PHARMACOLOGY OF BACTERIAL TOXINS

HELENA RAŠKOVÁ AND JIŘÍ VANĚČEK

Department of Pharmacology, Faculty of Pediatrics, Charles University, and Pharmacological Laboratory, Czechoslovak Academy of Science, Prague, Czechoslovakia

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I. INTRODUCTION

Bacterial toxins are interesting to pharmacologists but they have been too little investigated by them. The choice of material for this review has been biased by the authors' interests, and by the amount of current research in the field.

The effects of endotoxins have aroused more and more interest within recent years. Although several reviews have also appeared on this subject (see page 2) a variety of new facts justified reconsideration.

Shigella shigae toxin possesses interesting pharmacological properties. Streptococcal and staphylococcal toxins are of outstanding practical importance. Pharmacological research with *B. pertussis* vaccine and its toxin is a newly developing subject of special interest.

Some of the toxins reviewed are exotoxins, some endotoxins. But the prefixes exo- and endo- do not mean exactly that the toxins exist only in or out of the bacterial cell. The difference is rather in chemical structure and biological activity. Exotoxins are thermolabile proteins with marked biological activity, whereas endotoxins are complex phospholipid polysaccharides (8, 120, 337). The reviewers are pharmacologists, and this influenced to a considerable extent the presentation of the material, which excluded the more specialized microbiological and immunological work.

II. ENDOTOXINS

Van Heyningen divided toxins into exotoxins, which apparently have different pharmacological properties for each toxin, and endotoxins, which are consistent in their effects (119). But the more endotoxins are studied, the more new pharmacological properties are discovered. Numerous review articles have appeared on the subject; material already reviewed has been avoided when possible, and the reader is referred to this literature (5, 15, 21, 98, 314, 337, 338, 342).

A. Nature of endotoxin

Endotoxins are toxic principles of the cell wall of gram-negative bacteria (337). Endotoxin-like activity has been found also in products from gram-positive microbes. The most active endotoxins are water-soluble, complex polysaccharides of high molecular weight. The protein-free lipopolysaccharide has been assumed to be the essential part for pharmacological effects. It consists of various sugars, and, from the point of view of pharmacological activity, the lipoid component, lipoid A, has been reported to be the most important. An artificial lipoprotein was prepared of which only the lipoid A was of bacterial origin; this was pyrogenic and relatively toxic, whereas the polysaccharides were relatively inactive (186). Recently, however, the idea that lipoid A is the part of endotoxin responsible for full endotoxic activity has been challenged. In bioassay a lipid component from endotoxin had a very low activity, never more than $1/100$ that of the parent endotoxin. Endotoxin was inactivated by acid hydrolysis prior to liberation of water-insoluble lipid components. According to these investigations, in which a different chemical precedure was employed, the major endotoxin activity cannot be attributed to the lipid component, and is due to polysaccharide (175, 251, 252).

B. Toxicity

Endotoxins are lethal for various species including man. The course of intoxication is characterized predominantly by circulatory and respiratory disturbances, weakness, ataxia, hemorrhagic lesions in the intestine, and shock. The lethality of endotoxins varies according to species (348). The approximate relative LD75's are as follows: cat 1, rabbit 2.5, dog 8, guinea pig 20, rat 300, and mouse 500. The toxicity of endotoxin is also age-dependent. In newborn mice, coli endotoxin is five to ten times, and in infant mice six times less toxic than in adults (62, 290). Increased resistance of newborns to endotoxin was also reported for the

Flexner endotoxin (236). The high resistance of newborn animals to dysentery endotoxin is also apparent in the unresponsiveness of isolated organs.

In titrating endotoxins, the lethal potency in mice has a very good correlation to the primary skin inflammatory response induced by intracutaneous injection in rabbits. The ED50 and LD50 correlation is 1 to 1,000. Therefore, the LD50 determination as an indicator may be replaced for some purposes by this method of titration (177).

The lethality of endotoxin can be reduced in various ways. Inorganic iron preparations added *in vitro* decrease markedly the toxicity. Only lethality and tissue-necrotizing ability are affected, while pyrogenicity remains unchanged; this result points to different mechanisms (141). Adenosine triphosphate, ADP, and AMP (but not adenosine) possess therapeutic activity against various toxins (including endotoxin) and X-ray irradiation (240).

C. Fever

The most striking property of endotoxin is its pyrogenic activity. Fever may be induced by as little as 1 μg of purified endotoxin, and within a certain range the rise of temperature is dose-dependent. After intravenous injection of endotoxin, a febrile response develops after a latent period. The curve of elevated temperature has two peaks and within a few hours returns to normal levels. The endotoxin-induced pyrogenic reactions in cats, dogs, rabbits, horses, chimpanzees, and man have been evaluated quantitatively. The dose-effect curve in rabbits is generally flat. The threshold doses for fever are comparable in man and rabbit. With increasing doses man has a greater reaction, not only a fever, but also chills, nausea, vomiting, and hypotension. Chimpanzees and dogs have little febrile response to endotoxin, whereas even very small doses (micrograms) produce high fever in horses (15, 21, 154).

Pyrogenic response to endotoxin does not occur in anesthetized animals (102). Pyrogenic reactions depend on the temperature of the surroundings. Higher environmental temperature results in a more constant and elevated fever, whereas lowering the temperature abolishes the reaction altogether. The question of how fever develops after the administration of endotoxins is still not settled. In principle there are two views: 1) Endotoxin induces fever at least partly by direct action on the central nervous system (20, 21, 101). 2) In the latent period following endotoxin injection, granulocytes are damaged, an endogenous pyrogen is liberated, and fever develops by the action of the latter on thermoregulating centers (341).

Experiments have been quoted which show that the amount of endotoxin required to elicit fever by the intrathecal route is many times lower than for production of the comparable effect by the intravenous route (20). In contrast to the above quoted experiments, Fritze *et al.* reported that larger doses must be given intrathecally for febrile responses. Strangely, even minute amounts of endotoxin produced under their experimental conditions an initial fall of temperature. They did not observe any fall in granulocytes during the prolonged latent period before fever began. This finding favored the view that the primary fall in temper-

ature resulted from a direct action on the central nervous system, whereas a prolonged latent period indicated an indirect action (93). Intraventricular injections caused a very marked febrile response, and no tolerance could be evoked by this route of administration; whereas intrathecal injection of endotoxin caused slight responses, and it was possible to induce tolerance by repeated administration. Göing (101) has also shown that the question of endogenous pyrogen is rather complicated. Passive transfer of rabbit serum after three daily injections of endotoxin caused no pyrogenic reaction, whereas in the recipient animals injections of endotoxin were pyrogenic. The results of Sheth and Borison (279) speak in favor of direct hypothalamic action, and they attributed the differences between intrathecal and intraventricular injections to higher and lower sites of action. The authors were unable to demonstrate a febrile response in chronic low-cervical spinal cats on intrathecal application of endotoxin, but the response was still present on intraventricular application in such cats. Göing and Mike (102) observed pyrogenic reactions after intravenous injections in spinal rabbits. Negative results of previous workers may have been caused by neglect of the environmental temperature. After intravenous injection there is always emesis in cats by an action on the chemoreceptor trigger zone (279); this finding suggests that pyrogenic substances could act on other specialized receptor sites, including those which activate the thermoregulatory centers.

What new evidence is there in favor of the endogenous pyrogen hypothesis? Endogenous pyrogen from rabbits challenged with endotoxin, transferred passively, caused a short-lasting leucopenia shortly after injection. Supernatant fluid from peritoneal exudates induced fever but no leucopenia (118, 163). In dogs, a relation was found between leucopenia and the febrile response to endotoxin. Anesthesia abolished the febrile response but did not affect the leucocytic changes. Repeated daily administration of endotoxin produced a tolerance with respect to fever parallel to the tolerance with respect to leucopenia (118). The authors held the results to favor the concept of the dominant role of leucocytes in endotoxin fever. They suggested that changes in the ability of leucocytes to release pyrogens are concerned in the mechanism of tolerance. Recently Kaiser and Wood brought new support for this point of view (148). From leucocytes derived from exudates, they obtained much more pyrogen than from circulating cells. The pyrogen is probably not preformed, because it is not detectable in subcellular fragments. The production of pyrogen by granulocytes is blocked by arsenite, iodoacetate, *p*-chloromercuribenzoate, or N-ethylmaleamide; di*iso*-propyl phosphorofluoridate and dinitrofluorobenzene are even more potent inactivators of the pyrogenic molecule, and their effect cannot be reversed by pyridine-2-aldoxime methiodide, or by hydroxylamine. Glutathione activates proteolytic enzymes which degrade the pyrogen molecule. The authors suggested that this reaction may occur *in vivo*, as leucocytes are rich in glutathione (149). Fessler and co-workers were able to obtain the pyrogenic substance by various procedures from rabbit leucocytes, where it is released by incubation in saline, whereas they could not demonstrate pyrogen in human leucocytes (84).

The recent suggestion of Bennett that the solution of the nature of the febrile

response to endotoxin needs more refined neuropharmacological techniques is highly justified (20), and it can be added that a more pharmacologically oriented approach in general is desirable.

D. Changes in the formed elements of the blood

Changes in blood count. The effects of endotoxin on white and red blood cells have been reviewed in detail recently (15, 21). After intravenous injection of endotoxin, leucopenia develops and is followed by leucocytosis; this is now a classical description. Too little attention has been paid to the dose-effect relationship and to species differences. It has been stated that a small dose of endotoxin causes a very brief leucopenia, followed by leucocytosis with granulocytosis; with medium doses, the duration of leucopenia is dose-dependent, and a relative lymphocytosis with granulocytopenia precedes the granulocytosis; high doses induce leucopenia with marked relative lymphocytosis, due to the disappearance of granulocytes from the circulating blood. These effects persist until death in rabbits, but in rats the relative lymphocytosis is rapidly followed by relative leucocytosis (67). The species most sensitive to the pyrogenic and leucopenic endotoxin reaction seems to be the horse. The effects on the blood pattern observed in various animal species have been confirmed repeatedly in man (336). In the newborn and infants they include leucocytosis and increased leukergy (a form of movement of leucocytes) (184).

There is a slight and transient increase in the erythrocyte count 1 to 4 hours after endotoxin administration (282). A late, mild hemolytic anemia has been described in rabbits after repeated injection of endotoxin. Changes in osmotic or mechanical fragility of the red cells were not observed, and the anemia apparently was not dependent on the appearance of antibodies or the presence of the spleen (130). The number of circulating platelets is markedly diminished, the decrease beginning shortly after injection, with a peak usually at 1 to 4 hours or later. Recovery is slow and is not yet complete after 48 hours (283). Along with the phenomena described, increased amounts of 5-hydroxytryptamine (5-HT) occur in the plasma (139). The coagulation time of whole blood is significantly shortened (96, 97, 196). A Japanese group (284) also observed the occurrence of a platelet-agglutinating factor in rabbits treated with comparatively high doses of *Shigella flexneri* endotoxin. In the mechanism of blood changes the adrenals are supposed to play an important role (314). The influence of the sympathetic nervous system on blood count changes has been confirmed repeatedly (102, 131). But even in animals with depleted catecholamines, in which febrile reaction is almost abolished, the usual changes of leucocyte counts after endotoxin are present. Section of the cervical spinal cord does not prevent leucocytosis.

Endotoxin and metabolism of human leucocytes. Investigations *in vitro* have indicated that endotoxin added to leucocyte suspensions enhances glycolysis, and to a lesser extent respiration, but has practically no effect on the incorporation of pyrimidines and purines into nucleic acids (346). In leucocytes from patients treated with endotoxin, glycolysis falls very markedly during the first week, and uptake of glucose follows the same course. Endotoxin must be injected re-

peatedly to produce this result (34). Stimulation of glycolysis by endotoxin has been recently emphasized as a general principle of endotoxin effects in mammalian cells (342).

Leucocyte migration. There is no unanimity in views about the effects of endotoxin on leucocyte migration (21, 314). The whole question was reinvestigated by Watanabe and Tanaka (330). Even minute doses of endotoxin inhibit leucocyte migration in rabbits *in vivo*; a dose three thousand times higher had to be given to achieve analogous results *in vitro*. The time course of the inhibition of leucocyte migration *in vivo* is in good agreement with the initial leukopenia noted after endotoxin administration. Leucocytes from the peritoneal cavity or splenic fragments did not show the inhibition shown by leucocytes from blood. In preliminary experiments, administration of exogenous 5-HT, norepinephrine, epinephrine or reserpine also did not prevent the inhibition of migration. The same was reported for chlorpromazine, ACTH, cortisone, dihydroergotamine, atropine, lysergic acid diethylamide (LSD) and its bromo-analog (BOL). The authors considered that some released substances could be the cause of the observed inhibition (330). *In vitro*, very small doses stimulate, and higher doses inhibit leucocyte migration. Results depend on methods and endotoxin concentration (81). The effects are also dependent on the constituents of the nutrient medium. Sphingosine and glucofuranoside derivatives inhibited the migration, whereas glucosamine and salicylates enhanced it. The inhibitory action of adrenal corticoids on migration of leucocytes is well known (200, 267).

E. Local and generalized Shwartzman reaction

Endotoxin produces both a local and a generalized Shwartzman reaction (21, 314). It has been shown also that epinephrine can substitute for the local endotoxin injection in producing these reactions (315). The distribution of labeled endotoxin within the area of the Shwartzman reaction has been studied (309). While Cr^{51}-tagged endotoxin is not suitable, it has been reported repeatedly that in sensitized animals, P^{32}-labeled endotoxin has consistently the same distribution, with primary affinity to organs which contain reticuloendothelial (RES) cells (21, 223). Although the ability of the skin in the preparatory lesion to bind endotoxin is elevated early after the provoking injection of endotoxin, at the time of the appearance of the hemorrhagic lesion there is not an elevated amount of endotoxin in the skin. This finding speaks for an indirect mechanism of the reaction.

Changes in blood coagulation and activation of proteolytic mechanisms occur; fibrinogen is mainly involved (21). The intravenous injection of endotoxin by itself causes a sharp increase in fibrinogen levels within 24 hours. There is a decrease of coagulation time within 4 hours after the injection; 24 hours later it reaches normal values. The platelets drop to one half of the original number. The shortening of coagulation time is produced also by bacterial endotoxin *in vitro*. The effect is independent of leucocytes and red blood cells. Probably the *in vitro* mechanism differs from that *in vivo*. The second, or Shwartzman reaction-provoking, injection of endotoxin leads to a decrease in fibrinogen content within

4 hours. Twenty hours later it rises to twice the normal value, and coagulation time is again shortened (195, 196). Shortened coagulation time in rabbit blood seems to be produced by many antigen-antibody reactions *in vitro* (254). Proteolytic enzyme activation is an important factor in endotoxin toxemia, as has been repeatedly demonstrated recently using ϵ-aminocaproic acid and tolylsulfonylarginine methylester (plasminogen inhibitors). Protection against the lethal effects of endotoxin by these agents has been found in mice, rats, and dogs. The local changes induced by a combination of endotoxin and epinephrine could also be inhibited, but not the generalized Shwartzman phenomena (294).

F. Hemodynamic effects and endotoxin shock

Hemodynamic effects of endotoxin-induced shock are among the most important features of the host reaction and have been reviewed (98, 299). In the dog, an acute fall in blood pressure and a rise in portal vein pressure follow immediately after the endotoxin injection. Fifteen to 60 minutes after the injection the blood pressure reaches almost its original level. Then a gradual hypotension develops, accompanied by oliguria, anuria, acidosis, hemoconcentration, and death within 24 hours (334). There are marked species differences (172). Disturbances of the pulmonary circulation after endotoxin administration have been reported repeatedly (98, 189, 334). Pathological respiratory changes have been observed in animals (128, 233) and in man. With lethal doses of endotoxin, extreme renal vasoconstriction is followed by impairment of renal function (126). A combination of a small amount of nephrotoxic serum with a dose of endotoxin which is innocuous *per se* results in acute death with kidney changes which are reminiscent of the generalized Shwartzman reaction. This emphasizes the great importance of even minute doses of endotoxin as a potentiating pathological factor (14).

We should like to point out some conflicting features regarding the mechanism of action of the vascular changes induced by endotoxin. In the evolution of endotoxin shock, great importance has been attributed to the role of catecholamines. Zweifach and co-workers demonstrated an increased sensitivity to epinephrine of the vessels of the rabbit mesoappendix and isolated ear after endotoxin, and also suggested the importance of epinephrine in the local Shwartzman reaction (315, 349). Other investigators failed to confirm these findings (102, 204). The differences may be due to species, experimental conditions, and anesthesia. The general stress situation is important (9). Adrenergic factors in endotoxin effects have recently been confirmed. In rabbits an increased pressor reaction to epinephrine as well as to norepinephrine was observed after pretreatment with endotoxin. In isolated rabbit aortic strips, the response to norepinephrine could not be altered by several hours' pretreatment with endotoxin. Addition to the bath of centrifuged blood cells in a volume of 10 % initiates a sensitization (106). Since endotoxin releases 5-HT from circulating platelets, it has been suggested that the 5-HT immediately released from the platelets may act directly on the capillaries. Since the norepinephrine and epinephrine levels also increased in the plasma, one might expect a strong effect not only on the blood vessels but also on adjoining tissue through local circulatory disturbances leading to vaso-

constriction and edema. Hemoconcentration also may cause a variety of circulatory shock, the mechanism of which may be different from that resulting from simple intravenous injection of 5-HT (284). The role of plasma kinins remains to be elucidated. Kallikrein might be a factor important in endotoxin-induced vascular effects (332). Direct cardiotoxic activity of endotoxin on isolated mammalian hearts did not occur even with high doses (240, 317). Sublethal doses of lipopolysaccharide did not change the catecholamine content of the rabbit heart (193). *In vivo* the autonomic regulation of the heart is impaired (95).

The relation of *endotoxin* to *shock* has been repeatedly discussed recently (8, 298, 299). The conflicting present status will be mentioned briefly. One group (85) has put forward the hypothesis that the irreversible phase of shock is caused by endotoxin from bowel bacteria which penetrates into the general circulation through the increasedly permeable intestinal wall. Absorption of P^{32}-labeled endotoxin has been reported by Ravin *et al.* (249). This opinion has not been accepted unanimously. Einheber and co-workers were not able to induce shock passively by blood from irreversibly shocked animals (80). Sanford and Noyes used Cr^{51}-labeled endotoxin, which retained pyrogenicity, lethality, and other typical properties (266). After introduction into the gastrointestinal tract of normal dogs or dogs in shock, this endotoxin did not appear in the circulation. Broitman *et al.* (44) likewise did not find a transmural migration of bowel bacteria in animals intoxicated with endotoxin. In germ-free rats as well as in rats pretreated with chlortetracycline, lethal hemorrhagic shock had the same course as in normal rats (199). Kovách was not able to induce irreversible shock in normal animals by the administration of blood from irreversibly shocked animals. In some dogs and rabbits they were able to demonstrate "endotoxin-like" substances, *i.e.*, toxic substances, but chromatographic analysis did not reveal endotoxins (169). Nagler and Zweifach (212) came to the same conclusion. Further evidence was given that a toxic factor found in portal blood after ligation of the superior mesenteric artery was not endotoxin, and the effects could be explained by the increase of 5-HT in the blood.

The changes in irreversible hemorrhagic shock have been attributed to substances liberated from damaged tissues. The sympathomimetic action of endotoxin in the intestine was emphasized (181). Irreversible shock, including endotoxin shock, is a very complex mechanism varying not only from species to species but within the species itself, depending on the actual state of the organism in which shock occurs (348). We should not forget some new interesting points concerning endotoxin shock itself. Histamine is important (127). A very stimulating finding is that the combination of endotoxin with epinephrine in large doses, or bacterial infection leads to an increase in the activity of histidine decarboxylase (268). In endotoxin hypotension, an increase in histamine and in the ratio of histamine to histidine has been found. This increased formation of histamine favors the importance of histidine decarboxylase activation (125). Hence new formation of histamine seems to be another important factor in endotoxin shock. The whole explanation, however, should not be confined to one factor. The entire field of endotoxin shock should be the subject of much more research, without neglect of the pharmacological aspects.

In rats an injection of endotoxin caused intestinal congestion, hemorrhage, and edema within 60 minutes. Very early, before marked circulatory changes appeared, succinoxidase activity decreased. The damage, however, did not lead to emigration of intestinal bacteria, as mentioned above (44). The histamine liberator 48/80, caused congestion of the intestinal wall and damage to the epithelium within 15 minutes; these effects were maximal 15 to 30 minutes after injection. This could not be reproduced by exogenously administered histamine (83). It should be remembered that the changes produced by endotoxin in the intestine in the dog are accompanied by increased intestinal lymph flow. This is not related to the increase of venous portal pressure, and might have some relation to the release of active substances (16).

G. Tumor-necrotizing activity

The tumor-necrotizing activity of endotoxin is a well-established property (21, 314). It is used by some authors as an assay for endotoxin potency. The old question of the possibility of using endotoxin as an antitumor agent, alone or in combination with X-ray therapy, recently has aroused new attention (132). The only encouraging report has been from a study on subcutaneously implanted S-37 mouse sarcoma. Pretreatment with endotoxin ameliorated tolerance and survival of X-ray-treated tumor-bearing animals. Lipoid A, from *E. coli* endotoxin, also has a tumor-necrotizing effect, with a potency somewhat lower than that of the lipopolysaccharide from which it is derived (206). Extracts from gram-positive staphylococci have similar properties (205).

H. Endotoxin and abortion

Endotoxin induces abortion in mice (53, 253). This activity seems to be independent of the lethal effect. Neither cortisone nor progesterone prevents the abortion. Kass (152) suggested that endotoxin might be one cause of premature birth and abortion in human beings. Oxytocin contractions are, according to Kass, potentiated by endotoxin. Endotoxin induced abortion whether administered at the beginning of pregnancy, before implantation, or at the end (53). In this connection the tumor-necrotizing activity of endotoxin should be recalled. It is well known that a number of drugs used in the chemotherapy of malignant tumors also induce abortion. Further studies are indicated.

I. Metabolic effects

Studies of metabolic effects following endotoxin administration have been confined mainly to carbohydrate metabolism (265, 314). Initial hypoglycemia is followed by hyperglycemia, with depletion of glycogen from the liver (29). Hyperglycemia and glycogen depletion are the result of increased hepatic phosphorylase activity. Increased hepatic glycogenolysis persists after adrenalectomy, which causes a reduction of glycogenolysis in muscle. Endotoxin converts practically the total liver phosphorylase to the active form (114). Results depend on species and design of experiment; fed or fasting animals may give different results. In well-fed animals, after lethal doses of endotoxin the blood sugar remained normal to the time of death (101). High doses of cortisone prevented the

hepatic glycogen depletion. This was also reflected by increased incidence of survival (29, 31).

An endotoxin antagonism to ACTH induced a dose-dependent increase in nitrogen excretion; this phenomenon was developed into an endotoxin bioassay (30). There is a difference between changes in the glycogen content in the liver and those in muscle; this is probably explained by the distribution of endotoxin, which is markedly accumulated in the liver of rats, rabbits, and dogs (70, 263). The concentrations of pyruvate and lactate in the liver of mice vary biphasically after the administration of endotoxin. The alteration in preformed glycogen storage is primary, with a consequent increase in glucose and glycolytic intermediates. Following depletion of glycogen, the intermediates fall because there is no available source.

Endotoxin increased oxygen uptake by rabbit liver tissue; in kidney slices *in vivo* the oxygen uptake was diminished; but no changes were found in the brain and striated muscle. Endotoxin caused a decrease in the activity of succinic dehydrogenase (323). Cascarano and co-workers (51) found a differentiation between various endotoxins: *E. coli* lipopolysaccharide partially inhibited the succinic dehydrogenase in liver slices, with a histochemical pattern which indicated that the portal areas were mainly attacked; *S. typhi* endotoxin did not cause any alterations. In mitochondrial preparations of the liver in experiments *in vivo* and *in vitro*, a statistically significant stimulation of succinic acid dehydrogenase was found (4, 324). This again illustrates that results may depend on the conditions of individual experiments.

Recently an attempt has been made to put many changes observed as endotoxin effects on a common metabolic denominator (342). Endotoxin exerts in cells an insulin-like glycolytic stimulation. The glycolytic stimulation of tumor cells *in vitro* was parallel to the tumor-necrotizing potency *in vivo*. From the parallelism of several endotoxin effects with the glycolytic potencies of various endotoxins, a general scheme of endotoxin activity based on glycolytic stimulation was derived. Although the glycolytic stimulation is very important, a complex macromolecule like endotoxin has probably also other sites of action.

J. Nervous system

There are conflicting reports about the participation of the nervous system in the mechanism of endotoxin disturbances (98). The experiments of Penner and co-workers (231) emphasize the importance of the central nervous system in dysentery endotoxin intoxication. Their results have been challenged but the problem is still not solved.

The nervous system is involved in circulatory disturbances and shock produced by endotoxin. Penner and Bernheim (231) described intestinal lesions after intracerebral injection of toxin. Pretreatment by endotoxin leads to sensitization of the carotid sinus and other reflex areas to endotoxin and acetylcholine (4, 240).

In rabbits and cats various areas (carotid sinus, mesenteric area, rabbit ear) were vascularly isolated from the body and perfused with Ringer-Locke solution. Nervous connections remained intact. In these experimental conditions in some

animals after repeated injections of endotoxin into the peripheral artery of the area, instant (*i.e.*, reflex) changes of blood pressure, respiratory stimulation, and sudden death may occur. Intravenous control injections of endotoxin have no such effect. In some animals endotoxin abolishes the usual reflex changes of acetylcholine administration into the carotid sinus; in others endotoxin on the contrary evokes an increased acetylcholine response (240). It is also interesting (179) that in sensitized rabbits the extirpation of the carotid sinus, or its nervous block by local anesthesia, protects the animals against anaphylaxis. This has been attributed to the abolition of reflex vasomotor stimulation.

Pressor reactions induced by the stimulation of the sciatic nerve in rabbits were inverted or abolished after intravenous injections of endotoxin. Analogous pressor reflexes induced by distension of the urinary bladder or the rectum, however, were not affected (240). Endotoxin also evoked an increase in rheobase of the efferent site of a defense reflex (291). In children and animals with *Salmonella* and *Shigella* infections, the plasma cholinesterase activity in early phases and in severe cases was markedly lowered (4, 173). This could explain the increase in acetylcholine response observed after repeated toxic reaction and also on the pressor responses of intact rabbits (240).

Small doses of endotoxin, which did not change the general behavior of rats, inhibited positive conditioned reflexes. Higher doses caused general behavioral changes. Positive conditioned reflexes were completely inhibited, and it took several weeks before the pre-experimental state was regained (240). No change was found in the catecholamine content of the mouse brain (193). Extirpation of the superior cervical ganglion markedly enhanced intoxication caused by dysentery endotoxin even in previously immunized animals (233). All the above-described changes of the nervous system have a rather nonspecific character. The nonspecificity of general pattern of effects is of course the leading feature of endotoxins. Although humoral and local tissue factors are unquestionably of great importance, research should consider all possibilities and not be limited in approach.

K. Tolerance

A striking point of endotoxin effect is the capacity to induce increased resistance to pathological effects of various endotoxins subsequently administered. The properties affected include lethality, leucopenic activity, and tumor-necrotizing, hypotensive, antibody-enhancing, hyperglycemic, and Shwartzman-phenomena-inducing capacities (21). Change in these phenomena is now generally expressed as tolerance effects. However, as the term "tolerance" is generally used for diminished responses of the host against foreign tissue, the term "increased resistance" might be more appropriate as there indeed is increased resistance of the host to endotoxin. Since the basic work of Beeson it has been known that the increased resistance is not as effective as with specific immunization and that it is only temporary, disappearing a few weeks after the last endotoxin injection. It was claimed earlier that this tolerance is due entirely to stimulation of the RES (21).

Passive transfer of nonspecific tolerance has been described recently by Freedman. He increased resistance to homologous and heterologous endotoxin, measured on the basis of lethality and pyrogenicity, by passive transfer of serum from endotoxin-tolerant donors of homologous or heterologous animal species (89). When donors were made highly susceptible to endotoxin by RES blockade, their plasma still passively increased tolerance in other animals. However, he was not able to induce tolerance to the leucopenic effect of endotoxin by passive transfer. He has also stressed the importance of the type of schedule, showing that daily endotoxin injection is important. He noted no quantitative difference in the tolerance effect between 7 days' and 5 weeks' daily administration of endotoxin (90). This supports evidence that the tolerance effects are not due to specific antibodies (91). Donor serum mixed simultaneously with endotoxin gives no protection. Therefore, the effect seems not to be caused by direct, increased detoxifying properties of the serum, but this should be further investigated.

The toxicity and pyrogenicity of endotoxin in man are obstacles to the widespread use of endotoxin to increase nonspecific resistance in man. Therefore, numerous attempts have been made to prepare from endotoxin a substance with low toxicity and no pyrogenicity. It has been reported that Lipoid A, from *E. coli* endotoxin, is nontoxic in man and induces increased resistance (13). On the other hand, highly antigenic polysaccharide, which is free of lipid and nonpyrogenic for rabbits but induces tolerance to lethal as well as to febrile effects of endotoxin, has also been prepared (218). Freedman *et al.* have also attempted to prepare a detoxified endotoxin which can still induce tolerance (92).

As the tolerance factor, Freedman excluded properdin, adrenal cortical hormones, and phagocytosis-promoting factor (90). The former explanation of RES stimulation as the basis for tolerance has also been challenged from various sides. Glyceroltrioleate increases the activity of the RES, whereas ethylstearate depresses it. Although triolein stimulates the RES considerably, mice so treated were much more susceptible to endotoxin. Contrary to this, ethylstearate caused a blockade of the RES, but the susceptibility to endotoxin was not altered (56). Howard also excluded the RES as the responsible factor for a cross-tolerance found in mice infected with *Salmonella typhimurium* to *Salmonella paratyphi C* endotoxin (136). There is no clear parallelism between the tolerance of animals to infection and stimulation of the RES. In comparing the effects of endotoxin in two strains of mice, one genetically resistant, the other susceptible to *S. typhimurium* infection (33), it was found that the genetically resistant animals had a poorly responding RES, whereas the susceptible mice had a very active RES.

The pituitary-adrenal system has been invoked to explain tolerance to endotoxin. The higher susceptibility of adrenalectomized animals to shock is generally accepted and has been reviewed several times (347). But as tolerance to toxin is possible also in adrenalectomized animals, whether they are supported by cortisone or not (247, 347), it is difficult to explain tolerance only by stimulation of the RES and of the adrenals (43).

Hegemann and later an American group (116, 176, 287) have found, in the

sera of different species, including man, a factor which detoxifies endotoxin and is bactericidal. No reports of the relation between endotoxin-induced tolerance and changes of activity of this factor have appeared. Enzymatic processes seem to be involved in the decomposition of endotoxin (153). Whether the bactericidal factor is really present in circulating blood *in vivo* has to be reinvestigated, as there is some evidence that the appearance of the factor is dependent on substances released during the clotting mechanism (129).

Knowledge about cross-tolerance as well as the substances eliciting the endotoxin type of tolerance has widened beyond the range of gram-negative microbes within recent years. A cross-tolerance to *Shigella shigae* toxin (gram-negative), streptococcal filtrate (gram-positive), and *S. typhi* endotoxin could be induced; the tolerance involved impairment of conditioned responses, induced by *Salmonella typhi* endotoxin or streptolysin O, and disturbances of motor nerve conduction (240, 242). Endotoxin protects against gram-negative infection (133). Susceptibility to infection with gram-positive and acid-fast bacteria could be reduced significantly when sublethal doses of endotoxin were injected 1 month prior to challenge (77). Endotoxin increases resistance to influenza virus, viral encephalitis (133, 328), ectromelia virus, mouse hepatitis (100), and Newcastle disease virus (86). Endotoxin induces tolerance also against traumatic shock (350).

A wide range of the parameters of tolerance which characteristically result from administration of endotoxin has been reproduced using phenol (247). Repeated phenol injections in mice induce tolerance to various bacterial toxins and prolong survival in *Salmonella typhi* infection (322). They also prevent the impairment of motor nerve conduction induced by *Shigella shigae* toxin and streptolysin O (247). The induced resistance is dependent on the number of injections, and is transitory. No dependence on the RES was found (247). The same procedure can also increase tolerance to shock (246). Increased resistance to *Shigella shigae* toxin was induced by repeated drum-shock pretreatment (246). The range of resistance depended on the number of sublethal shock pretreatments. One pretreatment did not increase the resistance to shock, but rather decreased it; this may be the explanation for the earlier failure to see increased resistance (350). This increased susceptibility to endotoxin after a single pretreatment with shock has been repeatedly observed (10, 226). Recently Zweifach *et al.* have increased resistance to endotoxin by repeated shock pretreatment (348).

Increased resistance to bacterial toxin can be maintained during practically the whole lifetime of animals if phenol injections are repeated twice a month. When pretreatment with phenol in the rabbit induces a tolerance to the pyrogenic action of endotoxin, the degree of the tolerance in comparison with endotoxin-induced tolerance is reduced. Increased tolerance to the lethal effects of *Shigella shigae* toxin appears after passive transfer of the serum of phenol-treated mice. Infant mice from phenol-treated mothers have an increased resistance to *Shigella shigae* toxin. The same is true for animals whose mothers are treated with *Shigella shigae* toxin or endotoxin. The resistance induced by phenol is transferred mainly by the milk of the mothers (247). This excludes the RES as the only source of

increased resistance (42). However, the suggestion of Bennett and Cluff (21) that endotoxin-induced resistance should be similar in its mechanism to acquired resistance to opiates could not be confirmed, as establishment of tolerance to morphine did not protect against endotoxin. The only substances which have been found to increase resistance to endotoxin have in common irritating properties for the tissues: glyceroltrioleate (56), xerosine (133), calcium chloride, croton oil, sodium salicylate, and procaine. Among the tolerances induced by phenol, procaine, and endotoxin, a mutual analogy was found, including the passive transfer from mother to newborn (321).

Recent findings of Zweifach (347) showed that histamine release does not stimulate the RES but enhances resistance to shock and endotoxin and that repeated injections of phenol and other irritating substances induce the same effect (347). The great importance of liberated factors from host tissue can be concluded. Tolerance phenomena might therefore be partly the results of adaptation to various released compounds.

Some authors have suggested that endotoxin tolerance is caused by an elevation of specific antibody (338). But as the same phenomena can be produced by phenol, and repeated phenol injections increase tolerance to phenol itself (247), it is difficult to accept this explanation as the single factor in tolerance.

The possibility of contamination with minute amounts of endotoxin might be of great importance for serological and similar studies. It is somewhat overestimated in studies with toxin tolerance in general. In all experiments where tolerance to toxin and shock, including pyrogen-tolerance, was induced by repeated injections of phenol, control experiments were performed by injecting animals with the same amount of saline derived from the same source as for the phenol-treated animals. The possibilities of contamination were equal in both groups and therefore the negative results in control experiments concerning tolerance to lethality, pyrogenicity, *etc.*, appear to exclude the possibility that tolerance-positive effects obtained with simple chemical compounds can be due to endotoxin contamination. Endotoxin contamination as, so to say, the ubiquitous reason for endotoxin-like effects (21) was not confirmed by Antweiler (12). In analogous experiments Nicholls also could not attribute the slow-contraction activity with cotton dust to endotoxin contamination (217).

L. Enhanced susceptibility

Dubos *et al.* have shown that endotoxin-treated animals become more tolerant to BCG and to gram-positive microbes (73, 77). When the events were reversed, both Suter (308) and Halpern *et al.* (113) saw that after BCG mice became more susceptible to the lethal effects of endotoxin. It might be useful to recall also some results of Dubos and Schaedler. They showed that BCG vaccine or killed microbes injected several days prior to challenge with *Staphylococci* prolonged the survival of mice, but that BCG or purified endotoxin injected simultaneously or shortly after challenge with bacterial pathogens exacerbated the course of the infection. The same happened when such products were injected into animals with minor residuals of an acute infection (74). Experimental in-

fection with *Histoplasma capsulatum* increased the susceptibility of mice to endotoxin. On the other hand, repeated pretreatment with endotoxin increased the tolerance of animals to *Histoplasma* infection (40). These results may be due to what microbiologists call the "negative phase" in specific immunity (239). This has been described, for instance, for pertussis vaccine administered to mice with subsequent infection.

Endotoxin causes also a very early, transient increase in resistance to infection (41). One explanation could be that the administration of endotoxin, vaccine, or other agents of the group called by Westphal "Reizstoffe" causes a number of pharmacological and immunological events in the host. Hypersensitivity as well as hyposensitivity phenomena result.

In this connection it is useful to recall an observation concerning the course of events after the administration of lethal doses of bacterial endotoxin to rabbits (117). Ten minutes following injection, hypotension was present; there was neither depletion of catecholamines nor an increased plasma level, but sensitization to exogenously administered epinephrine was sometimes noted. Within 1 hour the blood pressure had returned to normal, but the sensitivity to exogenous epinephrine had diminished, and depletion of adrenal catecholamines began, and was followed by increased plasma levels of catecholamines. In the subsequent hours a gradual decline in blood pressure began; this was fully developed after 5 to 6 hours, at which period there was an increased level of catecholamines in the plasma. The final event of susceptibility or tolerance probably depends on the interaction of many factors. A small part of this complicated story has just been illustrated.

M. Release of substances from the host tissue

Histamine and 5-hydroxytryptamine (5-HT). The release of vasoactive materials has been referred to in section II, F. Endotoxin injection leads to release of histamine (127). Endotoxin incubated with rat plasma activates a histamine releasing factor (107). The *de novo* formation of histamine by endotoxin-activated histidine decarboxylase activity has already been mentioned (268). In spite of this, "pure" antihistaminic drugs have been comparatively little tested. The reason may be that a number of negative results has been reported, including some in man (44). Various antihistaminic agents were not able to prevent pyrogenic reactions in patients and rabbits induced by blood transfusion and pyrogens (119).

The increase of the 5-HT content of plasma has been reported in the anaphylactic reaction (146), as well as in endotoxin-treated animals (64, 284). In recent studies, the dynamics of 5-HT release from platelets and related phenomena induced by endotoxin was studied *in vitro* (66, 258). 5-HT is freed from platelets *in vitro* by incubation with endotoxin or bacterial cells. The release is preceded by an aggregation of platelets, as described for histamine (65, 283). Phase microscopic inspection revealed the process of aggregation and fusion to be roughly parallel to the release of 5-HT. The changes were dose-dependent. The type of anticoagulant used is important. Heparin produces spontaneous platelet aggre-

gation; hence citrate or EDTA is to be preferred. The phenomenon occurs at 37° but not at 4°C. Heating of plasma removes the capability for interaction between endotoxin and platelets. Indirect evidence suggested that phospholipid is released from the platelets (98).

Vasoconstriction, followed by vasodilatation, hypotension, and increase in portal and pulmonary vascular resistance (effects like those elicited by endotoxin) are certainly in many ways dependent on vasoactive factors like 5-HT or catecholamines. In this connection it is interesting that it was possible to increase vascular resistance in the perfused lung of the dog with whole blood or plasma, but not with dextran or gelatin (98). 5-Hydroxytryptamine and endotoxin also increased the effects of catecholamines on small blood vessels. Not only antagonists of catecholamines (193) but also 5-HT antagonists (66) protect against toxin.

Slow-contracting substances. It should be recalled that, as described for various toxins in this review, slow-contracting substances (shown not to be 5-HT, histamine, acetylcholine, or catecholamines) are also released. Traumatization of tissue also yields slow-contracting substances (240, 348).

The suggestion of Freedman (91), that in tolerance there appears in blood a lipid fraction which contains the protective factor against endotoxin and which stimulates the RES, is most interesting. Release of phospholipid from platelets by endotoxin (98, 258) should be further investigated.

The observation of increased nonspecific resistance to toxins induced by a factor present in milk from tolerant mothers focuses attention on the changes in resistance of mice to endotoxin produced by dietary factors, as the peroral route is common in both types of experiment. The resistance to some bacterial infection and endotoxins depends on dietary proteins and amino acids (75, 76). In view of the increased tolerance achieved by repeated injections of phenol, it would seem that increased resistance might not be a direct reaction (247).

N. Therapy of endotoxin disturbances

Adrenal hormones. There is some evidence of a difference in susceptibility between the adrenal cortex and medulla. The medulla is stimulated by very small doses of endotoxin, whereas for the stimulation of the cortex greater amounts are necessary (79). The release of corticoids may play a role in attenuating the course of events.

It is rational to expect general improvement by corticoids, and exacerbation by epinephrine and analogous pressor agents. Catecholamine-depleting substances or those which prevent catecholamine biotransformation should also ameliorate or exacerbate the course of events. As repeatedly pointed out in this review, a simple observed phenomenon is always only part of the story, and therefore the immediate experimental conditions, animal species, *etc.*, should decide whether the results will fulfill expectations. From this point of view, the sometimes conflicting results described below become more comprehensible. As in other stress situations, protective effects of corticosteroids were described comparatively early. The positive effects of suitable doses of glucocorticoids on endotoxin dis-

turbances has been observed in many animal species. The literature has been reviewed recently (98, 152).

Leukergy induced by endotoxin can be suppressed when corticosteroids are administered in a high dose for several days previously. The usual leucopenic reaction is also suppressed by cortisone. This is explained by the possibility that the suppression of leukergy prevents the leucocytes from being trapped in the pulmonary circulation; in this way, high doses of glucocorticoids may depress an important defense mechanism (87). Endotoxin increases serum transaminase, and this is in turn suppressed by cortisone (201). There is general agreement about the beneficial effect of corticosteroid administered after challenge with endotoxin, as proved recently in dogs (334). This does not mean that tolerance should be explained only by adrenal mechanisms, for, as mentioned before, evidence is steadily accumulating that tolerance to toxins may be induced also in adrenalectomized animals (247) by endotoxin or other irritating substances (54). Cortisone also protects hypophysectomized animals (229).

The protective effect of corticosteroids can be demonstrated in chick embryos and in 1- to 3-day old chicks. It has been confirmed in this species that hypoglycemia and fever are alleviated by cortisone (72). Various steroids have different anti-endotoxic effects, and their relative potencies compared with that of cortisol have been reported to differ markedly from those for the anti-inflammatory effects. Prednisone was less effective than cortisol in protecting young male adrenalectomized rats against the action of endotoxin (45).

In experimental infection, the beneficial or deleterious effect of corticoids depends on the infection, dose, host, and other factors (37, 38, 39). Susceptibility to *Salmonella typhimurium* infection was greatly enhanced by low doses of cortisone. An increase in environmental temperature further increased the susceptibility. The degree of cortisone-induced susceptibility is parallel to differences among strains with respect to natural resistance against infection. Cortisone does not alter parameters of acquired immunity. Pulmonary inflammation induced by endotoxin in mice is antagonized markedly by steroids, and only moderately by phenylbutazone (Butazolidin).

Another group of therapeutic experiments is related to the observed depletion of catecholamines from the adrenals, and their increased levels in the circulating plasma at certain periods of intoxication, and from activation of the sympathetic nervous system (21, 314). Reserpine causes catecholamine depletion. There are conflicting reports concerning its effect on the toxicity of endotoxin. No effects were noted when moderate doses of reserpine were administered 1 hour prior to challenge, but high doses enhanced the susceptibility to endotoxin (311). On the other hand, a prolongation of survival after the administration of reserpine has also been found (282). Pyrogenic effects in rabbits were suppressed by pretreatment with reserpine, and lethality in rabbits was reduced. Inhibitors of monoamine oxidase intensified catecholamine actions. Iproniazid has been found to potentiate the pyrogenicity and lethality of dysentery endotoxin (201, 281). No effect on the survival of mice after *E. coli* endotoxin was found after reserpine or iproniazid (106, 197).

The same conflicting situation has been reported with the administration of pressor drugs, mainly metaraminol (Aramine). Early hypotension in dogs challenged with endotoxin was opposed by this drug (333), but in the experiments of Lillehei and MacLean, pressor drugs, including metaraminol, injected into dogs before or together with endotoxin, enhanced lethality (182). Small doses did not increase the severity of shock, and increased survival, but higher doses were noxious. Increase of serum transaminase after endotoxin was used as an index of cellular injury; this was increased further by treatment by metaraminol (334).

Pressor agents in combination with hydrocortisone have been found to be useful in the treatment of endotoxin shock in dogs (293, 295). The endotoxin impaired renal function but renal blood flow remained unaffected (124). When these agents were combined with hydralazine, the renal blood flow, which in this case had been disturbed by endotoxin administration, was very much improved, but no definitive proof of increased survival by this drug combination was given (326). Dosage, species, kind of endotoxin, and time of administration probably influence the outcome of beneficial or adverse effects of the pressor drugs.

The role attributed to the stimulation of the sympathetic system and adrenergic mechanisms by endotoxin naturally led to numerous attempts to alter the course of endotoxin shock by the administration of adrenergic blocking agents. Dibenamine prevents lethality from *Salmonella typhi* endotoxin in rabbits (106, 315). Therapeutic administration, however, shortened the survival time in dogs (334). In mice, the effects changed with various toxins. With *S. dysenteriae*, and *A. aerogenes* there was protection by phenoxybenzamine (Dibenzyline); this drug did not influence the effects of *E. coli* endotoxin, but it increased susceptibility to *S. typhi* endotoxin (197, 224). Phentolamine (Regitine) was ineffective in prophylactic experiments in mice (197) and deleterious in therapeutic experiments in dogs (334). Also, 2,6-xylyloxyethyltrimethylammonium bromide was ineffective in mice. McLean and Berry found 3,4-dichloroisoproterenol (DCI) to be highly protective for endotoxin-treated mice (198). In none of the papers quoted was the distinction regarding adrenergic blocking activity to *alpha* or *beta* adrenoceptive sites taken into account. Vasoconstriction is an α-receptor effect of epinephrine, whereas vasodilatation is a β-receptor effect (7). Phenoxybenzamine is an antagonist of α-receptor effects and DCI of β-receptor effects. From this point of view it is of interest that high protection was found with DCI. The beneficial effect of phenoxybenzamine was attributed to a suppression of the α-receptor effects. More research, using a pharmacological approach, is needed in this area.

It has been reported several times that chlorpromazine, given before toxin or up to 2 hours after toxin administration, decreases the mortality from endotoxin (181, 224). The protective potency of chlorpromazine is superior to that of pentobarbital or hydroxydione (Viadril). Some protective activity is maintained in adrenalectomized mice. It was suggested that central actions of chlorpromazine on the reticular formation were responsible for the observed effects (1). Chlorpromazine acts on drug fevers induced either centrally or peripherally, and therefore its effects at both sites have been implicated (166).

It has been found that D-2-brom-lysergic acid diethylamide (BOL-148) or heparin (the latter in only a single injection), injected immediately after endotoxin challenge, decreased the intensity of pathological signs. The effect of heparin on survival was disappointing (281). Iproniazid increased the susceptibility of animals to *Shigella shigae* endotoxin. A high dose of 5-HT injected subcutaneously into mice increased to some degree the resistance to endotoxin. This was further enhanced by combination with hydrocortisone. Female mice were more resistant than male. Sex differences have repeatedly been reported to be important in susceptibility to drugs (104).

Since thyroxin elevates sensitivity to epinephrine on the one hand, and on the other increases the output of corticosteroids, its administration could be expected either to ameliorate or to enhance endotoxin shock. It has been reported that thyroxin (104) or triiodothyronine (202) enhances the susceptibility of mice to endotoxin. The suggested explanation that these compounds increase oxygen consumption and so enhance further epinephrine action, which by vasoconstriction further decrease the needed oxygen supply, seems logical.

Endotoxin and antibiotics. There were early reports of the anti-endotoxic properties of penicillin, which later were attributed to impurities in penicillin at that stage. Djeksenbayev found no influence on pyrogen-induced fever with penicillin or streptomycin, whereas the pyrogenic reaction was reduced by polymyxin (68, 207). Polymyxin B was also found to decrease the sensitivity of tumor to the necrotizing action of lipopolysaccharide derived from *Serratia marcescens*, but did not affect its pyrogenic activity, which was affected by lysozyme. As the results were achieved by previous incubation with both types of macromolecule, the results should be directly comparable. Both results indicate that macromolecular antibiotics may interact directly with endotoxin (207). The reduction in plasma albumin and globulin after endotoxin challenge can be prevented almost completely by previous administration of neomycin (335).

Endotoxins arouse wide interest as tools of research. Many of the conflicting views and results might be brought closer together by a more pharmacological approach.

III. *SHIGELLA DYSENTERIAE* TOXIN

Shigella dysenteriae produces both a heat-stable endotoxin whose properties fall into the range of the preceding chapter, and a thermolabile exotoxin, a simple protein with a molecular weight of about 82,000 (121). Only the latter will be considered here.

Toxicity. Its toxicity is like that of purified botulinum and tetanus toxins (121). A slow course, with a latency of 10 to 18 hours, characterizes the intoxication. Animals die within 3 to 4 days. Paralysis of striated and smooth muscle appears, as well as convulsions. Susceptibility is very much species-dependent (237). Taking rabbit susceptibility as one (this is the most susceptible animal), the decreasing sensitivity is as follows: monkey 5, hamster 40, mouse 700, rat 5000, and guinea pig more than 10,000 (52). This sequence of species varies from that indicated by Zweifach for *Shigella dysenteriae* endotoxin: cat, rabbit, dog, guinea

pig, rat (348). Susceptibility to the exotoxin increases with age (240), as with other toxins (109, 345). The most toxic route is the intravenous one, the intracerebral less so (135, 320).

Neurotoxicity. The typical pattern of response in rabbits is flaccid paralysis. From this observation is derived the alternative name for the toxin—*Shigella dysenteriae* neurotoxin. In the other species investigated, nervous effects have not been described. Rats have diarrhea and regurgitation of stomach contents; hamsters have bilateral serous effusions and minimal changes of the nervous tissue. Besides the rabbit, only the mouse has neurological signs; and in man, neurological complications of *Sh. dysenteriae* infections are rare. Traveling of the toxin up the nerve trunks seems to be unlikely. Histological studies point to a primary attack on the blood vessels in the brain; nervous disturbances are believed to appear as a consequence of vessel damage (52, 135).

After administration into the perfusion fluid of perfused carotid sinuses of the rabbit or cat, the toxin produces reflex changes of blood pressure. This is reproducible many times, and excitation by potassium chloride or cyanide remains intact whereas the usual acetylcholine response is suppressed. Small amounts of adenosinetriphosphate (ATP) restore the acetylcholine response and increase the response to the toxin (240). It may be that increased cholinesterase activity is the cause of suppression of the acetylcholine response (213). *Shigella dysenteriae* toxin impairs conduction in mammalian motor and autonomic nerves (240).

When injected into the lateral ventricle of the cat the toxin produces behavioral changes like those after intraventricular administration of 5-HT (244). After a latency period of several hours the animals become hesitant; they stay in an open cage and, when taken out, invariably return to it. Gradually an impairment of coordination, rapid respiration, trembling, perseverance in bizarre positions, convulsions, and death occur. As little as one microgram may be lethal. Surviving animals are inert and do not clean themselves.

Intravenous as well as intracerebral injections of toxin lead to a depletion of epinephrine, norepinephrine, and 5-HT content in the mouse brain. The intravenous route seems to be more effective, and this result points to an indirect mechanism (192, 193). The toxin also considerably lowers the convulsive threshold to pentylenetetrazole, electroshock, or audiogenic-seizure stimuli (138).

The behavioral changes after intracerebral administration and the changes in catecholamine and 5-HT content in the brain are typical for *Shigella dysenteriae* toxin. Other changes have been encountered with other toxins as well and must thus be considered of a rather nonspecific character.

Metabolic effects. Glycogen depletion in the liver and hyperglycemia occur as early as 1 hour after administration of the toxin. Gradually the content of labile phosphorus in plasma is reduced and ATPase activity increases, with a maximum 48 hours after toxin administration. In immunized animals the changes are not significant. Oxidative phosphorylation in rat brain is impaired (240). The toxin produces a cytopathogenic effect in tissue cultures (325).

Tolerance. In batches of *Shigella dysenteriae* used in the following experiments

no detectable endotoxin was present (292). Tolerance to the effects of the exotoxin could be induced by specific immunization or repeated injections of small amounts of phenol, procaine, other tissue-irritating substances, or crude streptolysin O (247). The tolerance could be passively transferred by plasma and transmitted from mother to young *via* the milk (321). Phenol-induced tolerance is nonspecific, *i.e.*, it protects also against other toxins. Repeated injection of drugs of the morphine group, histamine, epinephrine or many others, did not protect against *Shigella dysenteriae* exotoxin (247).

Effect of drugs. Adenosinetriphosphate, -diphosphate, and -monophosphate (but not adenosine) have a therapeutic effect. So have spleen homogenates (238). The same is true of papaverine. The interval between toxin and drug administration is important. A protective effect was reported with reserpine, pentamethonium, chlorpromazine, and Dibenamine, whereas iproniazid and pyrogallol enhanced the toxicity (193).

The pathogenic significance of the toxin in dysentery is not clear (296). Its pharmacological activity is, however, a tool for further research.

IV. STREPTOCOCCAL TOXINS

Streptococcus pyogenes produces a comparatively high number of extracellular products: streptolysin O, streptolysin S, erythrogenic toxin, proteinase, diphosphopyridine nucleotidase, streptokinase, deoxyribonuclease, ribonuclease, hyaluronidase, hyaluronic acid, and amylase (8). Intracellular products and components of the cellular wall are also being studied.

A. Streptolysin O

Most of the pharmacological work has been performed with streptolysin O. Its possible importance as a factor in the pathogenesis of rheumatic fever and other sequelae of streptococcal infection (26) has been suggested by most authors. Streptolysin O has been described as a thermolabile cellular product of *Streptococcus pyogenes*, active only in the reduced state, labile in the presence of oxygen, and hemolytic (119). Recently a highly purified product has been obtained (110). Most of the work quoted below, however, used crude streptolysin O.

Toxicity. Measured by the LD50 parameter, streptolysin O is relatively nontoxic. In rabbits the toxin causes a general depression, frequent and deep respiration, diarrhea, and pronounced pyrexia. A temperature fall below 36°C, diarrhea, and abrupt fall of blood pressure are signs of imminent death (112, 240, 286).

Hemolytic effects. In vitro, hemolysis is one of the basic effects of streptolysin O. The red cell sensitivity to streptolysin O decreases in this order: rabbit, mouse, and chicken. So does the toxicity (112, 137, 240). A relation between the age of rabbit erythrocytes and their susceptibility has been reported (203). Streptolysin O does not affect the osmotic resistance of erythrocytes, or the plasma volume. The hemolytic effect can be prevented by organic mercurial diuretics, iodoacetamide, or monoiodoacetate (285). *In vivo*, intravenously injected, streptolysin O produces hemolysis with a good dose-effect relationship (240, 277). A sharp rise

in plasma potassium level may be present (112). Heating of streptolysin O abolishes its hemolytic potency but some toxicity remains. Specific immunization does not prevent hemolysis *in vivo*, whereas other signs of toxicity do not appear. Hemolysis is changed by a number of drugs; it is increased by caffeine, camphor, and amphetamine, and decreased by hexobarbital, ether, thiopental, and nicotine. The intact toxin causes anuria and a lowering of creatinine clearance. The changes in hemoglobin levels after various drugs might be explained by effects on kidney circulation, by a direct action, or *via* the nervous system. High decortication does not influence hemoglobin levels, whereas shock or hypothalamic lesions produce a prolongation of high hemoglobin level in the plasma (240).

Shwartzman reaction. Administration of a product from rabbit skin infected with *Streptococci*, followed by streptolysin O injection, evokes a generalized Shwartzman reaction (274). Streptococcal infection in surviving animals induces rheumatic fever-like changes in the heart and joints (238, 259, 275).

Effects on heart and vessels. Many years ago Bernheimer and Cantoni described the depressant action of streptolysin O on the isolated frog heart (27). For the mammalian heart this was demonstrated later (155, 317). This is a property not shared by other known toxins (240). Suspensions of mitochondria from the myocardium of rabbits, containing as substrate citrate, fumarate, or *alpha*-ketoglutarate, react on the addition of streptolysin O by a sharp reduction or complete cessation of oxygen consumption (155). The suppressing enzyme has been identified as a diphosphopyridine nucleotidase. This product is present only in some streptococcal strains and has leucotoxic properties (28). A substance was found in crude streptolysin which, on the basis of biochemical properties, was supposed to belong to a group of diphosphopyridine nucleotidases (115). With modern chemical techniques it was possible to isolate DPNase from streptolysin O (50) and further purify the enzyme (228).

Repeated intravenous administration of streptolysin O in rabbits is followed by a decrease of the P and T peaks of the electrocardiogram, lowered voltage and lengthening of the QRS complex, and faster heart rate (286). Purified streptococcal extracellular products cause disturbances leading eventually to cardiac standstill (111). Crude streptolysin O produces a strong vasoconstriction in perfused muscle. This is preventable by 5-HT antagonists (240), one of which (UML-49) gives some protection against the lethal effect of the purified toxin (112).

Isolated organs. Crude streptolysin O added to the bath fluid produces after latency a slow contraction of isolated rat uterus (77). This is not antagonized by atropine or antihistaminics, and only partly by 5-HT antagonists. The nature of the slow-contracting material has not yet been identified (240). Goodwin and Richards described slow-contracting activity in the urine of animals infected with streptococci and identified the causative agents as polypeptides (103).

Nervous system. Sydenham's chorea has been associated with streptococcal infection (310). Intraventicular injection of crude streptolysin O into the lateral ventricles of cats is followed by numerous behavioral changes, somewhat like those of Sydenham's chorea. Various batches differ in the intensity of the effect

(240, 243). Doses of streptolysin O so small that they do not alter general behavior partly inhibit conditioned reflexes in rats. The second administration of the toxin after a 3-week interval makes the disturbances more pronounced, whereas repeated injections every 3 days lead to tolerance (242).

After previous sensitization, crude streptolysin O injected into the perfused carotid sinus of cats and rabbits evokes reflex changes of blood pressure and respiration. Repeated injections of the toxin and acetylcholine in such experiment eventually lead to sensitization, so that severe reflex circulatory disturbances appear (240, 319). Crude streptolysin O also impairs the conduction in the sciatic nerve of cats and rabbits *in situ*. Local administration of adenosinetriphosphate or -monophosphate, but not of adenosine restores conduction (240).

Tolerance to various streptolysin O effects, including nervous disturbances of all the kinds described, and also to death, can be achieved by specific immunization. Pretreatment with unrelated toxins also prevents the nervous disturbances. Increased resistance to the effects of the toxin can also be produced by repeated injections of small amounts of phenol (240, 247). This points to the nonspecific character of the observed phenomena.

Metabolic effects. In seriously intoxicated animals there is a drop in liver glycogen, increase in weight of the adrenals, and depletion of ascorbic acid from the adrenals. These effects depend on the toxicity of the batch used and must again be considered a rather nonspecific pattern (240).

It has long been known that the hemolytic activity of streptolysin O can be antagonized by cholesterol (27). It has been found that lipoid factors in the serum inhibit streptolysin O. This nonspecific inhibition can be prevented by a mucoprotein present in the α_1 globulin fraction (48, 49). Streptococcal extracts cause a selective liberation of specific lipoids from α_1 serum lipoprotein. The lipoid released accounts for 85 to 90 % of the lipoprotein, and esterified cholesterol constitutes 90 % of the total lipid released. The effect probably depends upon an enzyme reaction. In the presence of citrate and other intermediates of the Krebs cycle, streptolysin O enhances the uptake of oxygen in *Aerobacter aerogenes* (257).

On *cell cultures*, streptolysin O has a cytotoxic action. This is present also in a heat-inactivated commercial preparation of the toxin (232).

B. Other cellular components

Crude lysates of streptococci have properties like those of endotoxin (297). A component from sonically disrupted A *Streptococci*, containing polysaccharides and protein, induces chronic multinodular skin lesions (1, 272). This is due to the polysaccharide moiety (61, 272). The effect is similar to nonspecific injury produced by substances like methylcellulose (339) or dextran sulfate (19). Immune serum containing antibodies against somatic bacterial polysaccharide antagonizes and precipitates the toxic material. Therefore the toxic C polysaccharide complex should be investigated as another possible factor in the pathogenesis of the sequelae of streptococcal infections (255, 272). The group-specific C-carbohydrate and the type-specific M-protein are comparatively nontoxic to normal rats. In the adrenalectomized animal, however, the toxicity is considerable (288, 289).

A mucopeptide containing N-acetylglucosamine, N-acetylmuramic acid, and several amino acids has been found in streptococcal cells (171). An intracellular product obtained by sonic disintegration, when injected intradermally, produces an erythematous eruption persisting about a week and followed by desquamation. Recrudescence appears in a low percentage of animals. Capillary damage is present. Histamine, trypsin, and saline are without any analogous effect but sonic extracts of *Salmonella typhi* cause similar changes in about one third of the animals. Analogous extracellular products are without effect (59).

C. Hemolysins

Intracellular hemolysin, obtained from sonically disrupted group A streptococci, is distinct from streptolysin O and streptolysin S (278). It is lethal for mice, which die in convulsions; autopsy reveals hemoglobinuria, intravascular hemolysis, pulmonary hemorrhage, and edema. There is no constant relation between lethality and hemolytic effect. The hemolytic as well as the lethal potency is considerably diminished by heating. It seems that this intracellular hemolysin (278) is not bound to the cellular wall (270, 271, 272, 278).

It has been recently reported that a cell-bound hemolysin called streptolysin D (SLD) can be released from streptococcal cells into the surrounding medium by some surface-active materials (Tweens) or by crystalline albumin, but not by sonic energy. This product, as well as streptolysin O and streptolysin S, injures Ehrlich tumor cells (99). When these cells are pretreated with streptolysin O, D, or S, they lose the capacity to proliferate in mice after intraperitoneal injection (99, 168).

Products obtained are of special interest. An endotoxin has been described which enhances the lethal and tissue-damaging properties of gram-negative bacterial endotoxins and streptolysin O, with lethal, cardiotoxic, liver-necrotizing, and pyrogenic effects. The action is the same as that of the streptococcal erythrogenic toxin and is related to this. Group A streptococci produce two distinct pyrogenic toxins, one predominantly intracellular, the other mainly extracellular. Homologous tolerance is achieved by repeated injections of both toxins. By injecting the intracellular material it is possible to develop tolerance to *S. typhi* endotoxin in rabbits. No cross-tolerance exists between the extracellular product and *S. typhi* endotoxin (331). Animals treated with *S. typhi* endotoxin are tolerant to the effects of the intracellular product. This protection can be prevented by RES blockade (60). Whereas extracellular products enhance the toxicity of filtrates of *S. typhi* broth, the intracellular products have no such effects; hence the existence of two distinct pyrogenic streptococcal toxins has been claimed.

A toxic factor appears in cutaneous lesions from group A streptococci; this has been identified as thromboplastin derived from the tissue (273). An active principle has been found in crude acid extracts of several strains of group A streptococci which precipitate mammalian plasma and fibrinogen (151). From type 12 A streptococcal filtrates a polypeptide factor has been isolated which induces glomerulonephritis in monkeys (250).

Streptococcal infections and their sequelae are of outstanding importance.

Although little has been done up to now about the pharmacology of the individual streptococcal products, it is already evident that these products are pharmacologically active. This should encourage further research.

V. STAPHYLOCOCCAL TOXINS

Staphylococcal infections are nowadays one of the major menaces caused by microorganisms. This is because of the well-known resistance of these microbes to antibiotics. The toxins are important in the pathology of staphylococcal infections.

For the pharmacologist it is difficult to go through all the older conflicting evidence about various substances derived from *Staphylococcus aureus* (or, to be correct, *Micrococcus pyogenes* var. *aureus*). For this literature d'Antona's review may be consulted (11). Recently a survey of staphylococcal products has been presented (8). For the purpose of this review it is sufficient to remember that they may be divided into four groups: exotoxins (including *alpha* toxin), endotoxin (enterotoxin, the cause of food poisoning), leukocidin, and various enzymes and products which are not toxins.

A. Alpha toxin

Nature of toxin. In spite of attempts (47), the toxin has not yet been sufficiently purified to permit an exact chemical characterization. Recently it has been purified (28a). It is a thermolabile protein of high molecular weight, with different active centers for different biological qualities in the molecule (316). The effects of *alpha* toxin described below must be considered in the light of the fact that it is not a pure product.

Toxicity. Lethal effects of the *alpha* toxin have been described for rabbit, mouse, and guinea pig. Rabbits are killed within minutes, whereas in mice the course of intoxication is slow (78, 312). Death has been attributed to failure of the respiratory center, histamine release, or direct cellular attack (11, 215). Recently the lethal effect was explained by circulatory effects of the toxin (313). The course of intoxication in mice, rats, and guinea pigs is very similar; the intensity is dose-dependent (340). Almost immediately after intravenous injection, the animals have uncoordinated movements; they fall in side position and convulsions appear, the extremities being extended. The animals die within hours or days in respiratory distress. The toxicity depends on the temperature at which the preparation is stored: at $-10°C$ the toxin maintains its toxicity for 7 months; at $+2°C$ the toxicity is less after 3 months; and decrease in toxicity is even quicker at 20°C (340). Various batches have significantly different LD50's. The dependence of toxicity on the age of the animal is important. The lethality increases in infant rats with age up to the 18th day and young rabbits are not susceptible (114). Males are more susceptible and die more rapidly. The rat is less susceptible than the mouse. Guinea pigs die after intracardiac injections of toxin. The route of administration used (intramuscular) may explain the discrepancy with d'Antona's findings of the nonlethality of the toxin for guinea pigs (11, 340).

Hemolytic, lethal, and dermonecrotic properties have been attributed to the toxin. *Alpha* toxin (or it may be more correct to say, staphylococcal toxin rich in the content of *alpha* toxin) is a hemolysin. Hemolysis is a function of hemolysin concentration and of the period of contact (267). The velocity of hemolysis is maximal in the pH range from 4.4 to 5.2 (190). The only exact measurement of the hemolytic potency of a staphylococcus toxin is the ED50 (dose producing 50% hemolysis) (11). The susceptibility of erythrocytes of various species to *alpha* toxin decreases in the following sequence: rabbit, cattle, sheep, goat, man, horse, guinea pig. Wiegershausen (340) using the ED50 technique found, putting the ED50 for rabbits = 1, the following sequence: rabbit 1, cat 12, cattle 18, sheep 29, rat 70. Storage for 7 months at different temperatures (−10, +2, +20°C) had different effects on hemolytic activity. The relation was 1:6.4:15, as measured by ED50. The hemolytic activity of various batches of toxin varies considerably (340). The mechanism of hemolysis has not been elucidated. Some have considered adsorption and enzymatic processes to be responsible (240, 264). D'Aguanno (6) suggested that, under the influence of toxin, erythrocytes lose the ability to maintain the cation gradient across the membrane.

The combination of staphylotoxin with the toxin of *Clostridium perfringens* potentiates hemolysis, especially of human erythrocytes (180). The same was observed using the combination of staphylotoxin with tetanus toxin (164).

Dermonecrosis. The necrotizing effect of staphylotoxin in the skin has long been attributed mainly to the *alpha* toxin (119). The necrosis is usually attributed to primary vascular changes in the area into which the toxin is injected (220, 312) but necrotic changes have also been observed in explants of skin (178); the latter result speaks for a different mechanism of effect. Renal necrosis has been observed by many (see 11). Thal and Egner did not support older concepts of direct toxic effects on the kidney cell (312). They attributed the renal effect to vasoconstriction sufficient to stop renal circulation. The toxin also can produce hemorrhagic pancreatitis (57). Probably both the vascular and direct effects on cells should be taken into account. Free hemoglobin might also participate in the kidney. Moreover staphylotoxin was the first toxin shown to release pharmacologically active substances from tissues (82).

It is not clear whether the hemolytic, lethal, and dermonecrotic activity of the staphylococcal *alpha* toxin is really the effect of one substance. Sixty years ago it was postulated that the three effects are due to different factors (214). This concept still persists in the current German literature (158), but now the prevalent view is that one factor is responsible for all three effects (78, 313). Trypsin inactivates all three (167). Butler (47) in his study showed that these discrepancies of views may be explained by a varying sensitivity of the corresponding active groups of one large toxin molecule.

Circulation. Intravenous injection of *alpha* toxin into anesthetized cats causes an immediate fall of blood pressure, followed by transient recovery, which in turn is followed by terminal hypotension. The latter is accompanied by heart failure, which has been ascribed to obstruction of the pulmonary circulation. Isolated mammalian hearts react, according to Wiegershausen, first by increase, followed

after large doses of toxin by irreversible decrease of contraction of the isolated heart. Even large doses do not affect the heart of *Helix pomatia* (340). An increased resistance of the coronary vascular bed was described by Thal and Egner. The toxin remained without effect on electrically driven isolated auricles (313). The vessels of the isolated ear of the rabbit are contracted by the toxin (340). It also immediately contracts venous strips; within an hour, relaxation and complete unresponsiveness to epinephrine follow. In the whole rabbit, the toxin diminishes the size of the liver; the portal vein is dilated, portal pressure transitorily increased, and venous return to the right heart diminished. This evidence together with *in vitro* results speaks against a primary cardiac origin of the circulatory failure (313). Some discrepancies might be due to the age of the mammalian hearts used, as it has been demonstrated that the effect of *alpha* toxin on the isolated mammalian hearts is age-dependent (344).

Smooth muscle. The effect of staphylotoxin on isolated intestinal and uterine strips of various species has been repeatedly investigated (10, 23, 46, 82, 156, 313, 340). After a latency which depends upon dose, and which is shorter in intestinal than in uterine strips, a contraction appears. With higher doses, there is an irreversible contracture and the organ then is unresponsive to further stimuli. Only slight antagonism is produced by antihistaminics, atropine, sympathomimetic agents, caffeine, aminophylline, nitrites, LSD, chlorpromazine, or reserpine. Curare-like drugs and ganglion blockers are without effects. Papaverine is the only strong antagonist. This is in favor of a direct muscle effect (46, 108, 340).

The site of action on the smooth muscle cell has not yet been established. Some groups have suggested a direct site on the cytoplasmic membrane (46, 313); others have considered also the liberation of substances which cause the contractions (82, 340). The cytopathogenic effect of the toxin on various tissue cultures (178), the pyrogenic effect, and the fact that other products are yielded by the incubation of toxin with tissues (145) favor the second idea. Most probably both mechanisms are involved.

Striated muscles in general are less sensitive than smooth muscles to *alpha* toxin. Toxin administration does not modify the response of directly stimulated skeletal muscle (313). The toxin induces contracture in indirectly stimulated or denervated muscle. The acetylcholine response disappears. In the frog rectus muscle a contracture appears after a long latency. For this effect, calcium ions must be present. Boiled toxin is without effect (108).

Nervous system. Injections of the toxin into the ventricles of the brain in cats induces mydriasis and vomiting, strong salivation, miaowing, and paralysis of the extremities within the first four minutes. The cat is excited and tries to be aggressive. Peristalsis is violent and can be seen by simple observation of the bowel through the abdominal wall. Larger doses cause convulsions and death within 20 to 30 minutes. Intravenous injection also elicits vomiting (225). Sublethal doses of toxin abolish conditioned reflexes, and partial disturbances remain up to 110 days. Behavioral changes indicate subcortical effects (105). After intracerebral toxin administration, the perfusate from cat brains gains the ability to cause contraction of smooth muscle. This is not blocked by atropine, but is an-

tagonized by lysergic acid diethylamide (108). Nonlethal amounts of the toxin disturb the coordination of mice and prolong thiopental anesthesia. Susceptibility to pentylenetetrazol, strychnine, and audiogenic seizures is diminished (108).

Surprisingly few *metabolic* studies have been conducted with staphylococcal toxins. In lethal staphylococcal infections in mice, a thermolabile component causes a transient fall in blood sugar, increased serum transaminase and alkaline phosphatase activity, and a higher plasma level of inorganic phosphorus; and a lower plasma sodium level has been described (58).

Tolerance. Simultaneous or prior injection of the venom of the tiger snake protected mice against an LD50 to LD100 dose of a filtrate rich in *alpha* toxin (219). The opinion was expressed that the venom occupies the site in susceptible cells otherwise occupied by the toxin. Probably the protective effect is caused by phosphatidase A. Long-chain unsaturated fatty acids are liberated and lysophosphatide is formed (219, 220, 221). Ganglioside has the same protective effect against staphylococcal and diphtheria toxin. It has been suggested (222) that phosphatidase A plays a role in animal defense against some bacterial exotoxins. Lysolecithin itself has been found to have protective action. It might be that lysolecithin releases, from brain homogenates, compounds containing sialic acid, among which gangliosides predominate (222). Brain extracts have been effective in prevention and treatment of staphylococcal infection (24, 225). Extracts from the supernatant of some staphylococcal cultures protect mice against a number of infections (88). Repeated phenol injections increase the resistance of mice to the *alpha* toxin (108). Dóbiás, Balló, and Keményvári found a relation between the clinical course of staphylococcal empyemas in infants and the amount of *alpha* toxin produced. They assayed the amount of the toxin on the basis of its hemolytic potency and found the severity of the clinical course to increase with toxin production (69). Staphylococci cultivated *in vivo* in diffusion chambers produce a toxin with high hemolytic, lethal, and dermonecrotic activity (134). Undoubtedly pharmacological research with such a natural product would be of outstanding interest.

B. Endotoxin

Recently it has been suggested that the pathogenicity of staphylococci depends upon an endotoxin which possesses properties like those of endotoxins from gram-negative bacteria. Lethal effects were found in mice, and these were enhanced by adrenalectomy. Chick embryo lethality and tumor-necrotizing effects are present. Epinephrine, phenoxybenzamine, and mepyramine do not alter the response, but cortisone decreases lethality (123, 204).

Staphylococcal extracts obtained by methods similar to those used with gram-negative bacteria produce cutaneous lesions when injected with epinephrine in the manner described for the endotoxin from gram-negative bacteria (216). A combination of sublethal amounts of staphylococcal endotoxin and *alpha* toxin has lethal effects. Cortisol gives some protection against this mixture of endotoxins, but is ineffective against *alpha* toxin. Accordingly, both toxins could be important in the lethal outcome of staphylococcal infection (17).

C. Beta and delta toxins

Beta toxin is derived mainly from staphylococci of animal disease (11) but is present in some strains of human origin (119). Sheep, goat, and cattle erythrocytes are highly susceptible, and human only slightly, whereas erythrocytes of other species are quite resistant (191). Pharmacological interest has been aroused by the effects on isolated organs. Large amounts of toxin are necessary to cause the rabbit intestine to contract; with smaller amounts, only suppression of spontaneous motility is the typical pattern (10, 156). A possible overshadowing by the presence of *alpha* toxin has to be considered. The response to acetylcholine is blocked. A strong contraction, followed by relaxation, was described in the guinea pig ileum (25). Responses to 5-HT, acetylcholine, barium chloride, histamine, and nicotine are partially or completely blocked. Full blockade persists after the first washing. After repeated washing, contractions gradually reappear. The *beta* lysin possesses protozoacidal activity (25).

A *delta* toxin has been described, but nothing of special pharmacological interest has been reported (191).

D. Enterotoxin

Though there is much conflicting older literature as to whether the enterotoxin really exists (119), it is probable (23, 276) that this thermostable staphylococcal product exists as an entity. It has been described as a protein complex of a molecular weight of 15,000 to 25,000, with a high lysin content and a remarkable thermostability at a neutral pH (23, 122).

Human food poisoning is characterized by nausea, vomiting, diarrhea, shock-like symptoms from dehydration, and early recovery within 20 hours (276). The most striking effect in man is vomiting (119). Vomiting effects can be reproduced in monkeys and kittens (63). In the frog, enterotoxin produces spasm of the pylorus (256) and antiperistalsis. A lethal effect for chick embryos has been reported (157).

Within 6 to 8 hours after feeding enterotoxin to monkeys, the serum glutamic oxaloacetic acid transaminase activity is elevated to 2 to 3 times the normal level. This points to a cell-injuring effect of the enterotoxin (305). In cats a purified product regularly produces vomiting after intravenous, but not after intraventricular injection; injection by either route elicits fever. Vomiting persists after the ablation of the chemoreceptor trigger zone. Cats with chronic midbrain section continued to show a pyrogenic but not a vomiting reaction (55).

It has been shown in monkeys that subemetic doses of dihydroergotamine increase the incidence of vomiting. The site of action cannot be the same as that of the toxin, because DHE does not cause vomiting after the ablation of the medullary trigger zone (304), whereas enterotoxin does (55). Diphenhydramine, apomorphine, veratrum alkaloids, atropine, tetraethylammonium chloride, or adrenal cortical hormones did not affect the vomiting, but perphenazine and reserpine reduced it; and a peripheral site of action of these antagonists was excluded (306). Chlorpromazine had a doubtful effect. Vagotomy protects cats from enterotoxin vomiting (35). Bilateral destruction of the area postrema on the

floor of the fourth ventricle (chemoreceptor trigger zone) makes rhesus monkeys, in contrast to cats, completely refractory to the emetic effect of the enterotoxin (307). Thus, an important species difference in the site of action for cats and monkeys has been established.

Effects on the intestine. Anderson *et al.* described an increased activity of isolated segments of the gut after application of filtrates of staphylococci from food poisoning. Later, however, after reinvestigation, they came to the conclusion that increase in tone was caused by *alpha* toxin (10). Stolmakova investigated the effect of staphylococcal filtrates containing enterotoxin in various parts of the intestine of the anesthetized cat. Intravenous injection caused contractions, the intensity of which increased from stomach to large intestine. The same was seen in isolated intestinal strips from cats and rabbits. Control solutions and filtrates not containing enterotoxin, on the contrary, caused a transient relaxation. Relaxation due to epinephrine was not antagonized by enterotoxin. Ergotamine further increased the contracting action of the filtrates. Atropine decreased the enterotoxin effect. In whole animals, sympathomimetic drugs were without effect on the contractions. Ergotamine and dihydroergotamine did not interfere with peripheral effects of the toxin (300, 304). Crude filtrates supposed to contain enterotoxin and staphylococcal polysaccharides have been reported to contract isolated intestinal strips (143, 276). A partial but apparently nonspecific antagonism of 5-HT contraction occurs with enterotoxin in the isolated rat uterus (304). In cats Stolmakova described a fall of blood pressure (not caused by the broth), and the isolated cat heart stopped after administration of enterotoxin filtrates (300). As Sugiyama (304) did not see any blood pressure changes after partly purified endotoxin, what kind of toxin or interaction of toxins is responsible for the effects described above has to be the object of further investigation.

VI. *BORDETELLA PERTUSSIS* TOXINS

Three main toxic substances have been described: 1) *Bordetella pertussis* toxin, which contains protein and is thermolabile. Its activity is destroyed by trypsin. It does not contain carbohydrate (18). 2) A nontoxic, thermostable, and pyrogenic substance parent to the endotoxin from gram-negative microbes has been prepared (187). 3) A toxic lipopolysaccharide without immunizing properties has been isolated (165).

A. Pertussis vaccine

After an injection of some batches of pertussis vaccine, mice become more sensitive to histamine. The literature has been reviewed by Kind (159). According to accumulating evidence, it seems that there is no parallelism between histamine-sensitization and protective effects of pertussis vaccines (71, 159, 230, 303). Střížová and co-workers (303) compared protective agglutination and histamine sensitization in vaccines coming from producers in different countries; they found equal protection but differing ability to sensitize to histamine. The increased susceptibility to histamine develops within 3 to 5 days after challenge with pertussis vaccine and gradually declines within several weeks (159). It is species-

dependent, *e.g.*, reproducible in rats, but not in guinea pigs or rabbits (159). Even in the same species there are great differences depending on strain. Natural resistance is an important factor (2). The histamine-sensitizing potency of the vaccine is also dependent on age (and weight) of the animal (159, 235). Female mice are more susceptible than males to histamine, as measured by the LD50, and this sex difference is enhanced by pretreatment with pertussis vaccine (159, 234, 235). Difficulties with determinations of the LD50 of histamine led to an attempt to introduce, as an assay, not the LD50 of histamine, but the mortality after one dose of histamine with varying doses of vaccine (235). The LD50 of histamine seems, however, to be a better way of determination. With careful dilution histamine can be accurately assayed.

Various pharmacological patterns have been described after histamine administration in control rats and others challenged with pertussis vaccine (36). Pretreatment with vaccine increased the susceptibility of rats to the lethal action of histamine at least 25-fold. In control animals, sublethal doses of histamine caused salivation, urination, defecation, cyanosis, and paralysis of the extremities, whereas a quick intravenous injection of a lethal dose resulted in convulsions and almost instantaneous death. In animals challenged with vaccine, exactly the same signs were observed, but with much smaller doses. In rats vaccine enhances circulatory disturbances induced by histamine. After small doses of histamine, electrocardiographic changes were also pronounced in vaccinated animals, and daily treatment with 50 mg of histamine per kg did not alter the electrocardiographic changes from the third day after vaccination; this result shows that it was not possible, by previous histamine injections, to desensitize the animals to the sensitizing effect of the vaccine.

An immediate bronchospasm could be induced in rats by 100 mg of histamine per kg in normal as well as vaccinated animals. With lower doses control rats did not show any effect, whereas in vaccinated animals bronchospasm appeared after a latency of at least 5 minutes. Electroencephalographic patterns were the same in vaccinated and nonvaccinated animals. Histamine produced a flattening of cerebral electric activity. The duration of changes was much longer in vaccinated animals; further flattening occurred and finally there was absence of waves. There was a characteristic increase of response to the neuromuscular effect of histamine on masseter and diaphragm preparations in vaccinated rats. The reason for the increased sensitivity to histamine is by no means clear. The suggestion that pertussis vaccine could further alter the leak of potassium attributed to histamine (188) is worth further investigation.

In mice sensitized to *B. pertussis*, there is a lowering of blood sugar level in comparison to controls. The hyperglycemia usually seen in animals injected with histamine is not apparent; on the contrary, there is a further lowering of blood sugar.

Inactivation of histamine by tissue was inhibited by *B. pertussis* vaccination (159, 194). It was reported that the ability of the brain and of the liver of animals treated with *B. pertussis* to inactivate histamine was diminished. By means of *B. pertussis* vaccine, it is possible to increase histidine decarboxylase

activity in the rat lung (268). Wajda and co-workers have shown that *B. pertussis* vaccine, and other lipopolysaccharides lead to an increase in transglutaminase activity in the mouse liver. They also reported that in animals treated with lipopolysaccharide there is a marked incorporation of exogenous histamine; unfortunately, they did not mention any results with *B. pertussis* vaccine (329).

The sensitization produced by pertussis vaccine is not limited to histamine. Many investigations have confirmed an elevated sensitivity to 5-HT (150, 159, 208, 234). The course of the increased sensitivity to 5-HT is analogous to the histamine sensitization. It appears 24 to 48 hours after vaccine administration, reaches its peak after 2 days, and declines within the following week. But no sensitization appears toward catecholamines, carbachol, reserpine, or KCl (159, 185). Increased permeability of blood vessels occurs after *B. pertussis* vaccine; this might be related to sensitization to endogenous histamine and 5-HT (209). A sensitization to acetylcholine as well as histamine has been found *in vitro* on pretreatment of the isolated ileum of the guinea pig with pertussis vaccine. This is especially interesting because guinea pigs have been reported not to undergo sensitization to histamine after challenge with pertussis vaccine (269).

Sensitization to endotoxin, stress, and infection. Various reports agree that challenge with *B. pertussis* vaccine sensitizes animals to the lethal effect of endotoxin. According to Abernathy and Spink, increased susceptibility to endotoxin after pertussis vaccine has been found in mice. This phenomenon is not parallel to the histamine sensitization. Kind (159) found a parallelism between the increased susceptibility to *E. coli* endotoxin after challenge with pertussis vaccine and sensitization to histamine or 5-HT (160). Pertussis vaccine also sensitizes mice to cold stress, to radiation (260), and to infections (159). The importance of the interval between infection with *B. pertussis* and vaccination has been repeatedly stressed (170, 234). It depends on the experimental conditions whether sensitization or protective activity of pertussis vaccine to different infections appears (234).

Sensitization to anaphylaxis. Active anaphylaxis is enhanced by *B. pertussis* vaccine (159). Passive anaphylaxis with homologous antibody is also activated by pretreatment with *B. pertussis* (210). The enhanced susceptibility to anaphylactic shock when *Bordetella pertussis* vaccine and bovine serum albumin were simultaneously administered could be changed to increased resistance to anaphylactic shock when a first injection of the vaccine was made 5 days prior to the combined vaccine-serum injection (161).

Killed *B. pertussis* cells accelerated the appearance of the sensitivity of mice uteri, as demonstrated by the Schulz-Dale reaction (active sensitization). No influence was seen on the mouse uterus when the sensitization was performed passively (211). However, a sensitization to histamine was reported in isolated organs after the addition of pertussis vaccine (269). Enhanced susceptibility of pertussis-treated mice to pollen extracts has been also described (162). The sensitivity of mice to the lethal effect of peptone is also enhanced by *B. pertussis* vaccine (140).

B. pertussis increases the permeability of vessel walls to Evans blue between

the first and eighth day after challenge (209). Severe cutaneous hypersensitivity in rats could be produced by subcutaneous injection; an intensive swelling appeared. An injection of vaccine in another paw the following day caused the same intensive changes to appear there within 12 to 24 hours. Thus an induced cutaneous hypersensitivity was present (262). Although the vaccine sensitized the animals to intravenous histamine, preliminary sensitization with histamine, 5-HT, or 48/80 did not increase the edema-producing activity of histamine, 5-HT, or 48/80. In this type of experiment, rat mast cells in hypersensitive areas were not appreciably damaged. Antihistaminic as well as anti-5-HT drugs did not depress the inflammation induced by pertussis vaccine, whereas changes produced by 48/80 antagonized the inflammation.

B. pertussis vaccine and adrenal mechanisms. There is no evidence about the role of adrenal mechanisms in sensitization to histamine produced by vaccine (1959). Vaccines detoxified by long storage or centrifugation produce no changes in the content of ascorbic acid in the adrenals, whereas more toxic vaccines cause a decrease (230). Cortisone reduces the sensitivity to histamine in animals treated with pertussis vaccine (159).

B. Pharmacology of pertussis toxin

When not otherwise specified, the results were obtained either with thermolabile toxin or with whole bacteria; in the latter case, it is implied that the toxin might have been the cause. The toxin is sometimes called neurotoxin. Some of the severe neurological complications in man after pertussis vaccination might be attributed to it (22, 174). Changes of electromyographic patterns have been reported (174, 343). It has been shown that on intracerebral administration in guinea pigs, pertussis toxin causes a number of neurological abnormalities, which proceed to convulsions and death (94). Important vascular changes, hyperemia, edema, hemorrhages, spastic changes of vessels and bronchi, hypertrophy of the mucosa, and later peribronchial fibrosis have been described after intratracheal administration of toxin (32).

Toxicity and age dependence. Mice remain highly susceptible (227), whereas in rats the susceptibility decreases with age (301). In isolated lungs of guinea pigs, comparatively large doses of a crude thermolabile *B. pertussis* toxin cause a gradually developing bronchoconstriction. In animals pretreated with the toxin, the constriction appears quickly, usually within a few minutes after the challenge (302).

In cats under barbiturate anesthesia, intravenous injections of the toxin did not cause any spontaneous cough or increase in the reflex cough from irritation; on the contrary, there was some diminution of the latter. The same was true for unanesthetized cats. Respiration was changed; after intravenous injection of pertussis toxin it was deeper, and sighing, gasping, and transitory respiratory arrest appeared. These phenomena probably are of a nonspecific character, as similar effects were seen after *Shigella shigae* toxin (240), and diphtheria toxin (3).

Effects on isolated organs. On the isolated guinea pig ileum the toxin regularly

caused a typical slow contraction. In some experiments, there was a marked increase of histamine sensitivity; in others, the response to histamine was unaltered after toxin was added to the bath. The isolated lungs and distal ends of ileum of sensitized animals were assayed simultaneously and the responses to histamine were more frequent and intense (302). The slow onset of action and course of contraction of the isolated bronchi speak for a "slow-contracting substance" rather than for histamine. On the isolated uterus of the rat, in half of the experiments a very pronounced sensitization to acetylcholine was seen after toxin administration. The slow contraction again occurred regularly (302). Analogous results were obtained with pertussis vaccine; sensitization to histamine and acetylcholine was seen as well as the slow contraction. An alcohol extract of the vaccine treated with normal HCl and shaken into ether contained the factor which sensitized to histamine and induced the slow contraction (269).

It was shown previously that crude streptolysin O causes slow contractions (240). Goodwin and Richards reported the appearance of active polypeptides in the blood of animals infected with parasites and microbes (103). Lipid-soluble slow-contracting substances sensitize to histamine (see 327). The possibility of such sensitizing mechanisms has to be borne in mind. On the other hand it is known that, in guinea pigs, pertussis vaccine does not enhance the susceptibility to histamine (159). The doses of toxin used might be important to this result. Toxin sensitivity varies from organ to organ.

A crude thermolabile toxin was also assayed on the isolated perfused heart of various species (rabbit, cat, and hen). Ten to twenty minutes after the toxin injection, in all species investigated, there was a lowering of strength of contraction, followed by typical Luciani periods for a prolonged time (241). Some hearts recovered; others came to a standstill. In the frog heart, Lapin observed exactly analogous patterns after dinitrophenol (183); he concluded that the Luciani periods result from impaired oxidative phosphorylation. This should be a tool for further research with *B. pertussis* toxin, especially as it has been shown that other toxins impair oxidative phosphorylation in the brain (299).

The pharmacological activity of *B. pertussis* toxin and vaccine shows that this is an area of interesting research, which may help to elucidate the pathogenesis and therapy of whooping cough.

VII. CONCLUDING REMARKS

The purpose of this review has been to present information about current pharmacological knowledge related to several toxins. This turned out to be a very difficult task. Endotoxins were reviewed in this journal a few years ago (21). The number of new findings in the field made it necessary to cover it again. As the authors tried to avoid the older, previously reviewed literature, those unfamiliar with the earlier evolution of this field may find the chapter somewhat unbalanced and difficult to read. The task of the reviewers was easier with the other toxins.

The authors are among the few pharmacologists who have long considered

bacterial toxins to be a subject of special interest. They firmly hope that this interest will grow in the future, for the benefit of better understanding and combating of human disease, the basic aim of pharmacology.

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