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DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS

An Annotated Bibliography

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One hundred of the most frequently referenced and historically interesting reports related to the detection of staphylococcal enterotoxins are annotated.

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DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS

An Annotated Bibliography

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DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS

An Annotated Bibliography

INTRODUCTION

The development of sensitive, rapid, and simple methods for the detection, recovery, and quantitation of microbial toxins in food has been the goal of many researchers in the field of food microbiology. The enterotoxins produced by Staphylococcus aureus have been studied extensively, and numerous procedures for detecting the toxins have been developed. While performing research on a project for the development of new and unique methods for rapid detection of foodborne toxins, the author made an extensive search of the literature and has annotated 100 of the most frequently referenced and historically interesting reports related to this subject.

ANNOTATED BIBLIOGRAPHY

Baird-Parker, A. C., and R. L. Joseph. Fractionation of staphylococcal enterotoxin B. Nature 202:570-571 (1964).

A modification of Bergdoll's procedure for isolating and purifying enterotoxin B from Staphylococcus aureus strain S6 is presented. The protein was characterized by serologic procedures, ultracentrifugation, and electrophoresis; and the results compared with those obtained in the Bergdoll laboratory. Baird-Parker and Joseph obtained two protein bands when pure enterotoxin B was electrophoresed. These bands were very similar since they could not be resolved by serologic procedures, ultracentrifugation, or chromatography on carboxymethyl cellulose, but only one of the bands was biologically active.

Bayliss, M. Studies on the mechanism of vomiting produced by staphylococcus enterotoxin. J. Exp. Med. 72:669-684 (1940).

The emetic action of staphylococcal enterotoxin was tested on young and adult cats under numerous experimental conditions. The experiments indicated that the action of

staphylococcal enterotoxin on the peripheral sensory structures is more important in the initiation of emesis than is direct action of the enterotoxin on the vomiting center. It was concluded that the enterotoxin induces emesis by acting on peripheral sensory structures of the viscera. The nerve impulses pass to the vomiting center via the vagus nerve and to the diaphragm through the phrenic nerve.

Bergdoll, M. S. Methods of analysis of the enterotoxins. (15 pp.) Food Research Institute, University of Wisconsin, Madison, Wisc., 1968.

A critical review of the current methods employed for assaying staphylococcal enterotoxins is presented. The author feels that the most reliable bioassay for the enterotoxins is the feeding of samples to young rhesus monkeys. The microslide technic is the most desirable method for detecting very small amounts of enterotoxin in food extracts. The author also suggests that Silverman's reversed passive hemagglutination procedure may become the method of choice because of its sensitivity and rapidity.

Bergdoll, M. S. FRI's enterotoxin detection laboratory. (5 pp.) Presented at the Annual Food Research Institute Meeting, University of Wisconsin, Madison, Wisc., 1969.

A brief review is made of the history of the staphylococcal food poisoning research at the Food Research Institute. The paper was presented to a nontechnical audience and primarily explains the objectives and programs currently in existence at the Institute. The Institute has the capability of training people interested in enterotoxins, objectively evaluating current methods employed in enterotoxin detection, testing foods implicated in food poisoning outbreaks, and supplying laboratories with purified toxins and specific antisera.

Bergdoll, M. S., C. A. Borja, and R. M. Avena. Identification of a new enterotoxin as enterotoxin C. J. Bacteriol. 90:1481-1485 (1965).

Enterotoxin produced by staphylococcal strains 137 and 361 was purified and found to be antigenically dissimilar from enterotoxins A and B. This enterotoxin, designated "type C,"

has a similar toxicity for monkeys as types A and B. Staphylococcal strain 137 (ATCC 19095) was selected as the prototype strain.

Bergdoll, M. S., F. S Chu, C. R. Borja, I.-Y. Huang, and K. F. Weiss. The staphylococcal enterotoxins. Jap. J. Microbiol. 11: 358-368 (1967).

The discussion encompasses the history of enterotoxins; methods of purification; and physical, biologic, and chemical properties, including the amino acid composition of types A, B, C_1, and C_2. Alcohol precipitation at subzero temperatures, ammonium sulfate precipitation, hydrochloric acid precipitation, column chromatography, and starch gel electrophoresis were some of the methods originally used to purify the enterotoxins. The method of Dr. E. Schantz is now used for preparing multiple gram quantities of enterotoxin. All of the toxins are equal in potency: i. e., 5 μg intragastrically is required to cause emesis in 50% of 2- to 3-kg. young rhesus monkeys. The compilation of physical and chemical properties is quite complete, and some of the reasons for conflicting values are given.

Bergdoll, M. S., F. S. Chu, I.-Y. Huang, C. Rowe, and T. Shih. Staphylococcal enterotoxin B. III. The physicochemical properties and the N- and C-terminal amino acid sequences. Arch. Biochem. Biophys. 112:104-110 (1965).

The molecular weight of enterotoxin B was calculated by sedimentation, viscosity, and diffusion to be 30, 650. A molecular weight of 30, 000 ± 1, 000 was calculated from the amino acid composition, and the number of residues of each amino acid was based on this molecular weight. The C-terminal amino acid sequence was found to be -leucine-tyrosine-lysine-lysine-COOH, and the N-terminal amino acid sequence was found to be glutamic acid-serine-aspartic acid-lysine-.

Bergdoll, M. S., J. L. Kadavy, M. J. Surgalla, and G. M. Dack. Partial purification of staphylococcal enterotoxin. Arch. Biochem. Biophys. 33:259-262 (1951).

The report encompasses the purification of toxins, enzymes, and other proteins. This is one of the original attempts at purifying and characterizing staphylococcal enterotoxins. The authors

concentrated and partially purified the enterotoxin by dehydration in vacuo, dialysis, and precipitation with ammonium sulfate, acid, ethanol, and methanol. They tentatively concluded that the enterotoxin was a protein.

Bergdoll, M. S., H. Sugiyama, and G. M. Dack. Staphylococcal enterotoxin. I. Purification. Arch. Biochem. Biophys. 85:62-69 (1959).

A procedure for purifying enterotoxin from Staphylococcus aureus strain S-6 is outlined. It basically consists of acid precipitation, adsorption on alumina, precipitation with ethanol, chromatography on IRC-50, and starch gel electrophoresis. The final product was antigenically homogeneous and had only one peak when analyzed on the ultracentrifuge. One microgram nitrogen of this preparation caused emesis in monkeys. The greatest difficulty encountered in the purification was the loss of toxicity. The authors suggest using as few steps as possible for purification.

Bergdoll, M. S., H. Sugiyama, and G. M. Dack. The recovery of staphylococcal enterotoxin from bacterial culture supernatants by ion exchange J. Biochem. Microbiol. Technol. Eng. 3:41-50 (1961). (Now: Biotechnol. Bioeng.)

A method is described for the recovery of enterotoxin from bacterial supernatants of Staphylococcus strain S-6 by use of the ion exchange resin Amberlite XE-64 (IRC-50). The enterotoxin is adsorbed onto the resin which has been pretreated with sodium phosphate. The enterotoxin is recovered from the resin by washing with buffer and subsequent precipitation with ethanol, dialysis, and lyophilization. The commonly employed gel-diffusion technics are also discussed.

Bergdoll, M. S., M. J. Surgalla, and G. M. Dack. Staphylococcal enterotoxin. Identification of a specific precipitating antibody with enterotoxin-neutralizing property. J. Immunol. 83:334-338 (1959).

The enterotoxin-antienterotoxin precipitate was identified through the use of a highly purified enterotoxin preparation which gives a single precipitate band in gel diffusion. This identification of a specific precipitating antibody with the

antitoxin provided the technic for the first in vitro assay of staphylococcal enterotoxin. It also provided some additional evidence that different immunologic types of enterotoxin exist.

Bokkenheuser, V., M. A. Cardella, E. A. Gorzynski, G. G. Wright, and E. Neter. Effect of staphylococcal enterotoxin on dermal reactivity to epinephrine. Proc. Soc. Exp. Biol. Med. 112:18-21 (1963).

Intravenous or intradermal injection of staphylococcal enterotoxin into rabbits resulted in the appearance of dermal lesions at the site of subsequent intradermal injections of epinephrine. As little as 0.0001 μg of enterotoxin was effective. The enterotoxin was not destroyed by heating for 15 minutes at 60° C. or 100° C., and antiserum did not neutralize the toxic effect.

Borja, C. R. Staphylococcal enterotoxin C. I. Phenolic hydroxyl ionization. Biochemistry 8:71-75 (1969).

The ionization of phenolic groups of enterotoxin C was studied by spectrophotometric titration. Of the 21 tyrosyl residues per mole of protein, 5 residues were deduced to be located on the surface of the molecule and 16 were postulated to be buried and capable of ionizing only after unfolding of the protein molecule.

Borja, C. R., and M. S. Bergdoll. Purification and partial characterization of enterotoxin C produced by Staphylococcus aureus strain 137. Biochemistry 6:1467-1473 (1967).

Enterotoxin C produced by Staphylococcus aureus strain 137 was purified by column chromatography and gel filtration. The enterotoxin was found to have a molecular weight of 34,100, sedimentation coefficient of 3.0 S, and an isoelectric point of 8.6. Intragastric administration of 5 μg. of enterotoxin to rhesus monkeys (2-3 kg.) produced emesis within 2 to 5 hours.

Borja, C. R., and M. S. Bergdoll. Further work on the purification of enterotoxin produced by Staphylococcus aureus, strain 326. Food Research Institute, Madison, Wisc. DA 18064-AMC-534(A). U.S. Army Biological Center, Fort Detrick, Md., 1968.

The authors identified the specific antibody to the enterotoxin produced by S. aureus strain 326. Studies with polyacrylamide gel electrophoresis showed that the fastest moving band was the material responsible for causing emesis in monkeys. The use of Amberlite CG-50 as an adsorption material for removing enterotoxin from solution appears promising.

Borja, C. R., and M. S. Bergdoll. Staphylococcal enterotoxin C. II. Some physical, immunological, and toxic properties. Biochemistry 8:75-79 (1969).

Enterotoxin C undergoes considerable unfolding upon treatment with 5 M guanidine hydrochloride. The effect of 8 M urea is not as pronounced. Acetylation of 5 of the tyrosine residues does not affect the immunologic and toxic properties of enterotoxin C, suggesting that the free tyrosines are not required for emetic and serologic activities. Acetylation of all 21 tyrosines, on the other hand, results in an almost total loss of precipitating capacity and ability to induce vomiting in monkeys.

Bowden, J. P. A method for preparing small quantities of C^{14}-labeled staphylococcal enterotoxin B. 1B522301A059 Department of the Army, Fort Detrick, Md., 1968.

Small amounts of radioisotopically labeled staphylococcal enterotoxin B are produced by culturing the organism on medium containing C^{14}-labeled yeast protein hydrolyzate. The toxin is purified by adsorption on and elution from Amberlite CG-50 resin. The isotope concentration is 0.2 μc./mg.

Braymen, D. T. Resistance of bacteria to purified staphylococcus enterotoxin B. J. Bacteriol. 87:971 (1964).

A total of 170 bacteria, fungi, and actinomycetes were tested for susceptibility to 10 and 100 μg of enterotoxin B. All organisms tested were apparently resistant to the toxin.

Casman, E. P. Serologic studies of staphylococcal enterotoxin. Public Health Rep. 73:599-609 (1958). (Now: HMSHA Health Reports)

A dialyzable liquid medium for the production of staphylococcal enterotoxin is described. The author demonstrated the protective effects of antitoxin by injecting cats with enterotoxin from the same strain which elicited the production of antibodies and from other strains. There was good correlation between toxicity of staphylococcal extracts and serologic relatedness as measured by the Ouchterlony diffusion test.

Casman, E. P. Further serological studies of staphylococcal enterotoxin. J. Bacteriol. 79:849-856 (1960).

The author tentatively designated the enterotoxin produced by strain 196E as "type F " since a serologically identical toxin is produced by most of the food bacteria; and the enterotoxin produced by strain 243 as "type E" because of its production by most of the strains of enteritis origin. Actually he found that most of the strains from cases of enteritis produced both types of toxin. Again there was a strong correlation between biologic toxicity (cats, monkeys) and serologic relatedness. He also observed the production by some strains of a heat-susceptible, dialyzable, emesis-inducing substance.

Casman, E. P. Staphylococcal enterotoxin. In Whipple, H. E. (ed.). Ann. NY Acad. Sci. 128:124-131 (1965).

In 50 outbreaks of staphylococcal food poisoning studied, 90% were caused by strains producing enterotoxin A, 6% by strains producing type B, and 4% by strains producing both A and B. The author found strains which produced a toxic substance possessing many of the characteristics of both enterotoxins A and B, but they were serologically distinct. The predominance of type A enterotoxin was attributed to its incidence in staphylococci isolated from human sources. The enterotoxigenicity of types A and B was not associated with any phage pattern.

Casman, E. P. Staphylococcal food poisoning. Health Lab. Sci. 4:199-206 (1967).

This paper describes the procedures used by the Food and Drug Administration for the extraction, concentration, and detection of staphylococcal enterotoxins in food. The extraction and concentration procedure basically entails: (a) blending of the suspected food, (b) centrifugation to remove insoluble

materials, (c) chloroform extraction to remove lipids and some food proteins, (d) dialysis to concentrate extract, (e) passage of extract through a carboxymethylcellulose column, (f) dialysis, and (g) lyophilization. The concentrated extract is then diluted in a minimum amount of buffer, and the Ouchterlony plate or the microslide is used for detecting the enterotoxin. There is a brief description of other methods employed for assaying enterotoxin; the reasons for using the method are presented.

Casman, E. P., and R. W. Bennett. Production of antiserum for staphylococal enterotoxin. Appl. Microbiol. 12:363-367 (1964).

Methods for production of enterotoxin types A and B antisera and procedures for purifying enterotoxin A are presented. Rabbits were used for the production of antisera. A detailed description of times of inoculation and concentrations of enterotoxin used are included. The type B antisera required no adsorption and type A required little when used in the slide double diffusion test.

Casman, E. P., and R. W. Bennett. Detection of staphylococcal enterotoxin in food. Appl. Microbiol. 13:181-189 (1965).

The assay procedure for staphylococcal enterotoxins basically includes: (a) blending the suspected food in buffer, (b) centrifugation to remove insoluble materials, (c) dialysis against Carbowax to reduce the volume, (d) passage through a CM-cellulose column, and (e) concentration of eluates by dialysis against Carbowax. The microslide was used for detecting the enterotoxin in the concentrated extract. Casman (Health Lab Sci., 1967) has modified this procedure in order to increase the percent recovery of the enterotoxins. The modified method should be employed when attempting to detect small quantities of the toxins.

Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. A. Issa. Identification of a fourth staphylococcal enterotoxin, enterotoxin D. J. Bacteriol. 94:1875-1882 (1967).

Enterotoxin D was identified serologically with antiserum to a very crude extract from a strain of staphylococci

which also produces type C. Designation of the antigen as enterotoxin D was based on the following observations: (a) produced by strains which do not produce enterotoxins A, B, or C; (b) absent in growth products of nonenterotoxigenic strains; (c) capable of initiating emesis in cats; and (d) biologic activity neutralized only by its specific antibody and not by antibodies to enterotoxins A, B, or C. It is the second most prevalent type of enterotoxin associated with staphylococcal food poisoning. Staphylococcus aureus strain 494 (ATCC-23235) was selected as the prototype strain.

Casman, E. P., M. S. Bergdoll, and J. Robinson. Designation of staphylococcal enterotoxins. J. Bacteriol. 85:715-716 (1963).

The classification of enterotoxins as types E and F was changed to a sequential, alphabetic nomenclature, starting with A, in order to avoid any confusion and to conform with the procedure used in designating other bacterial toxins. Enterotoxins F and E were changed to A and B, respectively, and strains 196E (ATCC 13565) and 243 (ATCC 14458) were designated as the prototype strains. Additional enterotoxins will be designated as C, D, E, etc.

Casman, E. P., D. W. McCoy, and P. J. Brandly. Staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 11: 498-500 (1963).

The association of staphylococcal food poisoning with cooked meat in contrast to raw meat is related to the growth-supporting properties of the former. Since S. aureus does not compete well with other organisms, the increased growth on cooked meat may result from the relatively fewer organisms present on cooked meat as compared to raw meat. The production of enterotoxin was detected by gel diffusion.

Chang, S. L., and H. E. Hall. Resistance of free-living nematodes to staphylococcal enterotoxin. Appl. Microbiol. 11:365-367 (1963).

Staphylococcal enterotoxin B, at dosages ranging from 10 to 1,000 μg./ml., produced no toxic effects on five different free-living nematodes. Instead, the nematodes actually utilized the toxin protein as evidenced by their propagation.

Chu, F. S. Hydrogen ion equilibria of staphylococcal enterotoxin B. J. Biol. Chem. 243:4342-4349 (1968).

The ionizable groups in enterotoxin B found from the titration curves are consistent with the amino acid composition. From 5 to 6 free tyrosyl residues were found in toxin. No loss of biologic activity was noted when these free tyrosyl groups were acetylated, but the antigen-antibody reaction did diminish. The possible role of hydrophobic interactions for maintaining the structure of the molecule is discussed in reference to biologic activity.

Chu, F. S., K. Thadhani, E. J. Schantz, and M. S. Bergdoll. Purification and characterization of staphylococcal enterotoxin A. Biochemistry 5:3281-3289 (1966).

A method is described for purifying enterotoxin A in yields of 35%. The method includes chromatography on carboxymethyl cellulose and gel filtration with Sephadex G-100 and G-75. The simple protein was analyzed by ultracentrifugation and electrophoresis. It was found to have a molecular weight of 34,500 and an isoelectric point of 6.8. Five micrograms of toxin were required to produce emesis in monkeys when administered orally.

Crawley, G. J., J. N. Black, I. Gray, and J. W. Blanchard. Clinical chemistry of staphylococcal enterotoxin poisoning in monkeys. Appl. Microbiol. 14:445-450 (1966).

A purified preparation of staphylococcal enterotoxin B was administered intravenously to 90 monkeys, and the clincal manifestations were noted. The results suggested that the enterotoxin produced an early change in glucose metabolism, possibly related initially to catecholamine release and later to increased utilization of glucose and metabolic acidosis. The results also suggested tissue breakdown at undetermined sites and loss of endothelial membrane integrity.

Crawley, G. J., J. N. Black, I. Gray, and W. LeBlanc. A mechanism of action of staphylococcal enterotoxin poisoning. Proc. 1964 Army Sci. Conf., vol. I, pp. 207-220. U.S. Military Academy, West Point, N. Y., 1964.

The study was conducted by administering I^{131}-labeled enterotoxin to monkeys, rats, and rabbits. The animals were then sacrificed and the fate of the toxin determined. The results indicate that the toxin is initially bound to the white blood cells and albumin. These cells are then trapped in the lung, and an accumulation of interstitial fluid and loss of vascular fluid then follows. Rechallenged and resistant animals were not affected in the same manner. There was an increased accumulation of I^{131} in the liver and an increased clearance of I^{131} by the kidney and thyroid. The authors presented no information concerning the role of the central nervous system or the cause of emesis and diarrhea.

Crawley, G. J., I. Gray, W. A. Leblang, and J. W. Blanchard. Blood binding, distribution and excretion of staphylococcal enterotoxin in monkeys. J. Infect. Dis. 116:48-56 (1966).

Radioactively labeled (I^{131}) enterotoxin B was injected intravenously into rats, rabbits, and rechallenged monkeys; and its binding to blood fractions, disappearance from blood, and urinary excretion were studied. The results indicated that the enterotoxin bound initially to leukocytes (5%) and albumin (90%), toxin-bound leukocytes were localized in the lungs, and resistant animals exhibited slower disappearance of toxin from the blood and faster and greater excretion of I^{131} without the clinical signs of intoxication.

Dack, G. M. Food poisoning, pp. 109-158. Chicago, Ill.: The University of Chicago Press, 1956.

The book contains an excellent review of the history of staphylococcal food poisoning. The case descriptions presented in chronologic order are interesting when taken in the light of the state-of-the-art at that time. The review of known characteristics of staphylococcal enterotoxin up to the time the book was written is complete and includes discussions of the symptoms of staphylococcal food poisoning, treatment, laboratory diagnosis, control, enterotoxigenic staphylococci, production of enterotoxin, methods of purification, physical and chemical properties of enterotoxin, antigenicity, and the mode of action of the enterotoxins. The discussion describes early approaches toward elucidation of the characteristics of enterotoxin.

Dack, G. M. Staphylococcus enterotoxin: A review. Jap. J. Med. Sci. Biol. 16:1-12 (1963).

The review follows the work carried out at the Food Research Institute of the University of Chicago from 1946 to the time of publication. Significant contributions from other laboratories are also included. The discussion encompasses investigations of the cultural conditions required for production of enterotoxin, bioassays of enterotoxin, physical and chemical characteristics of the toxin, in vitro assays for enterotoxin, and attempts at elucidating the mode of action of the toxin. The author's comments are well substantiated, and his suggestions for solving some of the unknown features of enterotoxin food poisoning are logical.

Dalidowicz, J. E., S. J. Silverman, E. J. Schantz, D. Stefanye, and L. Spero. Chemical and biological properties of reduced and alkylated staphylococcal enterotoxin B. Biochemistry 5:2375-2381 (1966).

The single disulfide bridge in staphylococcal enterotoxin B was found to be nonessential for the biologic activity and conformation of the protein. The derivatives produced by reduction of the disulfide bridge and alkylation of the SH group had the same biologic and immunologic properties as native enterotoxin. Disrupting the conformation of the protein with 6 M guanidine did not affect the viscosity of the protein, and after removal of the guanidine, the enterotoxin had the same biologic activity.

Davison, E., G. M. Dack, and W. E. Cary. Attempts to assay the enterotoxic substance produced by staphylococci by parenteral injection of monkeys and kittens. J. Infect. Dis. 62:219-223 (1938).

The presence of hemolytic and dermonecrotic toxins in the supernatants of S. aureus cultures presents a problem when trying to biologically assay for enterotoxins. The authors felt that boiling or formaldehyde treatment probably destroyed some of the enterotoxin present. Their method of neutralizing the interfering toxins with immune serum prepared from a nonenterotoxigenic strain proved to be useful.

They also found intravenous injection of enterotoxins into cats and monkeys a more sensitive assay than oral administration.

Denny, C. B., and C. W. Bohrer. Improved cat test for enterotoxin. J. Bacteriol. 86:347-348 (1963).

The cat test performed by injecting extracts from bacteria grown in food or cultural media was found to be nonspecific for enterotoxin. Proteus and Escherichia coli produced a material which is emetic for cats. The ways of separating enterotoxin from these materials were investigated. A procedure involving ultrafiltration and heating of the extract was found to be the most desirable means of separating the enterotoxin from emetic factors of other bacteria.

Denny, C. B., P. L. Tan, and C. W. Bohrer. Isolation and purification of enterotoxin A by polyacrylamide gel electrophoresis. J. Environ. Health 29:222-230 (1966).

A rapid procedure for fractionating enterotoxin A from cultural supernatants is outlined. The fraction was identified serologically and by the cat test. The method is faster than the carboxymethylcellulose or Sephadex methods now used widely for separation of enterotoxins. The applicability of this method for extracting enterotoxin from food is doubtful, and the percent recovery of toxin is not mentioned.

Dolman, C. E. Ingestion of staphylococcus enterotoxin by human volunteers with special reference to staphylococcic food poisoning. J. Infect. Dis. 55:172-183 (1934).

Bacteria-free filtrates containing staphylococcal enterotoxin were obtained from 200 strains of staphylococci. Forty-two human volunteers drank these extracts and were observed for signs of gastrointestinal disturbance. The susceptibility of the volunteers as well as the potency of the extracts varied. The author felt that the incidence of staphylococcal food poisoning would not be high because of the small percentage of staphylococci strains which are enterotoxigenic.

Dolman, C. E. Bacterial food poisoning. II. Staphylococcus food poisoning. Can. J. Public Health 34:205-235 (1943).

Many of the manifestations of staphylococcal food poisoning are discussed. Milk and milk products were the primary vehicles for the transmission of the enterotoxin, and procedures for eliminating these means of transmission are included. The characteristics of the enterotoxigenic cultures, the relationships between cultural conditions and quantity of toxin produced, the clinical manifestations, and bioassays for detecting the enterotoxin are discussed in great detail. The author feels that the control of staphylococcal food poisoning depends on the enlightened efforts of food handlers and consumers to reduce to a minimum the chances of staphylococci contamination and multiplication in foods.

Dolman, C. E., and R. J. Wilson. The kitten test for staphylococcus enterotoxin. Can. J. Public Health 31:68-71 (1940).

The Dolman kitten test was one of the most reliable means of assaying for enterotoxin. A representative portion of a food sample believed to contain enterotoxigenic staphylococci was emulsified and placed in nutrient broth or a semisynthetic medium consisting of proteose peptone and salts. After incubation, the bacterial-free extract is boiled for 30 minutes in order to destroy the alpha and beta toxins. The extract is then injected intra-abdominally into kittens. The kittens are observed for three hours, and any reactions to the extract are noted. In a typically positive reaction, the kitten displays lassitude, weakness, and unsteadiness, followed shortly by increased peristaltic movement, retching, vomiting, and diarrhea.

Dornbusch, K., H. O. Hallander, and F. Löfquist. Extrachromosomal control of methicillin resistance and toxin production in Staphylococcus aureus. J. Bacteriol. 98:351-358 (1969).

The authors analyzed 38 strains of S. aureus which had produced enterotoxin B. They were all coagulase-positive, methicillin-resistant, lipase-negative, and resistant to a number of antibiotics. Acriflavine treatment of 4 of these strains resulted in the loss of enterotoxin production and elimination of methicillin resistance. Because of the mode of action of

acriflavine and the transducibility of these markers, it was felt that these features are controlled by episomes.

Evans, J. B., L. G. Buettner, and C. F. Niven, Jr. Evaluation of the coagulase test in the study of staphylococci associated with food poisoning. J. Bacteriol. 60:481-484 (1950).

A total of 24 coagulase-negative staphylococci isolated from foods suspected of causing food poisoning failed to produce enterotoxin. Enterotoxin was produced by 4 out of 5 coagulase-positive staphylococci isolated from frozen foods, and by 5 out of 7 isolated from humans with staphylococci infections. The monkey feeding test was used for all assays.

Friedman, M. E. Inhibition of staphylococcal enterotoxin B formation in broth cultures. J. Bacteriol. 92:277-278 (1966).

A variety of compounds were found to be capable of inhibiting the production of enterotoxin B by staphylococci. These compounds included potassium phosphate, potassium chloride, cobalt chloride, sodium fluoride, acriflavin, phenethyl alcohol, streptomycin sulfate, chloramphenicol, spermine phosphate, spermidine phosphate, and Tween 80. The effect of most of these compounds was reversible. The author concluded that the enzymatic synthesis of enterotoxin B requires magnesium ions, and that the activity of most of the inhibitors may be selective toward alpha-lysin or coagulase.

Friedman, M. E. Inhibition of staphylococcal enterotoxin B formation by cell wall blocking agents and other compounds. J. Bacteriol. 95:1051-1055 (1968).

The production of enterotoxin B was inhibited by Tween 80, sodium desoxycholate, oleic acid, penicillin, bacitracin, D-cycloserine, and sodium lauryl sulfate. The susceptibility of Staphylococcus aureus strains S6 and 243 to these agents was not consistent

The authors felt that the data indicated that the cell surface may contain sites important for the synthesis of enterotoxin B.

Friedman, M. E., and I. D. White. Immunofluorescent demonstration of cell-associated staphylococcal enterotoxin B. J. Bacteriol. 89:1155 (1965).

Strain S6 of S. aureus was stained with an antiserum conjugated with fluorescein isothiocyanate. A brilliant fluorescence was found in the interstitial spaces between packed cells. Very little fluorescence was present on the peripheral surface of the packed cells. The authors believe that their results supported the view that enterotoxin is a water-soluble protein, rich in lysine and resistant to trypsin, and that it is located primarily at the cell surface.

Fulton, F. Staphylococcal enterotoxin--with special reference to the kitten test. Br. J. Exp. Pathol. 24:65-72 (1943).

The author investigated the validity of the kitten test for detecting enterotoxin. He concluded that the intraperitoneal kitten test could not be relied upon to detect enterotoxin because many other substances were found to produce the typical reactions to enterotoxin, a kitten-positive extract did not affect a human volunteer, and an extract toxic for the volunteer did not affect the kitten. He found that the alpha and beta lysins were evoking the reactions in kittens, and since these materials are hard to destroy or remove and do not affect man, the problem of assaying for enterotoxigenic S. aureus should be reinvestigated. The author felt that at present the human volunteer was the only reliable subject available.

Genigeorgis, C., and W. W. Sadler. Immunofluorescent detection of staphylococcal enterotoxin B. I. Detection in culture media. J. Food Sci. 31:441-449 (1966).

Two methods for detecting enterotoxin in the presence or absence of bacterial cells were developed. The first method involved staining fixed smears with antiserum conjugated with fluorescein isothiocyanate. A minimum of 15 μg./ml. of enterotoxin B is required to obtain positive results. The second method involves precipitation of the enterotoxin by mixing conjugated antiserum with a drop of culture medium. The precipitate is collected on a Millipore filter and impression slides are made.

As little as 1 μg./ml. of enterotoxin B can be detected by this method.

Genigeorgis, C., and W. W. Sadler. Immunofluorescent detection of staphylococcal enterotoxin B. II. Detection in foods. J. Food Sci. 31:605-609 (1966).

Enterotoxin B antiserum conjugated with fluorescein isothiocyanate was used successfully to detect enterotoxin B in food smears or slurries without utilizing extraction procedures. Impression smears of food were stained directly, or a slurry of the food was made and mixed with the conjugated antiserum. In the later procedure the precipitated enterotoxin was collected on a Millipore filter and an impression slide was made. The authors claim that 0.05 μg./ml. of enterotoxin B could be detected.

Gilbert, C. F. Effects on staphylococcal enterotoxin B on the coagulation mechanism and leukocytic response in beagle dogs--A preliminary study. Thromb. Diath. Haemorrh. 16:697-706 (1966).

Ten young beagle dogs were given intravenous doses of enterotoxin B ranging from 50 to 100 μg./kg. body weight and were observed and bled at intervals during a 55-day period. The immediate effect associated with the toxin was an abrupt decrease in the total white blood cell count. This initial leukopenia was follwed by leukocytosis which persisted for two weeks. The time required for clotting increased approximately 4 min. The results suggested that the plasma factors are primarily involved.

Graves, R. R., and W. C. Frazier. Food microorganisms influencing the growth of Staphylococcus aureus. Appl. Microbiol. 11: 513-516 (1963).

Approximately 870 cultures of predominating microorganisms were isolated from a variety of foods. The isolates were screened by means of spot-plate tests and broth cultures for their ability to affect the growth of S. aureus strain 196E. Approximately half of the cultures isolated affected the growth of S. aureus. Over half of these organisms were inhibitory--these included most of the lactic acid bacteria (Streptococcus, Leuconostoc, Lactobacillus), Escherichia freundii, and E.

intermedia. Aerobacter and Paracolobactrum isolates were mostly stimulatory. The organisms which were inhibitory on spot-plates were inhibitory in broth, but not all of the organisms which were stimulatory on spot-plates were stimulatory in broth.

Hall, H. E., R. Angelotti, and K. H. Lewis. Quantitative detection of staphylococcal enterotoxin B in food by gel-diffusion methods. Public Health Rep. 78:1089-1098 (1963). (Now: HMSHA Health Reports)

Oudin and Oakley gel-diffusion tubes were used to quantitatively measure the amount of enterotoxin B produced by strains of S. aureus grown in various culture media and food slurries. The Oudin method detected as little as 1 μg. of enterotoxin B per ml. The Oakley method detected as little as 0.05 μg. per ml., but one week of incubation at room temperature was required. Growth of S. aureus in brain heart infusion broth resulted in the highest level of enterotoxin production. Slurries of shrimp, lobster, crabmeat, and scallops yielded high levels of toxin; whereas perch and custard slurries yielded levels detectable only by the Oakley test. Preliminary work with enterotoxin A indicated that it produces similar results as type B in the gel-diffusion technics.

Hall, H. E., R. Angelotti, and K. H. Lewis. Detection of the staphylococcal enterotoxins in food. Health Lab. Sci. 2:179-191 (1965).

Extraction, concentration, and detection technics for assaying for the presence of enterotoxins in foods are outlined. Extraction and concentration basically entail: blending of the food with buffer, incubation at room temperature to allow for precipitation of inorganic phosphates, centrifugation to remove insoluble food particles, passage through an Amberlite CG50 column, and concentration of eluate by dialysis in polyvinylpyrrolidone. This concentrate is then used as an antigen in both Oudin and Oakley gel-diffusion tubes. To determine the percent recovery of enterotoxin, 900 μg. was added to 30 gm. of food. In the final concentrate, 27% of enterotoxin A and 42% of type B were recovered.

Hallander, H. O. Purification of staphylococcal enterotoxin B. Acta Pathol. Microbiol. Scand. 67:117-132 (1966).

A procedure is outlined for purifying enterotoxin B by gel filtration on Sephadex G-100 and preparative electrophoresis. The overall yield of immunologically pure enterotoxin was 23%. The purified toxin caused emesis in cats at 0.1 to 0.3 μg. of protein per kilogram body weight and gave a precipitation line in gel diffusion (microslide) at a concentration of 0.04 to 0.06 μg. of protein. Antiserum prepared from rabbits produced a single arc when tested immunoelectrophoretically against crude toxin. The antiserum also neutralized the effect on the cat.

Hammon, W. M. Staphylococcus enterotoxin: An improved cat test, chemical and immunological studies. Am. J. Public Health 31:1191-1193 (1941).

The intravenous cat test was found to be more applicable than the intra-abdominal test for detecting enterotoxin. Some physical and chemical tests were performed. The investigator discovered the heat stability of enterotoxin and its resistance to both trypsin and pepsin. The enterotoxin was precipitated with ammonium sulfate and alcohol and was not soluble in chloroform, ether, or alcohol. The author felt that enterotoxin was not a protein and was not antigenic.

Hayama, T., and H. Sugiyama. Diarrhea in cecectomized rabbits induced by staphylococcal enterotoxin. Proc. Soc. Exp. Biol. Med. 117:115-118 (1964).

Rabbits from which had been removed the appendix and all of the cecum except the proximal three segments were challenged intravenously with staphylococcal enterotoxin B. Diarrhea was induced in these rabbits at doses ineffective for normal rabbits. The reaction to the enterotoxin was noticeable 1-3/4 hours after injection, and complete recovery from diarrhea took 24 hours. Weekly injections of the toxin resulted in the development of resistance, and pretreatment with atropine or pyribenzamine afforded some protection against the action of the enterotoxin.

Hibnick, H. E., and M. S. Bergdoll. Staphylococcal enterotoxin. II. Chemistry. Arch. Biochem. Biophys. 85:70-73 (1959).

Some of the physical and chemical properties of staphylococcal enterotoxin were calculated. The enterotoxin was found to have a molecular weight of 24,000 ± 3,000 and an isoelectric point of 8.6. The amino acid sequence was determined. The molecule did not contain any lipids or carbohydrates.

Hodoval, L. F., M. I. Rapoport, and W. R. Beisel. A radiobioassay for staphylococcal enterotoxin B antitoxin. J. Lab. Clin. Med. 68:678-685 (1966).

The half disappearance time of I^{131}-labeled staphylococcal enterotoxin B from the blood of albino rats was investigated as a means for assaying for enterotoxin B antisera. The half disappearance time of the I^{131} increased when the rats were passively immunized with enterotoxin B antiserum. This increase in time was found to be proportional to the concentration of antiserum used. The assay appeared to be specific since no change in the half disappearance time of I^{131} was observed when the rats were injected with gamma globulin, human sera, burro sera, guinea pig sera, monkey sera, saline, or heparin.

Hopper, S. H. Detection of staphylococcus enterotoxin. I. Flotation antigen-antibody system. J. Food Sci 28:572-577 (1963).

Froth flotation was used to concentrate and remove staphylococcal enterotoxin from aqueous solutions. An anionic wetting agent and enterotoxin B antiserum complexed with Rhodamine B were added to a toxin solution. Compressed air was blown through the mixture and as the foam was produced, a dye-toxin complex (red color) located in the top layer separated from the rest of the foam. This fraction was collected and two drops were mixed with latex particles on a slide. The rate of clumping of these particles was proportional to the concentration of enterotoxin present. The procedure takes only a few minutes, and the author feels the system is specific for the antiserum added to the solution.

Johnson, H. M., H. E. Hall, and M. Simon. Enterotoxin B: Serological assay in cultures by passive hemagglutination. Appl. Microbiol. 15:815-818 (1967).

Bis-diazotized benzidine hemagglutination with formalinized sheep erythrocytes was used to rapidly and specifically detect staphylococcal enterotoxin B. The results obtained with 8 enterotoxin B-positive cultures, from a total of 68 staphylococcal cultures, agreed with gel-diffusion reactions. The sensitivity of hemagglutination inhibition equals or exceeds that of gel diffusion, and the procedure takes only a few hours. Problems with hemagglutinins for sheep erythrocytes found in many staphylococcal culture fluids are discussed.

Jordan, E. O., and J. McBroom. Results of feeding Staphylococcus filtrates to monkeys. Proc. Soc. Exp. Biol. Med. 29:161-162 (1931).

These investigators found that the use of lower animals (kittens, puppies, and monkeys) in the testing of staphylococcal enterotoxin was less than satisfactory. In these tests the animals gave either negative results or results dissimilar to those found in humans.

Joseph, R. L., and A. C. Baird-Parker. Fractions of staphylococcal enterotoxin B. Nature 207:663-664 (1965).

These researchers had found in the past that staphylococcal enterotoxin B could be separated into two fractions by starch gel electrophoresis. Since the publication of these facts, questions have been raised as to the possibility of artifacts in this procedure. In order to validate their results they compared the opacity of their sample to a standard during purification, measured the denaturation of the protein during electrophoresis, and compared toxicity of the fractions in pigs and young cats. After reviewing their findings they concluded that the two protein fractions are very similar, with the only differences being charge and toxicity. They found that the two fractions differ in secondary and tertiary protein configuration and that they do not appear to be artifacts of the purification procedure.

Kent, T. H. Staphylococcal enterotoxin gastroenteritis in rhesus monkeys. Am. J. Pathol. 48:387-407 (1966).

The pathologic features of intragastric administration of staphylococcal enterotoxin B in rhesus monkeys were investigated. The author found that with a 30-μg. dose of enterotoxin acute gastroenteritis was well developed at 2 hours, peaked at 4 to 8 hours, and regressed to near normal at 72 hours. Gastritis was found to be most severe in the antral and fundic mucosa and less severe in the area of the stomach containing parietal cells. The alteration of the small intestine was of decreasing severity from the jejunum to the ileum. The pathology of the jejunum showed epithelial damage with distended villi, and crypts extending nearly to the surface. The ileum showed only minor alterations. After daily administration of the enterotoxin, about 50% of the monkeys showed severe alteration, while approximately one-third showed only mild alterations.

Kienitz, M., and R. Preuner. On the demonstration of staphylococcal enterotoxin. Zentralbl. Bakteriol. 174:56-70 (1958).

The authors consider large research animals not ideal for measuring the biologic activity of staphylococcal enterotoxin because of expense and space. For this reason the investigators used chick embryos. The results of their investigation showed that a 9- to 10-day-old chick embryo could be injured or killed with 0.15 ml. of a toxin-containing filtrate. They found that the sensitivity of the chick embryos was much greater in the spring of each year than in the fall. The size of the egg was another variable which had to be considered. Owing to these variations the authors felt that the test should only be used as a qualitative procedure.

Kupehik, G. J. Some cultural and biochemical characteristics of enterotoxigenic staphylococci. J. Infect. Dis. 61:320-324 (1937).

The author considered the Dolman method of injecting kittens intraperitoneally with a boiled filtrate of staphylococcal strains the best procedure for detecting enterotoxin. A number of morphologic and physiologic tests were performed with enterotoxic strains of staphylococci, and no distinctive

reaction was observed which could distinguish between enterotoxic and nonenterotoxic strains.

Lal, H. Distribution studies of staphylococcal enterotoxin. IIT Res. Inst. Chicago, Ill. DA-18-064-AMC-84(A). U.S. Army Biological Laboratories, Fort Detrick, Md., 1964.

Staphylococcal enterotoxin was labeled with C^{141} and found to be identical to the unlabeled toxin when tested by electrophoresis, ultracentrifugation, gel diffusion, and biologic activity in cats and monkeys. In mice intravenously injected with C^{141}, enterotoxin and radioactivity were excreted by the kidney, salivary gland, and stomach, but retained by the liver, spleen, and thyroid. Radioactivity was also found in the blood and urine when the toxin was administered orally. In cats, radioactivity was primarily accumulated in the lung and spleen, and lesser amounts were found in the liver and adrenal gland.

Matheson, B. H., and F. S. Thatcher. Studies with staphylococcal toxins. I. A reappraisal of the validity of the "kitten test" as an indication of staphylococcal enterotoxin. Can. J. Microbiol. 1:372-381 (1955).

Filtrates from 5 enterotoxigenic strains of staphylococci were injected intraperitoneally into young cats. When boiled filtrates were used, consistent and characteristic reactions were obtained with 315 injections. No false positive reactions were obtained when nonenterotoxigenic culture filtrates were employed. The authors feel that the kitten test provides reliable means for detecting the presence of an emetic principle in staphylococci cultures.

McCoy, D. W., and J. E. Faber. Influence of food microorganisms on staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 14:372-377 (1966).

A total of 44 microorganisms were studied for their influence on staphylococcal growth and enterotoxin production. The environmental conditions markedly affected the influence of the food microorganisms on staphylococci. Of the organisms tested, 12 inhibited the growth of staphylococci. Only *Bacillus cereus* stimulated growth and toxin production.

McLean, R. A., H. D. Lilly, and J. A. Alford. Effects of meat-curing salts and temperature on production of staphylococcal enterotoxin B. J. Bacteriol. 95:1207-1211 (1968).

The effect of time, temperature, and curing salts on the production of enterotoxin B was investigated. The maximum amount of enterotoxin was produced at the end of the log phase as determined by gel diffusion. Lowering the temperature and increasing the concentration of curing salts reduced toxin production more rapidly than cell growth.

Merrill, T. G., and H. Sprinz. The effect of staphylococcal enterotoxin on the fine structure of the monkey jejunum. Lab. Invest. 18:114-123 (1968).

The intragastric administration of staphylococcal enterotoxin in monkeys produced a lesion confined to mitochondria of villus and crypt epithelial cells and of diverse cells of the tunica propria of the jejunal mucosa. These changes developed within 2 hours and reached a maximum at 4 to 8 hours. The damage produced by the enterotoxin appears to be repairable.

Milone, N. A. Studies on the use of tissue culture for the bioassay of staphylococcal enterotoxin. J. Food Sci. 27:501-507 (1962).

The author attempted to develop an assay for staphylococcal enterotoxin based on the induction of cytopathogenic effects in various strains of tissue culture cells. Several different preparations of enterotoxin, varying in purity, were used. The cruder preparations were found to contain a thermolabile cytotoxic substance, but no effect could be attributed to the enterotoxin itself.

Morse, S. A., and R. A. Mah. Microtiter hemagglutination-inhibition assay for staphylococcal enterotoxin B. Appl. Microbiol. 15:58-61 (1967).

The procedure consists of adding a sample suspected of containing enterotoxin to a mixture of erythrocytes sensitized with 10 μg./ml. enterotoxin and antiserum. Within 3 hours it was possible to identify samples containing enterotoxin. The enterotoxin could be quantitated and was accurate

within a range of a factor of 2. As little as 1.6 μg. of toxin per milliliter of sample could be detected. The procedure is rapid, requires only small quantities of reactants, is easy to read, and is reproducible.

Normann, S. J., R. F. Jaeger, and R. T. Johnsey. Pathology of experimental enterotoxemia. The in vivo localization of staphylococcal enterotoxin B. Lab. Invest. 20:17-25 (1969).

The clearance of enterotoxin B from the blood and its distribution in the body were studied in rats and monkeys injected intravenously with purified toxin. The toxin was removed rapidly from the bloodstream principally by means of the kidney. Lesser amounts were also found in the liver, lung, and gastrointestinal tract. It was concluded that the renal proximal tubules are the predominant site of enterotoxin localization (about 75%). The liver and lung may also be important sites.

Olson, J. C., E. P. Casman, E. F. Baer, and J. E. Stone. Enterotoxigenicity of Staphylococcus aureus cultures isolated from acute cases of bovine mastitis. Appl. Microbiol. 20: 605-607 (1970).

Cultures of staphylococci identified as causing bovine mastitis were tested for potential involvement in human intoxications. The cultures were examined microscopically, characterized biochemically and physiologically, and tested for phage type and enterotoxin production. Of the 160 cultures tested, 157 were identified as Staphylococcus aureus and 23 of these produced enterotoxins. A direct correlation between staphylococcal food poisoning and staphylococcal mastitis was not established, but the results indicated that mastitic cows are a reservoir of enterotoxigenic S. aureus.

Raj, H. D., and M. S. Bergdoll. Effect of enterotoxin B on human volunteers. J. Bacteriol. 98:833-834 (1969).

Two volunteers were fed 50 μg. of an enterotoxin B preparation (50% purity), and the symptoms of enterotoxemia were studied for 13 hours. The results showed that man is sensitive to 0.4 μg. enterotoxin B per kilogram of body weight.

Read, R. B., Jr., and J. G. Bradshaw. Thermal inactivation of staphylococcal enterotoxin B in Veronal buffer. Appl. Microbiol. 14: 130-132 (1966).

Purified staphylococcal enterotoxin B was suspended in Veronal buffer, and the thermal inactivation was measured by the double gel-diffusion technic. Enterotoxin in the amount of 30 μg./ml. was reduced to 0.7 μg./ml. within a range of 103.0 to 12.0 minutes at temperatures ranging from 96° to 126.7° C. Some limited studies with crude enterotoxin showed it to be more thermostable.

Read, R. B., Jr., and J. G. Bradshaw. Irradiation of staphylococcal enterotoxin B. Appl. Microbiol. 15:603-605 (1967).

Enterotoxin B was suspended either in Veronal buffer or in milk and sealed in borosilicate vials. The irradiated enterotoxin was titrated by use of gel-diffusion and cat assay procedures. With Veronal buffer and milk solutions, the doses required to inactivate 90% of the enterotoxins were 2.7 and 9.7 Mrads (at 21° to 26° C.), respectively.

Read, R. B., Jr., J. Bradshaw, W. L. Pritchard, and L. A. Black. Assay of staphylococcal enterotoxin from cheese. J. Dairy Sci. 48:420-424 (1965).

An assay procedure for enterotoxins A and B from cheese was developed by use of single or double gel-diffusion methods. To detect enterotoxin levels greater than 1 μg., the cheese was blended in buffer, centrifuged, acidified, recentrifuged, neutralized, heated, centrifuged; and the supernatant used as an antigen in single diffusion. Detection of lower quantities required that the supernatant be concentrated further by dialysis and purified by chloroform extractions. When this latter procedure and double diffusion were used, levels of enterotoxin A and B as low as 0.02 and 0.05 μg. per gram of cheese were detectable.

Read, R. B., Jr., W. L. Pritchard, J. Bradshaw, and L. A. Black. In vitro assay of staphylococcal enterotoxins A and B from milk. J. Diary Sci. 48:411-419 (1965).

The procedure for assaying staphylococcal enterotoxins A and B in milk is basically the same as the method for cheese. The procedures were applied to raw, skim, pasteurized, homogenized, condensed, and dry milks. When the extract was concentrated and the double diffusion test employed, as little as 0.015 and 0.03 μg. of enterotoxins A and B, respectively, per milliliter of milk were detectable.

Schaeffer, W. I., J. Gabliks, and R. Calitis. Interference by trypsin in the interaction of staphylococcal enterotoxin B and cell cultures of human embryonic intestine. J. Bacteriol. 93:1489-1492 (1967).

Trypsin treatment of human embryonic intestine cell cultures rendered them resistant to enterotoxin B for 48 hours. The amount of resistance increased proportionally to the time of exposure to trypsin. The authors feel the effect is enzymatic. They discuss a number of hypotheses to explain the phenomenon.

Schantz, E. J., W. G. Roessler, J. Wagman, L. Spero, D. A. Dunnery, and M. S. Bergdoll. Purification of staphylococcal enterotoxin B. Biochemistry 4:1011-1016 (1965).

A method was developed for extracting enterotoxin B in a highly purified state from the culture supernatants of Staphylococcus aureus. The procedure consisted of removal of the toxin with CG-50 resin and purification by chromatography on carboxymethyl cellulose. The dose of purified toxin required to produce emesis in monkeys was 0.1 μg. intravenously and 0.9 μg. orally per kilogram of body weight.

Silverman, S. J. Serological assay of culture filtrates for Staphylococcus enterotoxin. J. Bacteriol. 85:955-956 (1963).

Estimations of enterotoxin B were made by the gel-diffusion and quantitative precipitin tests. Analysis of the results by the t-test showed no significant difference between the results obtained with the two tests at the 95% level.

Silverman, S. J., A. R. Knott, and M. Howard. Rapid, sensitive assay for staphylococcal enterotoxin and a comparison of serological methods. Appl. Microbiol. 16:1019-1023 (1968).

Reversed passive hemagglutination was used to assay enterotoxin B in culture filtrates and in food samples. Formalin-preserved sheep red blood cells were treated with tannic acid prior to sensitization with enterotoxin antiserum. The enterotoxin solutions were incubated for 2 hours with the sensitized erythrocytes, and the amount of agglutination was determined. As little as 0.0015 μg. of enterotoxin per milliliter caused distinct hemagglutination. Since 0.3 μg. enterotoxin could be detected in 100 gm. of food, there was no need for concentrating the food extract before assaying. In tests with several foods to which 1 μg. enterotoxin B was added to 100 gm., no difficulty was encountered. It is not possible to compare a known enterotoxin with an unknown directly; however, this method is very advantageous for detecting small quantities of enterotoxin in food extracts.

Spero, L., D. Stephanye, P. I. Brecher, H. M. Jacoby, J. E. Dalidowicz, and E. J. Schantz. Amino acid composition and terminal amino acids of staphylococcal enterotoxin B. Biochemistry 4:1024-1030 (1965).

The complete amino acid composition of enterotoxin B is presented. The protein is composed solely of amino acids, has no free sulfhydryl groups, and has only one disulfide bridge. Aspartic acid and lysine are present in the highest concentrations. Based on the amino acid composition, the molecular weight is 35,380, the isoionic point is 8.70, and the partial specific volume is 0.731. These values agree well with experimentally determined values. One N-terminal residue (glutamic acid) and one C-terminal residue (lysine) per mole of protein were found, indicating that enterotoxin B is a single polypeptide chain.

Staab, E. V., J. Niederhuber, D. A. Rhoda, C. S. Faulkner II, and W. R. Beisel. Role of the kidney in staphylococcal enterotoxemia. Appl. Microbiol. 17:394-398 (1969).

This study attempted to determine whether the localization of enterotoxin B in the kidney was of importance in the development of lethal shock following intravenous administration of toxin to monkeys. No evidence was obtained to suggest that the kidney converted purified enterotoxin to a more potent toxin. The authors felt that the accumulation of enterotoxin in the kidney occurred as a nonspecific result of its moleculer size and might serve as a means of reducing the quantity of toxin reaching some other site of toxic activity.

Stark, R. L., and P. R. Middaugh. Immunofluorescent detection of enterotoxin B in food and a culture medium. Appl. Microbiol. 18:631-635 (1969).

The production of enterotoxin B in food and culture medium was demonstrated rapidly by using the fluorescent antibody technic. The authors feel that an estimation of the concentration of enterotoxin can be made by observing the degree of cell fluorescence. The production of enterotoxin was also determined under various nutritional and environmental conditions.

Sugiyama, H. Research relative to the mode of action of staphylococcal enterotoxin. Food Research Institute, University of Chicago, Chicago, Ill. DA-18-064-404-CML-490. U.S. Army Biological Laboratories, Fort Detrick, Md., 1963.

This report attempts to discern the mode of action of staphylococcal enterotoxin by several approaches. A summary of some of the more significant results follows. Monkeys developed a tolerance rather than a permanent immunity to the emetic action of enterotoxin. Monkeys treated with Thorotrast (colloidal thorium dioxide) were more sensitive to enterotoxin. The pretreatment of mice and rabbits with enterotoxin resulted in increased susceptibility to the lethal action of endotoxins. The enterotoxin was capable of inducing enterocolitis in chinchillas. Studies with rabbits indicated that the enterotoxin was incapable of changing the norepinephrine level in their organs. The results of an

electrophysiologic study on receptor sites for centrally acting emetics were inconclusive.

Sugiyama, H. Endotoxin-like responses induced by staphylococcal enterotoxin. J. Infect. Dis. 116:162-170 (1966).

A biphasic change in the reticuloendothelial system of the rabbit, depression in the rate of carbon clearance followed by granulopectic activity, was noted following intravenous injections of purified enterotoxin B. These changes are similar to those following administration of endotoxin, although the onset of changes is earlier with enterotoxin. Trasylol, and antiprotease polypeptide, affords a significant protective effect against the emetic effect of enterotoxin. The Trasylol does not suppress the vomiting reflex, but has a specificity for the emetic action of the enterotoxin. It is suggested that proteolytic enzymes may alter the walls of the gastrointestinal tract, permitting the indigenous microflora capable of producing endotoxin to evoke endotoxin-like responses. The Trasylol may inhibit this reaction.

Sugiyama, H., M. S. Bergdoll, and R. G. Wilkerson. Perphenazine and reserpine as antiemetics for staphylococcal enterotoxin. Proc. Soc. Exp. Biol. Med. 103:168-172 (1960).

Perphenazine was the most effective of four drugs tested against the emetic activity of enterotoxin. Reserpine was also effective, but chlorpromazine and cyclizine had little or no protective activity. The authors suggest that the area postrema of monkeys is an important site for the emetic action of enterotoxin.

Sugiyama, H., K. L. Chow, and L. R. Dragstedt II. Study of emetic receptor sites for staphylococcal enterotoxin in monkeys. Proc. Soc. Biol. Med. 108:92-95 (1961).

Vagotomy at the level of the diaphragm and bilateral destruction of the chemoreceptor trigger zone protect monkeys from the emetic activity of enterotoxin. Cats were only refractory

following vagotomy. It is possible that cats have a forebrain site for emesis which is lacking in monkeys.

Sugiyama, H., and E. M. McKissic, Jr. Leukocytic response in monkeys challenged with staphylococcal enterotoxin. J. Bacteriol. 92: 349-352 (1966).

Monkeys fed enterotoxin demonstrated a leukocytosis within 0.5 hour after challenge. When the enterotoxin was injected intravenously, there was an initial leukopenia followed by a neutrophilic leukocytosis. With intravenous challenges less than the ED_{50}, a variation in the responses was obtained, but all animals demonstrated a neutrophile-predominant white blood cell population.

Sugiyama, H., E. M. McKissic, Jr., M. S. Bergdoll, and B. Heller. Enhancement of bacterial endotoxin lethality by staphylococcal enterotoxin. J. Infect. Dis. 114:111-118 (1964).

Rabbits and mice pretreated with enterotoxin were more susceptible to the lethal action of bacterial endotoxins. Rabbits appeared to be more sensitive than mice. The enterotoxin alone produced no observable effect. A number of possible explanations for these results are discussed.

Sullivan, R. Effects of enterotoxin B on intestinal transport in vitro. Proc. Soc. Exp. Biol. Med. 113:1159-1162 (1969).

Enterotoxin B at a concentration of 2 μg./ml. was found to severely inhibit the net transport of water, Na, K, Cl, glucose, and lactate. A section of the small intestine in rats was used for the tests. Chloride transport was affected the most; water, Na, K, and glucose were next in order; and lactate was least affected.

Surgalla, M. J. A study of the production of staphylococcal enterotoxin in chemically defined mediums. J. Infect. Dis. 81:97-111 (1947).

The production of enterotoxin was studied in synthetic media consisting of vitamins, glucose, inorganic salts, and amino acids. Enterotoxin was produced in media containing

0.2% to 20% glucose. No other carbohydrates were investigated. Amino acids necessary for growth were the only ones required for enterotoxin production. The simplest medium used successfully contained the amino acids cystine and arginine. The amount of enterotoxin produced appeared to be dependent upon the amount of available nitrogen.

Surgalla, M. J., M. S. Bergdoll, and G. M. Dack. Some observations on the assay of staphylococcal enterotoxin by the monkey-feeding test. J. Lab. Clin. Med. 41:782-788 (1953).

Approximately 1,000 monkeys were injected intragastrically with staphylococcal enterotoxin. A wide variation in susceptibility was found. For estimations of doses, satisfactory results were obtained only when new animals were used.

Thatcher, F. S., and B. H. Matheson. Studies with staphylococcal toxins. II. The specificity of enterotoxin. Can. J. Microbiol. 1: 382-400 (1955).

The authors concluded that enterotoxin by itself is capable of eliciting an emetic response in cats. The Dolman kitten test can be made more specific by boiling the filtrates to destroy the lysins and then incubating the specimen in the presence of ascorbic acid. The existence of antigenically distinct enterotoxins was also demonstrated by immunizing cats with specific enterotoxic filtrates.

Thatcher, F. S., and J. Robinson. Food poisoning: An analysis of staphylococcal toxins. J Appl. Bact. 25:378-388 (1962).

The authors considered the cat test to be the best assay procedure but felt the need for a more specific and sensitive procedure. A hemagglutination procedure appeared promising. Three emetic toxins produced by staphylococci were found to be nonhemolytic, nonlethal, and resistant to proteolytic enzymes. They are poor antigens but can be detected by hemagglutination and by the precipitin reaction.

Wagman, J., R. C. Edwards, and E. J. Schantz. Molecular size, homogeneity, and hydrodynamic properties of purified staphylococcal enterotoxin B. Biochemistry 4:1017-1023 (1965).

Enterotoxin B demonstrates a high degree of molecular homogeneity and has a partial specific volume and infrared spectral absorption typical of simple proteins. The molecular weight was found to be 35,300 and the density 1.286 gm./cm.^{3} The sedimentation behavior was stable over a wide pH range (5-10).

Weirether, F. J., E. E. Lewis, A. J. Rosenwald, and R. E. Lincoln. Rapid quantitative serological assay of staphylococcal enterotoxin B. Appl. Microbiol. 14:284-291 (1966).

A rapid assay technic was developed based on the Oudin single gel-diffusion procedure. The rapid quantitation of the Oudin procedure is based on the linear relationship between the length of the band of the precipitate and the logarithm of the concentration of the antigen. Unknown antigen concentrations can be determined by precipitin band measurements within 16 hours, and estimates can be made in 4 hours. If small concentrations are to be detected (2 μg./ml.), a 0.2 M salt buffer solution must be employed.