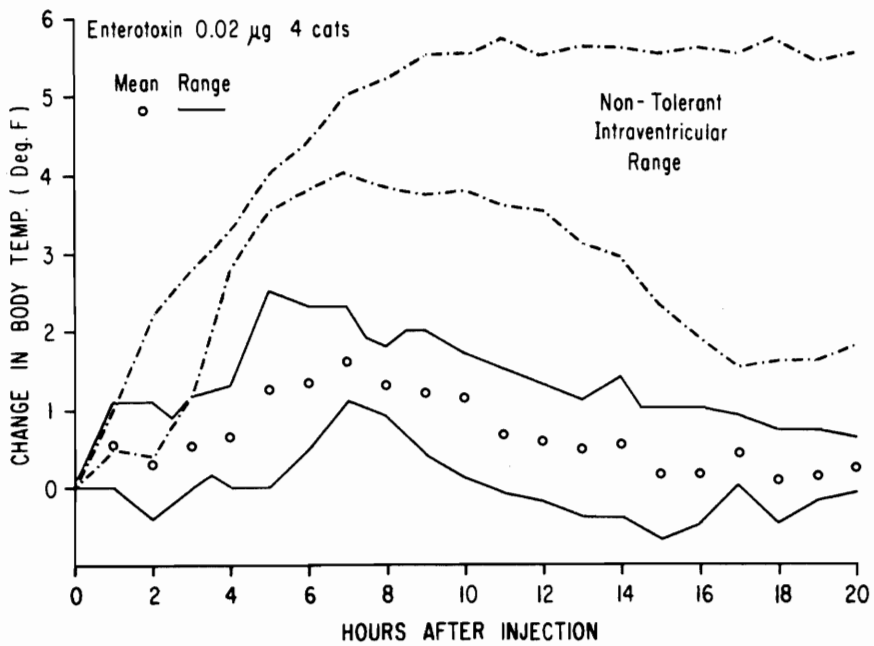
Figure 8. Cross-tolerance between intraventricular and intravenous enterotoxin induced by repeated intraventricular administration of the toxin in three cats. Individual responses to final intraventricular injections in tolerant animals and to initial intravenous injections are shown. Compare the diminished responses after intravenous enterotoxin with the range of responses (dotted lines) in non-tolerant animals.

Figure 9. Cross-tolerance between intravenous and intraventricular enterotoxin induced by repeated intravenous administration of the toxin in four cats. The solid lines indicate the range of initial responses to intraventricular enterotoxin. Compare the diminished effect of intraventricular enterotoxin in these cats with the range of responses (dotted lines) in non-tolerant animals.

INITIAL I.V. ENTEROTOXIN AFTER TOLERANCE TO INTRAVENT. ENTEROTOXIN



INITIAL INTRAVENTRICULAR ENTEROTOXIN AFTER TOLERANCE
TO I.V. ENTEROTOXIN



was induced by repeated injections of enterotoxin in doses up to 2.0 µg. The solid lines depict the responses to intravenous injections of 1.0 µg/kg of enterotoxin after intraventricular tolerance development. For comparison, the usual range of initial responses to this dose of intravenous enterotoxin is shown in Figure 8 by the dotted lines (taken from Figure 2). The intravenous responses were reduced in both phases, but perhaps more in the second. Thus, tolerance to intraventricular enterotoxin is accompanied by partial tolerance to intravenous enterotoxin.

Initial response to intraventricular enterotoxin after the development of tolerance to intravenous enterotoxin. - The converse of the above experiment was also carried out. In this case four cats previously made tolerant to 1.0 µg/kg of intravenously administered enterotoxin were tested with enterotoxin injected into the lateral ventricle. The mean and range of their responses to 0.02 µg of intraventricular enterotoxin are shown in Figure 9. The dotted lines enclose the usual range of initial responses to 0.02 µg of intraventricular enterotoxin (taken from Figure 5). The response to 0.02 µg of intraventricular enterotoxin was greatly reduced in these animals.

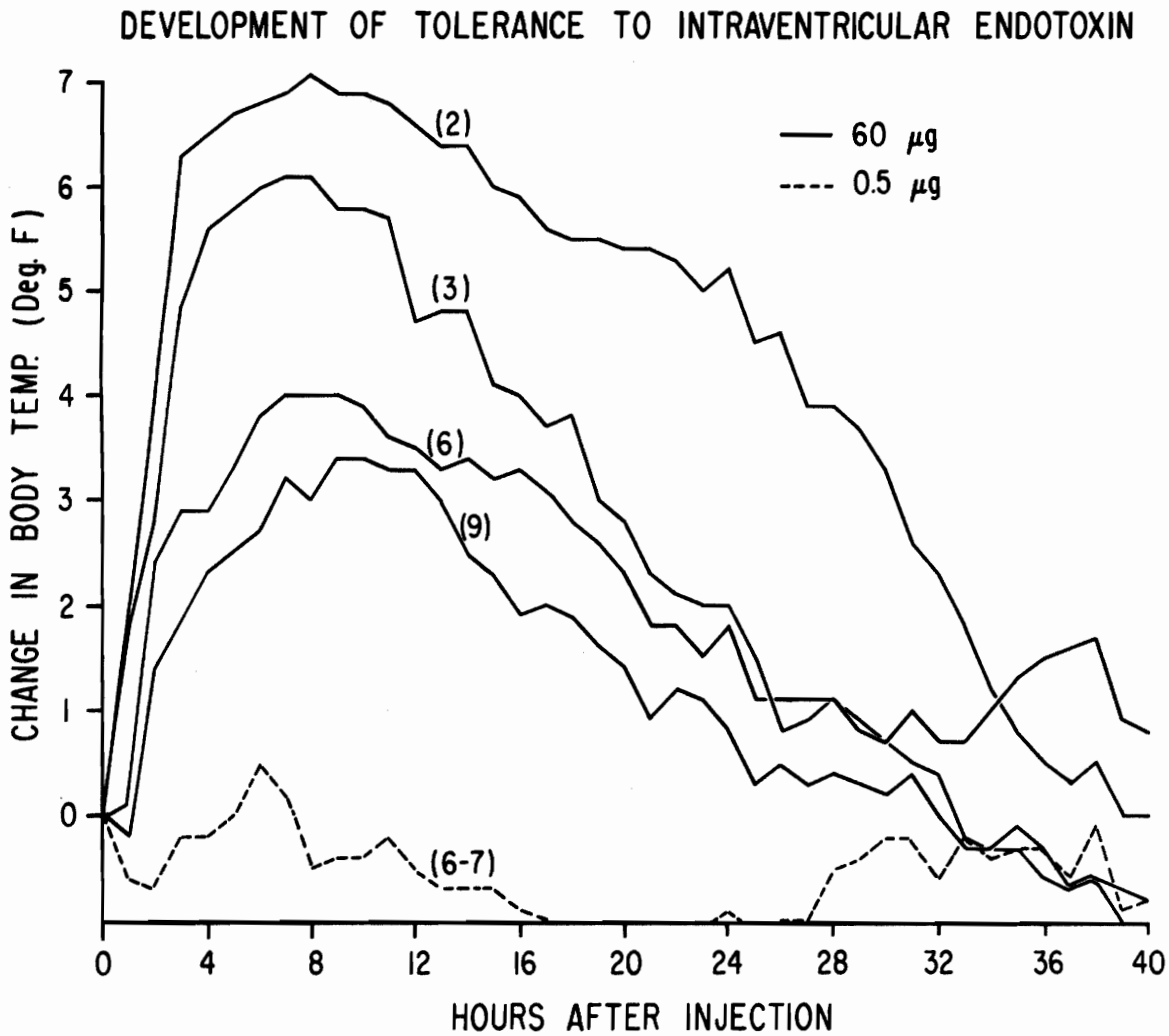
Thus, cross-tolerance occurs in both directions between the intravenous and lateral ventricular routes of administration of enterotoxin.

2. Endotoxin

a. Development of tolerance to intraventricular endotoxin

Sheth and Borison (1960) found that 0.6 µg of S. typhosa endotoxin injected into the lateral ventricle of a cat in a volume of 0.25 ml produced a maximal rise in body temperature although larger doses gave more prolonged responses. In keeping with the practice of following lateral ventricular drug injections with a saline flush, 0.5 µg of the same batch of endotoxin injected in 0.20 ml of saline followed by 0.05 ml saline was taken as the standard dose of endotoxin for lateral ventricular administration. In general, repeated intraventricular injections of 0.5 µg of endotoxin (up to nine injections in 10 days) did not result in the development of tolerance. However, in one cat a gradual diminution in the peak response to less than half of the initial response with a correspondingly decreased duration was obtained after 11 such injections over 34 days. In one cat complete tolerance to 0.5 µg intraventricular endotoxin was induced by repeated injections of the massive dose of 60 µg of endotoxin. The marked development of tolerance to this large dose of endotoxin is shown in Figure 10. Again the number of each response in the sequence is indicated by the number in parenthesis. The response to the first 60-µg dose is not shown but reached the same absolute peak as the second response. Four days were required for complete recovery from the first injection.

Figure 10. Representative responses from a series of intraventricular injections of 60 µg of endotoxin in one cat. The numbers in parentheses indicate the position of each response in the series. Note the complete tolerance to the standard dose (0.5 µg) of endotoxin after six injections of the larger dose.


DEVELOPMENT OF TOLERANCE TO INTRAVENTRICULAR ENDOTOXIN
CHANGE IN BODY TEMP. (Deg. F)
HOURS AFTER INJECTION
60 μg
0.5 μg
(2)
(3)
(6)
(9)
(6-7)
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40

Thereafter, the effectiveness of the same dose in producing fever decreased as indicated by the increased latency and the decreased peak and duration. After recovery from the sixth 60-µg injection, 0.5 µg of endotoxin gave essentially no response as shown by the dashed line. During the course of these injections, the animal's weight fell from 3.6 kg to 2.3 kg due to greatly decreased food intake over the 5-week period.

b. Passage of intraventricular endotoxin into the systemic circulation

Several observations indicated that intraventricular endotoxin can cross into the systemic circulation. Firstly, the cat that had received an initial 60-µg injection of endotoxin vomited after 6 hours. Endotoxin does not ordinarily induce emesis when administered intraventricularily (Sheth and Borison, 1960). Secondly, a diminished pyrogenic response was produced in the same cat by an initial intravenous injection of 1.0 µg/kg of endotoxin after tolerance to intraventricular endotoxin had been attained. Both the first and second phases of the response were reduced, reflecting perhaps both tolerance to a direct central effect of endotoxin and increased activity of the reticulo-endothelial system induced by endotoxin which had gained access to the circulation. Finally, another cat that had had a number of intraventricular injections of 0.5 µg of endotoxin, but which still gave a good pyrogenic response to that dose, gave a good first peak when intravenous endotoxin was

initially administered, but showed no second phase. This again suggests stimulation of the reticulo-endothelial system by endotoxin which has leaked into the blood stream.

3. Cross-Tolerance between Enterotoxin and Endotoxin Administered by the Same Route

Experiments involving the injection of either endotoxin or enterotoxin after the development of tolerance to the other were explored as a possible means of differentiating the two toxins.

a. Intravenous administration

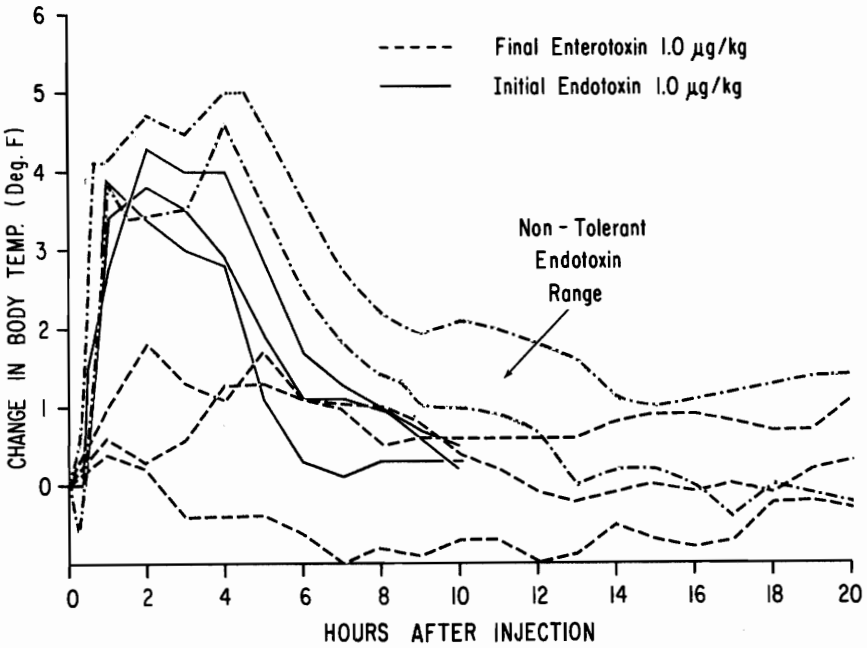
Initial intravenous endotoxin after the development of tolerance to intravenous enterotoxin. - Three cats were tested with 1.0 µg/kg of endotoxin administered intravenously after tolerance to 1.0 µg/kg of intravenous enterotoxin had been induced by an average of 12 injections. Their responses to both the initial endotoxin injections and to the final enterotoxin injections are shown in Figure 11. The dotted lines enclose the range of pyrogenic responses obtained from four other cats tested initially with 1.0 µg/kg of endotoxin without any previous enterotoxin injections. After enterotoxin tolerance, the second phase of the endotoxin response is reduced somewhat, both in maximum rise and in duration.

Initial intravenous enterotoxin after the development of partial tolerance to intravenous endotoxin. - Tolerance to the second phase of the response to 1.0 µg/kg of endotoxin was induced in three cats by an

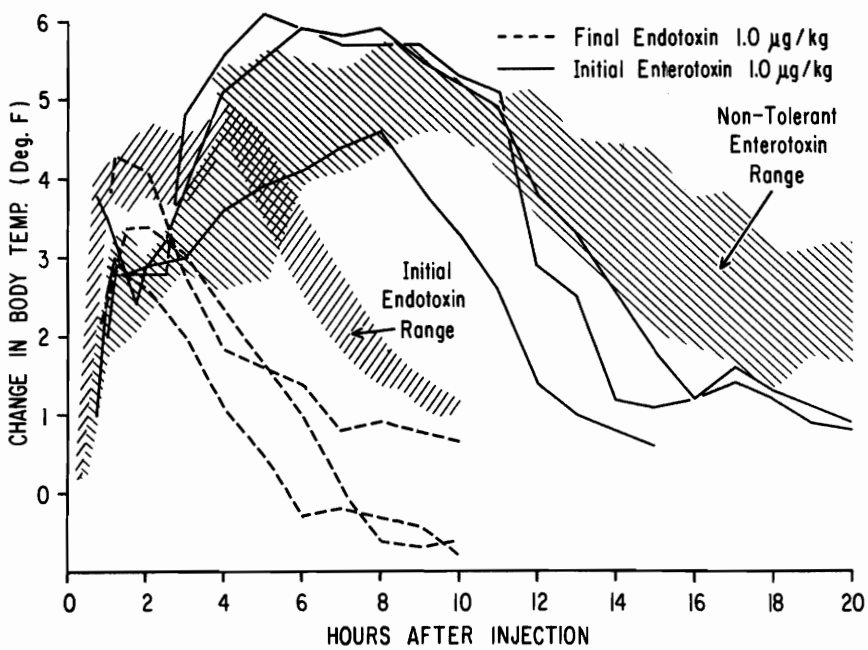
Figure 11. Minimal cross-tolerance between intravenous endotoxin and enterotoxin in three cats. The duration of the responses to initial intravenous injections of endotoxin after tolerance had developed to intravenous enterotoxin was shorter than expected when compared with the range of responses in non-tolerant animals (dotted lines).

Figure 12. Minimal cross-tolerance between intravenous enterotoxin and endotoxin. After tolerance to endotoxin had developed to the extent that the second pyrogenic phase was no longer evident, only the duration of the response to initial injection of enterotoxin was shortened when compared with the range of responses in non-tolerant animals.

INITIAL I.V. ENDOTOXIN AFTER TOLERANCE TO I.V. ENTEROTOXIN



INITIAL I.V. ENTEROTOXIN AFTER PARTIAL TOLERANCE
TO I.V. ENDOTOXIN



average of six injections. Figure 12 shows the range of responses in these animals to the initial endotoxin injections and the final individual responses after tolerance to the second phase had developed. Injections of 1.0 µg/kg of enterotoxin then produced the responses shown as solid lines. The duration of these responses was somewhat shorter than usual when compared with the non-tolerant range, also shown in Figure 12.

Thus, in both types of cross-tolerance experiments, the duration of the response to enterotoxin or endotoxin was shorter than usual after a number of injections of the other. However, this minimal change in duration was probably less than the self-induced tolerance that would have been produced by one previous injection of the same material. Since initial injections of 1.0 µg/kg of enterotoxin produced fever of a longer duration it might be argued that this dose of enterotoxin was more potent than 1.0 µg/kg of endotoxin (see Figure 12), and that the decrease in duration of the second enterotoxin-induced phase was equivalent to the decrease in the second phase produced by endotoxin. On the other hand, the fact that 1.0 µg/kg of endotoxin produced a more rapidly developing, higher first peak than enterotoxin indicates that endotoxin was not necessarily less potent than enterotoxin, but rather that the patterns of the responses were determined by differences in the characteristics of the two agents, such as ability to gain access into the central nervous system, ability to release endogenous pyrogen, metabolic pathways or rate of inactivation,

etc. These considerations in turn suggest that the agents responsible for producing the two types of fever patterns are different in their physical and/or chemical properties and are likely to belong to different chemical groups.

b. Intraventricular administration

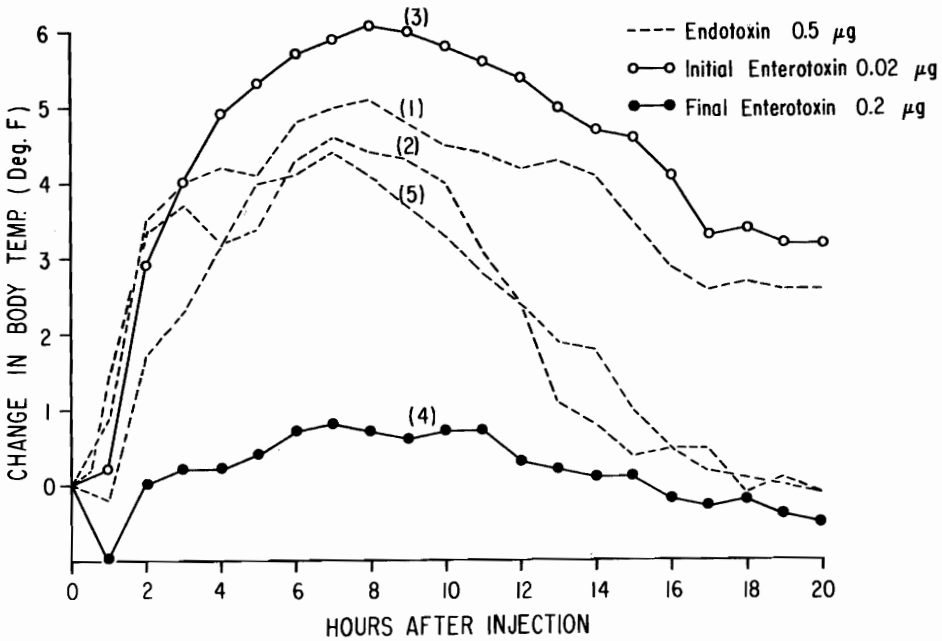
Initial intraventricular endotoxin after the development of tolerance to intraventricular enterotoxin. - An essentially normal response to intraventricular endotoxin after development of tolerance to enterotoxin is shown in Figure 13. Response (1) was initially obtained with 0.5 μg of endotoxin. This response agreed very well with those obtained by Sheth and Borison (1960). Another injection of the same dose 2 days later gave response (2) which differed from the initial response in that it showed a shortened duration and a slightly lower peak, which may reflect either tolerance development or normal variation. A test with 0.02 μg of enterotoxin then gave response (3). By repeated injections of 0.02, 0.2 and 2.0 μg of enterotoxin, this animal was made completely tolerant to ten times the original dose of enterotoxin (response 4). The following day 0.5 μg of endotoxin produced response (5) which was very little changed from response (2). A subsequent test with 0.2 μg of enterotoxin again showed no fever.

Three cats, including the one above, were tested with 0.5 μg of endotoxin after tolerance to 0.02 μg of enterotoxin. Their mean

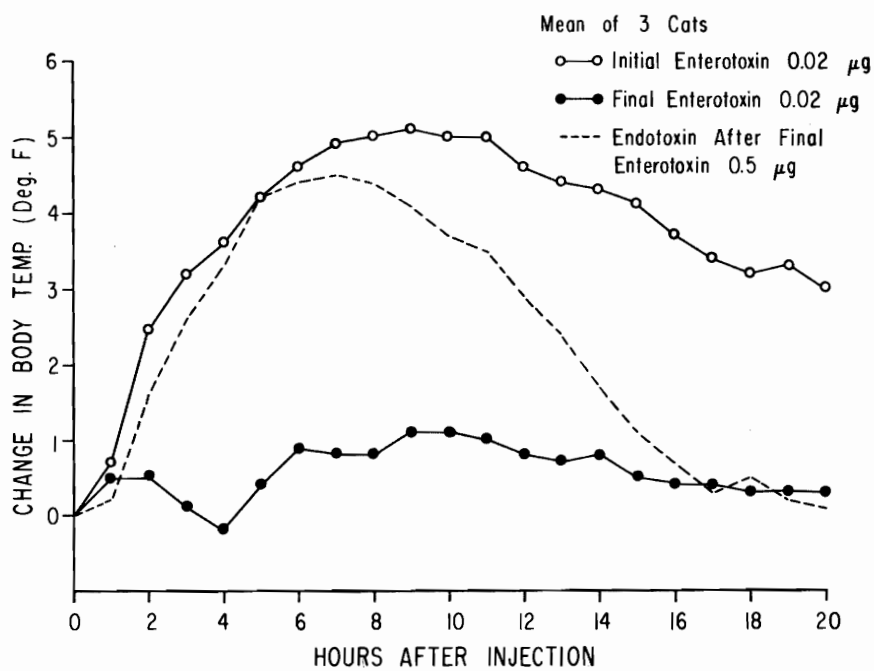
Figure 13. Absence of tolerance to intraventricular endotoxin after development of tolerance to intraventricular enterotoxin in one cat. The numbers in parentheses indicate the sequence of representative responses. Note the essentially undiminished response to endotoxin after the development of tolerance to ten times the initial dose of enterotoxin.

Figure 14. Absence of tolerance to intraventricular endotoxin after development of tolerance to intraventricular enterotoxin in three cats. Compare the mean responses in these cats with those for the single cat shown in Figure 13.

INTRAVENTRICULAR ENDOTOXIN AFTER TOLERANCE TO ENTEROTOXIN



INTRAVENTRICULAR ENDOTOXIN AFTER TOLERANCE TO ENTEROTOXIN

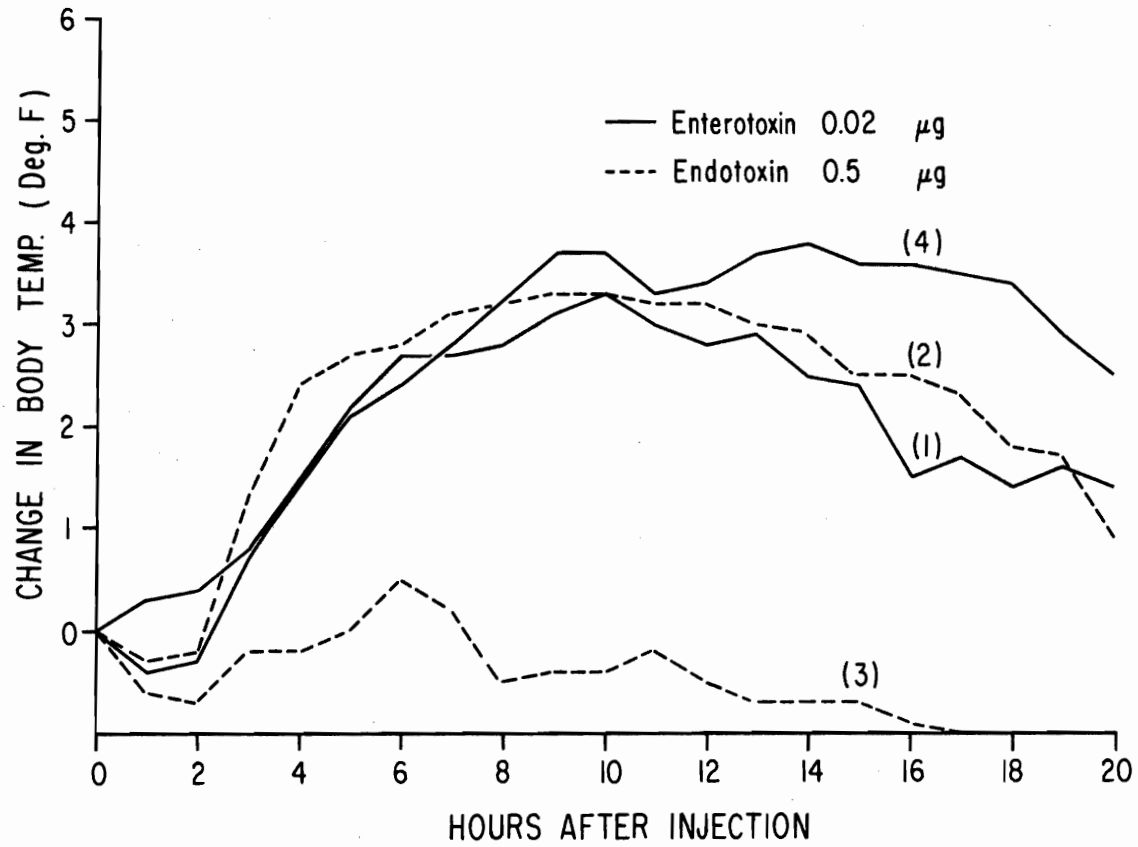


response to the injection of endotoxin following enterotoxin tolerance is shown in Figure 14. Also shown are the mean responses to initially injected enterotoxin, indicating that these animals were normal in their response initially, and to the same 0.02-µg dose of enterotoxin when tolerance had developed and before testing with endotoxin. These results demonstrate a lack of cross-tolerance between endotoxin and enterotoxin administered by the lateral ventricular route.

Initial intraventricular enterotoxin after the development of tolerance to intraventricular endotoxin. - The converse of the above, that is administration of intraventricular enterotoxin after tolerance to intraventricular endotoxin, was also examined (Figure 15). Response (1) was produced by the second enterotoxin injection interspersed among injections of 60 µg of endotoxin in the same cat as shown in Figure 10. The first enterotoxin injection, not shown in Figure 15, fell within the range for initial injections of 0.02 µg of enterotoxin, with a peak of 5.2°F at 9 to 11 hours, even though a considerable degree of tolerance had developed to 60 µg of endotoxin. Following response (1) to enterotoxin, 0.5 µg of endotoxin produced response (2). Further injections of 60 µg of endotoxin produced complete tolerance to 0.5 µg of endotoxin, response (3). After three more injections of 60 µg of endotoxin, administration of 0.02 µg of enterotoxin produced response (4), which if anything shows some recovery from tolerance over the 18-day interval between (1) and (4).

Figure 15. Absence of tolerance to intraventricular enterotoxin after tolerance to intraventricular endotoxin in one cat. The numbers in parentheses indicate the sequence of the responses. Note possible improvement in the response to enterotoxin.

INTRAVENTRICULAR ENTEROTOXIN AFTER TOLERANCE TO ENDOTOXIN



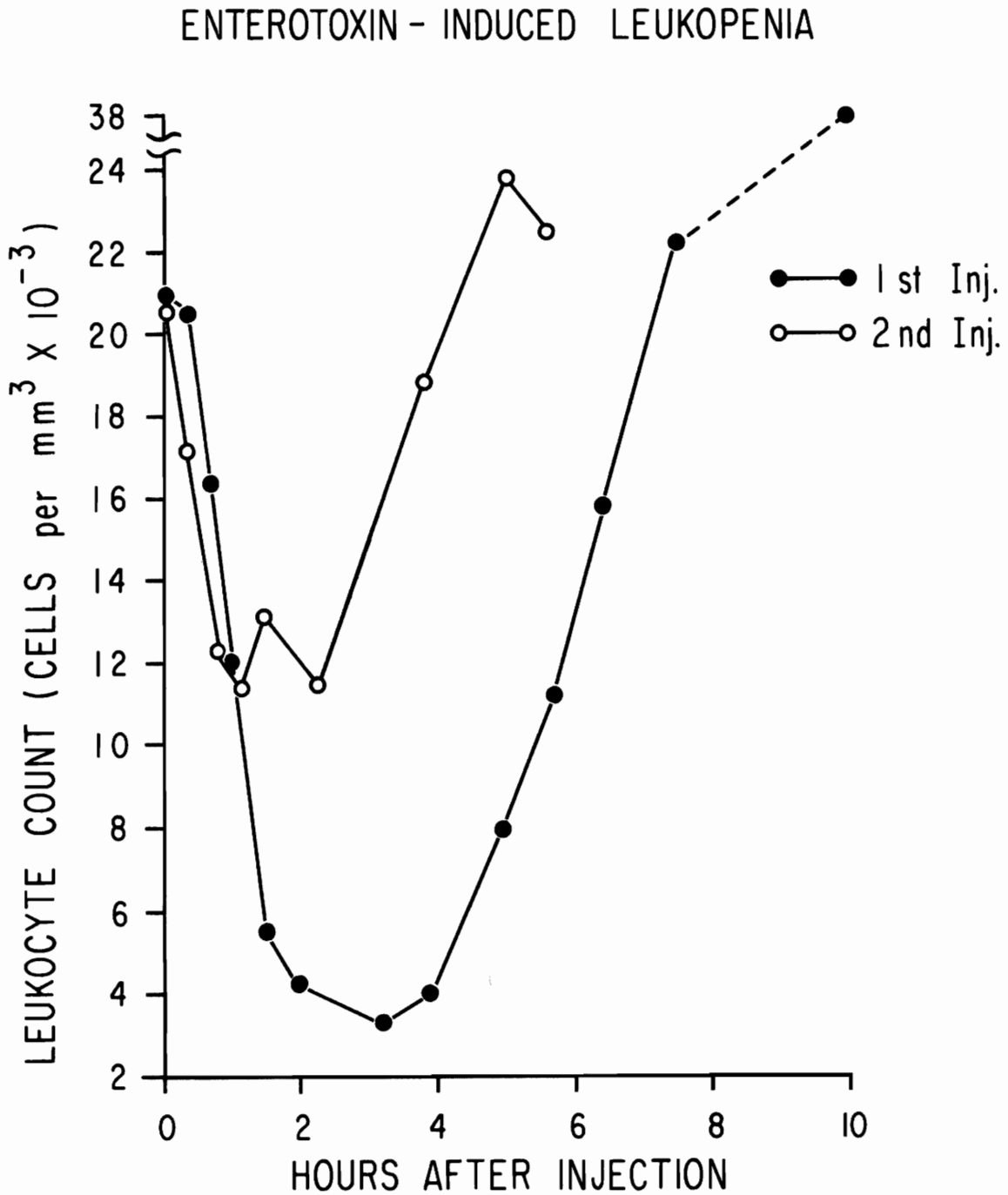
The above results indicate that the development of tolerance to either enterotoxin or endotoxin administered via the lateral cerebral ventricular route is not associated with tolerance development to the other. This is further evidence of the distinction between enterotoxin and endotoxin.

B. Leukopenia and Endogenous Pyrogen

1. Enterotoxin-induced Leukopenia

Throughout these experiments, stress has been laid on obtaining evidence that endotoxin contamination could not account for the results obtained. Since the production of leukopenia by pyrogenic doses of endotoxin is well known, the lack of such an effect after pyrogenic doses of enterotoxin would have demonstrated the absence of sufficient endotoxin contamination to account for the observed pyrogenic responses. Seven cats were tested with intravenous doses of enterotoxin ranging from 0.1 to over 5 µg/kg. In all cases marked leukopenia accompanied the production of fever. Thus, it was not possible to differentiate between endotoxin and enterotoxin on this basis. Figure 16 shows two leukopenic responses in a single cat to approximately 0.25 µg/kg of enterotoxin. The interval between injections was 9 days. Tolerance, as manifested by a diminished degree of leukopenia and a shorter duration, developed in this case. The responses obtained were entirely equivalent to a leukopenic

Figure 16. Leukopenic responses after intravenous injection of enterotoxin in one cat. Note prominent leukocytosis after the first injection and partial tolerance to the second injection.


ENTEROTOXIN - INDUCED LEUKOPENIA
LEUKOCYTE COUNT (CELLS per mm3 X 10-3)
38
24
22
20
18
16
14
12
10
8
6
4
2
0
2
4
6
8
10
HOURS AFTER INJECTION
1 st Inj.
2 nd Inj.

response obtained by intravenous administration of endotoxin in one cat. Leukopenia was followed by leukocytosis.

2. Attempts to Detect Endogenous Pyrogen in Plasma after Intravenous Enterotoxin Administration

A number of plasma transfers were made in an attempt to demonstrate the presence or absence of endogenous pyrogen (EP) in blood following enterotoxin administration. The procedure used was somewhat modified from that usually used by others which was described in the INTRODUCTION. Between 20 and 45 ml of blood were withdrawn into a heparinized syringe from the donor through the chronic jugular catheter. Withdrawals were made when the donor's body temperature was near the maximum of the second phase. The blood was immediately centrifuged and 5 to 12 ml of plasma was injected into a recipient, approximately 30 minutes after withdrawal. Sterile precautions were used throughout. In two experiments plasma from donors injected with 1.0 µg/kg of endotoxin produced fevers with short latencies (less than 15 minutes) in the recipients, which indicates that EP could be detected with this procedure. Two recipients previously untested with enterotoxin, that received plasma from donors injected with 1.0 µg/kg of enterotoxin, developed high fevers (up to 4°F), but the long latencies (at least 40 minutes) suggest that they were responding to transferred enterotoxin rather than to EP. Six transfers were made from donors injected with 1.0 µg/kg of enterotoxin to

recipients tolerant to that dose of enterotoxin. Rises in temperature from 1.1 to 2.8°F were obtained, but the latency in only one of these cases was less than 30 minutes. In addition, it was not possible to distinguish these responses from those produced by transfers of plasma from donors not injected with enterotoxin. If EP is released by enterotoxin, the possibility of detecting it would be increased by increasing the amount of plasma transferred and/or by increasing the dose of enterotoxin given to the donor, thereby presumably increasing the amount of EP released. Transfers from donors injected with 5.0 μg/kg of enterotoxin in two cases caused short-latency fevers in enterotoxin tolerant recipients. This may indicate that enterotoxin does release EP, but that it is less potent in this regard than S. typhosa endotoxin. However, on the basis of the relative durations of the second phase of the pyrogenic responses to these toxins, enterotoxin should be the more potent releaser if EP is the cause of the second phase after enterotoxin. It is hoped that a definite answer as to whether or not enterotoxin releases EP can be obtained in future experiments with the use of more extensive controls, larger doses of enterotoxin in the donors and transfers of larger volumes of plasma.

C. Emetic Responses to Enterotoxin

The effectiveness of enterotoxin in causing emesis in cats after administration by the intraventricular or intravenous routes and after various surgical procedures is summarized in Tables 2 and 3. Animals were observed for emesis up to 5 hours after enterotoxin injections. Like endotoxin (Sheth and Borison, 1960), enterotoxin did not cause emesis after intraventricular administration, even with doses which were emetic when given intravenously. In addition to the negative observations with initial tests, no emesis was seen after over 80 subsequent intraventricular injections.

A dose of 1.0 μg/kg of enterotoxin was a uniformly emetic intravenous dose, whether given to previously untested animals or to cats previously injected with small doses (0.02 μg) of enterotoxin intraventricularily. One cat previously given up to 2.0 μg of intraventricular enterotoxin vomited to 1.0 μg/kg of intravenous enterotoxin but only after a latency of over 2 hours. Previous intravenous endotoxin injections did not alter the response to an initial injection of enterotoxin. Increasing the initial dose to 5.0 μg/kg or higher decreased the latency of the response. Tolerance to the emetic action of enterotoxin developed rapidly as a result of the first injection. Thus, although a total of 21 emetic responses were obtained with 21 initial injections of 1.0 μg/kg of enterotoxin,

only two emetic responses were obtained after 35 subsequent injections of this same dose in these animals.

Ablation of the medullary emetic chemoreceptor trigger zone did not alter the emetic response to intravenous enterotoxin in cats. On the other hand, transthoracic vagotomy was effective in raising the threshold for emesis. From 5 to 28 days were allowed for recovery from surgery before enterotoxin was injected. Only three of the seven vagotomized cats vomited to large doses of enterotoxin. Those vagotomized animals which did not vomit usually exhibited various prodromal signs such as salivation, forward licking and defecation. Of three cats tested after recovery from high thoracic cord section as well as vagotomy, none vomited to 5.0 µg/kg of enterotoxin. That all were capable of vomiting was shown by emetic responses to intravenous lanatoside C. These animals did forward lick occasionally after enterotoxin, but ate willingly the following morning.

TABLE 2

EMETIC TESTS WITH INITIAL INTRAVENTRICULAR INJECTION OF STAPHYLOCOCCAL ENTEROTOXIN

Dose		Responses / Tests
µg	ml	
0.02	0.2	0/8
2.0	0.2	0/11
4.0	0.2	0/1
16.0	1.0	0/2

TABLE 3

EMETIC RESPONSES TO INITIAL INTRAVENOUS INJECTION OF STAPHYLOCOCCAL ENTEROTOXIN

State of Cat	Dose µg/kg	Responses / Tests	Latency (min) Average	Range
Normal	0.1	0/4		
	1.0	12/12	48	34-130
	1.0	6/6*	32	15-57
	1.0	3/3†	45	17-90
	⩾5.0	5/5	23	14-35
CTZ Ablated	1.0	5/6	52	10-186
	2.0-10.0	5/5	58	19-110
Transthoracic Vagotomy	2.0-5.0	3/7	42	17-82
Transthoracic Vagotomy and Spinal Cord Section (T1 or T3)	5.0	0/3‡		

*After intraventricular enterotoxin injections.

†After repeated intravenous endotoxin injections.

‡All vomited to intravenous lanatoside C.

IV. DISCUSSION

A. Advantages and Disadvantages of the Method Used to Study Pyrogenic Responses

The method used in the present experiments was developed by Borison and colleagues (Sheth et al., 1960). This method has the following advantages: (1) The animals remain in their usual familiar environment during the tests, are essentially unrestrained and free to carry on their usual activities. (2) The position of the recording portion of the thermocouple is fixed with relation to tissues within the animal. (3) Injections may be made by two routes without pain to the animals. (4) Core temperature can be recorded essentially continuously for long periods of time if desired, and the response can be seen immediately without graphing. (5) It is not necessary to have an observer present to record the temperatures; and, in fact, it is desirable not to have an observer since his activity might have some influence on the experiment.

The harnesses and thermocouples did not seem to cause any annoyance or disturbance to the animals. This setup was especially useful when used for numerous tests in an individual animal, as in the tolerance studies. Since an animal's temperature was usually monitored even on days when it was not being tested, knowledge of its body temperature gave additional information as to its general state of health.

The primary disadvantage in this method was the necessity to carry out the surgical procedures involved. This was a problem only for experiments involving single tests in a number of animals, as in determining dose-response relationships to initial injections of enterotoxin. In acute experiments, measurement of rectal temperature would have had the advantage of simplicity.

B. Can Endotoxin Contamination Account for the Pyrogenic Responses Attributed to Enterotoxin?

Early experiments in this series indicated that enterotoxin-induced fever resembled the pyrogenic response produced by endotoxin. Intravenous injection of enterotoxin was followed by a biphasic fever. Tolerance to this response was manifest by a rapid decrease in height and duration of the second phase and disappearance of this phase while the first phase remained relatively unchanged. Leukopenia, followed by leukocytosis, was also produced. Recovery from tolerance after long periods without enterotoxin injections did occur, although this was not studied carefully. If injected intraventricularly, less than one hundredth of an effective intravenous dose of enterotoxin was needed to produce a comparable fever.

These observations led to consideration of the possibility that endotoxin contamination rather than enterotoxin per se was causing the pyrogenic responses. The use of the precautions described

under METHODS excluded any possibility of contamination in this laboratory. It was still possible that endotoxin was an impurity in the purified enterotoxin received from Chicago. However, four types of evidence indicate that endotoxin, if present at all, did not contribute significantly to the responses obtained after injection of purified enterotoxin.

First, despite their many similarities, the pyrogenic responses to enterotoxin and endotoxin were not identical. As noted in Figure 12, the initial response to endotoxin was characterized by a shorter latency and a more rapid rise to a higher first peak than the initial response to enterotoxin even though the duration of the second phase was longer after enterotoxin. These differences in the patterns of the responses may indicate some physical or chemical differences between the toxins. In addition, tolerance to intraventricular enterotoxin developed easily, whereas tolerance to intraventricular endotoxin could ordinarily be induced only by repeated injections of extremely high doses. Finally, tolerance to intraventricular enterotoxin could even be induced by repeated intravenous injections. This was not the case with endotoxin (Sheth and Borison, 1960), which may again reflect the difficulty of inducing tolerance to intraventricular endotoxin.

A second type of evidence which favors the view that the pyrogenic effect of enterotoxin cannot be attributed to endotoxin contamination is the minimal or no cross-tolerance between enterotoxin

and endotoxin observed in the tolerance experiments performed in the present study. If the absence of cross-tolerance between S. typhosa endotoxin and enterotoxin solutions is to be valid evidence that the enterotoxin solutions were not contaminated by endotoxin, cross-tolerance must exist between endotoxins of different bacterial strains. Non-specific cross-tolerance between endotoxins would be expected because of the many other similarities in their actions. It has been shown that such cross-tolerance does exist between endotoxins administered intravenously (Bennett and Cluff, 1957). However, cross-tolerance between endotoxins injected intraventricularly has not been studied since tolerance by this route has not been previously observed. If specific tolerance were developed to a particular endotoxin after intraventricular administration, as might be the case if antibody formation were involved, cross-tolerance might not be shared with other endotoxins.

The third type of evidence is derived from experiments carried out primarily by Martin and Marcus (personal communication). They prepared enterotoxin-containing filtrates from cultures of staphylococcal strain S6 using rigorous precautions to prevent contamination by other bacteria or endotoxin. These crude filtrates were pyrogenic in both rabbits and cats. This strain was also found not to produce a pyrogen which could be extracted using either trichloroacetic acid extraction (Boivin preparation) or alcohol-acetone extraction (Roschka-Edwards preparation), common methods

for extracting endotoxin. Thus, crude enterotoxin-containing S6 filtrates contain a pyrogenic material which is not an endotoxin. Whether this material is enterotoxin or not remains to be demonstrated. In addition, heating purified enterotoxin in a boiling water bath for 60 minutes, a procedure which did not decrease the pyrogenic activity of endotoxin in rabbits, decreased both the ability of enterotoxin to induce fever in rabbits or cats and its emetic activity in cats.

The fourth type of evidence, which suggests that enterotoxin is itself pyrogenic, relies on presently available quantitative data. Bergdoll and associates were unable to detect either lipid or carbohydrate in enterotoxin preparations of even less than 90% purity (personal communication). No impurity, as determined by gel diffusion, made up more than 1% of the 90% enterotoxin preparation. If it is assumed that 1% of the purified enterotoxin preparation is the most pyrogenic endotoxin known, the material would have to produce a rise in temperature of $5^{\circ}F$ after intravenous administration at a dose of 0.01 µg/kg. No reports of an endotoxin preparation of this potency have been found in the literature. S. enteritidis endotoxin has been calculated to produce a rise of approximately $1.1^{\circ}F$ at a dose of 0.0001 µg/kg in the rabbit (Keene et al., 1961), but 0.01 µg/kg caused only about a $2^{\circ}F$ rise. In the same study, 1 µg/kg of S. typhosa endotoxin, which was more potent than S. enteritidis endotoxin, produced a fever of the order of $5^{\circ}F$. It was found in the

present experiments that enterotoxin was approximately 25 times as potent a pyrogen as S. typhosa endotoxin when injected into the lateral ventricle. For contamination to account for the fever produced by the purified enterotoxin preparation, the contaminating agent would have to account for over 10% and up to 100% of the purified material or the available endotoxin preparations would have to be of low purity or activity.

These four types of evidence, taken together, strongly support the contention that enterotoxin is itself pyrogenic when given intravenously or intraventricularily, and that the effects observed were not due to contamination with endotoxin.

C. Mechanism and Site of the Pyrogenic Action of Enterotoxin

Enterotoxin produces fever when injected either intravenously or into the lateral cerebral ventricle. Intraventricular enterotoxin or a metabolite must exert its pyrogenic action locally within the central nervous system since a dose which was highly effective by this route (0.02 μg) was ineffective when given intravenously. The latency of over 1 hour associated with the response to this dose of intraventricular enterotoxin suggests that diffusion to a site within the brain substance may have been necessary before the pyrogenic effect was elicited. Tolerance to the pyrogenic effect of intraventricular enterotoxin developed with repeated injections. Whether

tolerance was produced by decreased sensitivity in the system, immunological inactivation, or some other process is not known.

Intravenous enterotoxin could possibly exert its pyrogenic action in at least four general ways, including: (1) a peripheral action on efferent or afferent components of the thermoregulatory system, (2) a central action, (3) an indirect action involving release of endogenous pyrogen, or (4) some combination of the first three possibilities.

During the rapid development of the first phase of the pyrogenic response to intravenous enterotoxin, the animals exhibited a co-ordinated effort to increase body temperature as though their temperature were initially low or as if they were reacting to a cold environment. Shivering was prominent during this phase. In many instances, the animals also curled up as if to conserve heat. No panting was observed. This type of response would be expected to occur only if the afferent components or the central integrating mechanisms involved in temperature regulation were affected. Although the environmental temperature was not carefully controlled, it was usually maintained below 80°F. The "neutral" zone of temperature regulation in the resting cat is approximately 80 to 84°F (Euler, 1961). It is therefore unlikely that cessation of sweating or panting, or peripheral vasoconstriction would have contributed much to the pyrogenic responses. Since shivering was prominent, it must have been an important mechanism for producing heat and increasing

body temperature in these animals.

Production of fever solely by a peripheral action on efferent heat-producing systems, such as by induction of shivering (if this is possible by a peripheral action), would warm the blood flowing to the central thermodetectors. Heating of these detectors would activate the heat-loss mechanisms of peripheral vasodilatation, sweating and panting, and also inhibit shivering to attempt to return the body temperature to the normal range. The absence of panting while core temperature was elevated indicates that heat-loss mechanisms were not activated and that, therefore, the action of enterotoxin cannot be explained entirely on the basis of an action on an efferent component of the thermoregulatory system.

An action of enterotoxin to stimulate peripheral cold detectors or to inhibit discharge from peripheral warmth detectors would be expected to produce a co-ordinated response to increase temperature. However, as body temperature increased, increased discharge from the central thermodetectors would have increasing influence over the integrating mechanisms and would prevent large increases in core temperature. In addition, there is no reason why tolerance to an action on the peripheral thermodetectors should affect the response to intraventricular enterotoxin. However, tolerance to intravenous enterotoxin was accompanied by tolerance to intraventricular enterotoxin.

Since a large co-ordinated pyrogenic response cannot be pro-

duced by actions on the peripheral components, either efferent or afferent, of the thermoregulatory system, or, probably, by any peripheral mechanism as long as the thermoregulatory system is functioning properly, a central action must be involved in the pyrogenic response to intravenous enterotoxin. Although the possibility that both central and peripheral actions are involved cannot be excluded, a more simple explanation would be that the agent which induces the fever acts either on the central thermodetectors, or on the integrating mechanisms. This possibility will now be considered.

After tolerance to intraventricular enterotoxin, the initial response to intravenous enterotoxin was considerably less than that expected for a cat previously untested with enterotoxin. This could be due to tolerance at a central site common to the pyrogenic action of both intravenous and intraventricular enterotoxin and/or to tolerance to a peripheral action of enterotoxin induced by passage of intraventricular enterotoxin from the cerebrospinal fluid into the blood. The fact that complete tolerance to intravenous enterotoxin did not develop with repeated intraventricular injections of enterotoxin suggests that both central and peripheral mechanisms may be involved.

Whether the fever produced by enterotoxin administration is caused by enterotoxin itself, a metabolite, or endogenous pyrogen, the active agent must cross the blood-brain barrier into the central nervous system. It is generally thought that proteins are unable to

penetrate this barrier (Dobbing, 1961). However, radioactive albumin has been shown to enter the cerebrospinal fluid in dogs (Fishman, 1953) and in man (Sweet et al., 1956). Thus, it is possible that the smaller enterotoxin molecule can also enter the central nervous system. One observation that supports the possibility that enterotoxin does enter the central nervous system was that of tolerance to intraventricular injection of the toxin after the development of tolerance to intravenous injections. To produce tolerance to intraventricular enterotoxin by a peripheral action would require that the peripheral efferent pathways involved in the pyrogenic response to intraventricular enterotoxin be blocked. However, intravenous endotoxin was still effective in producing fever in animals tolerant to intravenous enterotoxin. This demonstrates that the efferent mechanisms for producing fever were still functional. Since the intravenous doses of enterotoxin were large compared with the intraventricular doses, entry into the central nervous system of less than 1% of the enterotoxin could account for part or all of the fever produced by intravenous enterotoxin. The fact that the onset of fever was more rapid after intravenous enterotoxin than after injection of an equivalent dose of enterotoxin intraventricularily is not necessarily incompatible with a central action since this may be explained by more rapid passage of enterotoxin from plasma to the central site than from cerebrospinal fluid to that site.

Additional experiments are needed to determine the relative

contributions of central and peripheral mechanisms in producing pyrogenic responses after enterotoxin. Various tolerance experiments would be useful in this regard. For instance, if repeated intravenous injections of 0.02 μg of enterotoxin do not induce tolerance to 1.0 μg/kg doses, tolerance to intravenous enterotoxin induced by repeated intraventricular enterotoxin injections would indicate a common central site. On the other hand, a peripheral site of action would be indicated if complete tolerance to intravenous enterotoxin can not be induced by a longer series of larger doses of intraventricular enterotoxin.

Since the pyrogenic responses to initial intravenous injections of both endotoxin and enterotoxin were biphasic, and since endogenous pyrogen is an important factor in endotoxin-induced fever, the possibility exists that it also contributes to the pyrogenic response to enterotoxin. Attempts to demonstrate the presence of EP in the plasma of cats after intravenous injections of enterotoxin were equivocal. However, since EP can be released by a number of unrelated agents besides endotoxin, it would not be surprising to find that enterotoxin can also release EP.

D. Relationship of Enterotoxin to Clinical Fevers

It would be interesting to know what role the pyrogenic action of parenterally administered strain S6 enterotoxin plays in such clinical disorders as staphylococcal food poisoning, enteritis

and pseudomembranous enterocolitis. As mentioned in the INTRODUCTION, fever does not seem to be a prominent symptom in staphylococcal food poisoning. This might be explained in at least four ways: (1) Fever is really present, but is overlooked. (2) Unlike purified S6 enterotoxin, enterotoxin of the 196E type implicated in food poisoning does not have pyrogenic activity. (3) Fever may be produced only by larger doses than needed to produce other symptoms. (4) Fever is not produced by oral enterotoxin. These explanations will now be considered in turn.

(1) The staphylococcal food poisoning syndrome has been observed by the victims, by physicians, and by investigators specifically studying this syndrome in human volunteers. It seems highly unlikely that most of these people would consistently overlook fever if it were present. Fever has not been overlooked in otherwise similar syndromes including salmonella-induced food poisoning, and staphylococcal enteritis and enterocolitis.

(2) Purified strain S6 enterotoxin is of the type (243) known to be associated with enteritis and pseudomembranous enterocolitis, but not with food poisoning. Intravenous injection of a crude filtrate containing 196E enterotoxin into one cat indicated that this type of enterotoxin also induces fever, but this should be investigated further.

(3) On the contrary, in the present studies it was found that fevers were produced by intravenous administration of doses

of enterotoxin too small to produce emesis. However, the relative doses needed to produce various symptoms may be different by the oral route, in different species or with different types of enterotoxin.

(4) No studies have been made of the fate of oral enterotoxin. It is not known whether enterotoxin is absorbed from the gastrointestinal tract. It is, likewise, not known if the entire protein molecule is necessary for production of the emetic and pyrogenic activities or whether smaller polypeptide units retain these activities. It is highly likely that gastrointestinal enzymes alter enterotoxin to some extent. Conflicting results have been obtained regarding the ability of trypsin and pepsin to destroy or partially inactivate enterotoxin (Dack, 1956). Perhaps following the action of these enzymes, enterotoxin is no longer capable of producing fever, but retains its emetic and other activities. Since enterotoxin-induced emesis is mediated in part by vagal afferents, it is also possible that orally administered enterotoxin can gain access to the vagal receptors involved, without systemic absorption which is probably necessary for pyrogenic activity. The fact, however, that enterotoxin is emetic whether given orally or intravenously indicates that it can pass through gastrointestinal membranes (or that more than one receptor site exists). Thus, while enzymatic alterations may account for a lack of pyrogenic activity by the oral route, it is less likely that poor absorption, per se, is responsible.

Staphylococcal enteritis and pseudomembranous enterocolitis are both characterized by fever. Since enterotoxin is such a potent pyrogenic agent, it seems likely that systemic absorption of enterotoxin could be at least partially, if not wholly, responsible for the fever exhibited by patients with these diseases. The fact that fever is prominent in these intestinal diseases, but not in food poisoning, may suggest either that the types of enterotoxin involved differ in their pyrogenicity or that the pyrogenicity of ingested enterotoxin is decreased by the action of gastrointestinal enzymes.

E. The Site of Emetic Action of Enterotoxin

Among the results of the emetic studies described above were two new observations: (1) In cats ablation of the CTZ, which is a receptor for the emetic action of a number of agents (Borison and Wang, 1953), did not affect the emetic response to intravenous enterotoxin. This result in cats differs from that obtained in monkeys by Sugiyama, Chow and Dragstedt (1961) who suggested that a species difference may account for the results obtained in the two studies. These authors also considered the other possibility, namely, that interruption of vagal afferents by the ablation procedure might have accounted for their results.

(2) Intraventricular administration of enterotoxin did not cause emesis. Complete absence of emesis to enterotoxin after brain

stem transection (Bayliss, 1940) suggested the possibility of cortical or hypothalamic receptors for the emetic action of enterotoxin. If such receptors exist, it would be expected that intraventricular administration of enterotoxin would evoke emesis. The fact that vomiting did not occur after intraventricular enterotoxin may indicate (1) that enterotoxin does not diffuse from the ventricular and subarachnoid spaces to the receptor site, (2) that a metabolite of enterotoxin, but not enterotoxin itself, produces emesis centrally, (3) that enterotoxin evokes emesis through peripheral receptors and that essential afferent pathways through the brain anterior to the midbrain are interrupted by the transection, or (4) that facilitation by forebrain structures is necessary for enterotoxin-induced emesis.

Transthoracic vagotomy raised the threshold for emesis to enterotoxin. This was in agreement with the results of Bayliss (1940) in cats and of Sugiyama and co-workers (1961) in monkeys. None of the three cats tested with enterotoxin after both transthoracic vagotomy and high spinal cord section vomited. While the negative emetic tests in these three cats were not enough to show a statistically significant decrease in emesis over vagotomized cats, the situation in enterotoxin-induced emesis appears similar to that in emesis induced by both X-radiation (Borison, 1957) and nitrogen mustard (Borison et al., 1958). Supradiaphragmatic vagotomy plus spinal cord section at T4 completely abolished emesis to these stimuli while neither procedure alone abolished the emetic response

uniformly. In addition, mid-collicular decerebration or frontal lobectomy raised the threshold for emesis after administration of nitrogen mustard or X-radiation. Thus, it is probable that, in the cat at least, the emetic response to enterotoxin, like that to X-radiation and to nitrogen mustard, is mediated through visceral afferents in both the vagi and spinal cord and that this response is facilitated by supracollicular activity, or utilizes long recurrent pathways through the forebrain.

V. SUMMARY

The pyrogenic and emetic actions of a highly purified preparation of staphylococcal strain S6 enterotoxin were examined in cats. Intravenous administration of enterotoxin produced a biphasic pyrogenic response and leukopenia, similar to those produced by intravenous endotoxin. Tolerance to the pyrogenic response upon repeated administration of enterotoxin was manifested by diminished responses in both phases. The first phase was the more resistant to tolerance development and persisted nearly intact until after the second phase had disappeared. Crude culture filtrates of various other staphylococcal strains also produced fever.

Intraventricular administration of enterotoxin produced a monophasic pyrogenic response to which tolerance developed with repeated injections. Cross-tolerance developed between intraventricular and intravenous enterotoxin.

Tolerance to intraventricular S. typhosa endotoxin was induced by repeated injections of massive doses.

Minimal or no cross-tolerance was demonstrated between endotoxin and enterotoxin by either intravenous or intraventricular administration. Attempts to demonstrate the presence of endogenous pyrogen in plasma after enterotoxin administration were suggestive but inconclusive.

Evidence was marshalled that the pyrogenic responses produced by enterotoxin administration were caused by enterotoxin per se and

not by endotoxin contamination. Possible correlations of this pyrogenic action to food poisoning, staphylococcal enteritis and pseudomembranous enterocolitis were considered. The possibilities that enterotoxin acts at central and/or peripheral sites were also analyzed.

Intraventricular administration of enterotoxin did not produce vomiting. Chemoreceptor trigger zone ablation in cats did not affect the emetic response to intravenous enterotoxin. Transthoracic vagotomy raised the threshold for enterotoxin-induced emesis. The possibility was considered that in cats the emetic action of enterotoxin may involve afferent pathways in common with X-radiation- and nitrogen mustard-induced emesis.

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THE PYROGENIC AND EMETIC EFFECTS OF STAPHYLOCOCCAL ENTEROTOXIN

by

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Characteristics of pyrogenic responses in cats produced by intravenous and by lateral cerebral ventricular injection of staphylococcal enterotoxin were determined. A highly purified preparation of staphylococcal strain S6 enterotoxin, obtained from M. S. Bergdoll of the Food Research Institute of the University of Chicago, was used for most experiments. Body temperature was monitored continuously using thermocouples implanted chronically in the retroperitoneal space.

The following observations were made: (1) Intravenous enterotoxin produced a biphasic pyrogenic response. (2) Tolerance, characterized by progressively decreasing responses to repeated injections of a given dose, developed to the pyrogenic action of intravenous enterotoxin. Tolerance to the second phase of the response developed more rapidly than did tolerance to the first phase. (3) Intraventricular injection of enterotoxin produced a monophasic pyrogenic response. (4) Tolerance also developed to intraventricular enterotoxin with repeated injections. (5) Cross-tolerance developed between the pyrogenic actions of intravenous and intraventricular enterotoxin. (6) Minimal or no cross-tolerance developed between the pyrogenic actions of enterotoxin and endotoxin by either the intraventricular or intravenous routes of administration.

In addition, it was found that intravenous enterotoxin produces leukopenia followed by leukocytosis. Attempts to demonstrate the presence of endogenous pyrogen in the plasma of animals injected with enterotoxin were inconclusive.

After consideration of available evidence, including the results of the present study, it was concluded that contamination with endotoxin could not account for the fever produced by enterotoxin and, therefore, that enterotoxin itself was responsible for the pyrogenic responses obtained. Possible sites for the pyrogenic action of enterotoxin were discussed as well as possible roles of enterotoxin as a pyrogen in staphylococcal food poisoning, enteritis and pseudomembranous enterocolitis.

The site of emetic action of enterotoxin was also investigated. The following observations were made: (1) Emesis was not caused by intraventricular administration of enterotoxin. (2) The emetic response to intravenous enterotoxin was not affected by chemoreceptor trigger zone ablation. (3) Supradiaphragmatic vagotomy raised the threshold for emesis to intravenous enterotoxin. The possibility was considered that the emetic response to enterotoxin may involve pathways in common with X-radiation and nitrogen mustard-induced emesis.