ENDOTOXIN SUSCEPTIBILITY AND ENDOTOXIN HYPERSENSITIVITY

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INTRODUCTION

The aim of this monograph is to point out the connections between the different endotoxin effects and to discuss the possibility whether endotoxin apart from its toxicity has also a special hypersensitizing ability. Although the latter problem will be discussed most extensively, because of its close relationship the toxicity of endotoxin is also discussed in the first two chapters.

One of the special aims of this work is to coordinate the results of different authors as far as possible. The species of animals, the kinds of endotoxin, the doses and experimental methods used are mostly different, so that a proper comparison between the experiments is not easy. However, such comparison and coordination seem to be important. Thus, in the „concluding remarks" an attempt is made to evaluate the experiments quoted from this point of view, and to put forward questions and hypotheses for discussion.

There are many excellent reviews on endotoxin. Among them is Landy and Braun's „Bacterial Endotoxins" (1964, Rutgers Univ. Press) which may be considered as a leading one. It contains 58 contributions to an endotoxin symposion held in New Brunswick. In spite of the divergence of data and opinions, this book represents a most extensive review of the experiments carried out in endotoxin research. A further considerable contribution to this problem appeared in Volume 133 of Ann. N. Y. Acad. Sci. in 1966 entitled: Molecular Biology of Bacterial Lipopolysaccharides.

From the results given in the literature and those of our experiments we tried to make a general classification and scheme of the toxic effect of endotoxin on an anatomical and physiological basis. The role of the autonomic nervous system, of the vascular system and of humoral agents in connection with an allergic basis: the allergen (antigen) nature of endotoxin, the immunospecificity of reactions, and the humoral agents of hypersensitivity will be discussed.

An attempt is made to fit the different effects of endotoxin suitably into the scheme given in the contents. On the basis outlined above we tried to devise a uniform concept for all the endotoxin effects.

In view of the possible deleterious effect of endotoxin to human beings, especially in relation to damage by pathogenic microorganisms or to symbiosis with endotoxin-producing microorganisms, interest is also focused on this problem.

Our work shows that endotoxin can damage the organism in two directions:

1. Endotoxin has a non-specific primary toxicity which relates to endotoxin itself. We suggest the term „endotoxin susceptibility" to denote the mode of reaction of the organism in response to this property.

2. Endotoxin is also an antigen (allergen). It is proposed to denote the reactivity of the organism to this antigenic property „endotoxin hypersensitivity". This condition includes all those features of hypersensitivity which occur in all early, anaphylactic and delayed hypersensitivity reactions. The main purpose of this monograph is to prove the existence of endotoxin hypersensitivity. In addition, two connected problems will be dealt with:

a) The characteristic vascular effects of endotoxin which are due in most instances to endotoxin susceptibility together with endotoxin hypersensitivity. Therefore, the vascular effects of endotoxin will be discussed from a dual point of view in a separate chapter.

b) Finally endotoxin resistance will be discussed as an altered state of the organism to endotoxin. Endotoxin resistance is the absence of endotoxin susceptibility and of the anaphylactic and early part of endotoxin hypersensitivity. Whether resistance or desensitization develop to the delayed component of endotoxin hypersensitivity is questionable. Because of the role of endotoxin and endotoxin resistance in various disorders and in therapy this problem will also be discussed in this chapter.

We assume that all vertebrates living together with endotoxin-producing microorganisms have a natural endotoxin hypersensitivity. During life this hypersensitivity will be enhanced owing to the acquired endotoxin hypersensitivity. Nevertheless, it is difficult to distinguish clearly the manifestations of hypersensitivity because these are always mixed with those of susceptibility e. g. with massive haemorrhages and fever.

This particular type of hypersensitivity, due probably to the bacterial symbiosis, may be considered to have arisen over innumerable generations and is thus not analogous to the usual types of hypersensitivity reactions.

Although the two endotoxin reactions of the organism, i. e. susceptibility and hypersensitivity run parallel and influence each other's features, their separation is feasible.

It should be noted that not all the effects of endotoxin are deleterious. For example, hyperglycaemia and hormone release must be considered at least to a certain degree rather as beneficial than harmful effects. Very small doses of endotoxin are also nontoxic and if given repeatedly they induce resitance to endotoxin and to some infections. Small doses of endotoxin can potentiate the specific antibody formation to protein antigens.

Because of the complexity of endotoxin effects and the close connections between them, some repetition is inevitable in the text.

Finally, it should be mentioned that it is not the aim of this book to deal with the chemistry of endotoxin. The reader interested in these problems is referred to special reviews.

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The author is indebted for his encouragement throughout this work to the late professor M. JANCSÓ and for their medical advice to Dr. A. KOVÁCH and to Dr. R. BACKHAUSZ. He would also like to express his gratitude to Dr. P. VÉGH for his collaboration in the experimental work and to Mrs. I. BERTA and Miss M. DOBÓ for their technical assistance.

Dr. J. H. HUMPHREY (Head of Immunological Division of National Institute for Medical Research, Mill Hill, London) supervised and corrected this monograph. I am greatly indebted for his careful and generous help.

Szeged, 1966.

T. G. Kováts

DEFINITIONS

Endotoxin. Although endotoxin is a part of the bacterial cell wall and easily excreted into the media the original denotation endotoxin is used most extensively in the literature. Synonyms are: bacterial pyrogen, lipopolysaccharide, Shwartzman active toxin, tumour necrotizing toxin, Boivin toxin.

Somatic O antigens are the part of the endotoxin complex which represents the main part of the cell wall of Gram-negative enterobacteria. The whole somatic antigen contains protein, lipid and polysaccharides — the latter being responsible for the antigenic specificity. The lipopolysaccharide part of the endotoxin complex may be extracted without the protein. Its molecular weight is about 10^6. By hydrolytic treatment it may be degraded to about $2x10^5$ molecular weight without losing its toxicity.

Endotoxin susceptibility means a state of the organism apparent during the nonspecific injurious effects of endotoxin manifested predominantly by the adrenergic (sympathetic) nervous features. Primary toxicity of endotoxin is the toxic property of endotoxin. Its equivalent is endotoxin susceptibility on the part of the organism.

Independently of their origin all kinds of endotoxin produce almost identical toxic effects, but some bacterial exotoxins and other agents also produce somewhat similar effects. Hence, these primary toxic effects are nonspecific. (Exotoxins may also exert specific neurotoxic effects on some cerebrospinal nerves.)

Susceptibility or primary toxicity may be assayed by the intensity of the reactions observed after endotoxin injections. In general these reactions run parallel to the dose.

Endotoxin hypersensitivity is due to the antigenic (allergenic) nature of endotoxin. To a limited degree, it shows immunological specificity. It involves similar features to those found in the anaphylactic, early and delayed hypersensitivity reactions. This type of hypersensitivity is not strictly analogous to other allergic manifestations because of its special characteristics and its parallelism with the features of susceptibility, or primary toxicity. It resembles bacterial hypersensitivity in some respects. Whether toxic and antigenic characters of endotoxin depend on different or identical chemical groups is uncertain.

Endotoxin reactivity. Because of the occasional use of this term in the literature, it should be defined. Endotoxin reactivity is the normal response of the organism to endotoxin in general. If one wants

to define the state of susceptibility and hypersensitivity together the term endotoxin reactivity seems adequate.

Endotoxin hyperreactivity. It is proposed to use this denotation when one wants to express the increase is both susceptibility and hypersensitivity to endotoxin simultaneously. Marked changes in this state may be observed, e. g. during infections and after vaccine treatment.

Endotoxin resistance is the reduction or abolition of toxic and anaphylactic *plus* early hypersensitivity effects after the repeated injection of amall doses of endotoxin.

Instead of „resistance" the term „tolerance" is commonly used in the literature. In our view, however, tolerance is rather a state caused by substances which act exclusively in a pharmacological manner.

The term „endotoxin desensitization" is also inadequate because desensitization relates only to the anaphylactic and early parts of endotoxin hypersensitivity. Desensitization in respect of the delayed part of endotoxin hypersensitivity is questionable and the difficulty of producing it will be discussed. The term „endotoxin immunity" is also unfortunate. In resistance there is never complete protection against endotoxin, on the other hand, endotoxin immunity should be related to O-antigen and antibacterial immunity.

Cellular targets of endotoxin. Vascular endothelial cells, leucocytes, mastocytes, macrophages and other RES-cells are damaged by larger doses of endotoxin. The consequence of this damage is the destruction of cell membranes and the release of vasoactive agents, endogenous pyrogen, lysosomal enzymes and other unknown mediators of endotoxin-caused injury. The cellular targets include also the platelets. It is assumed that this type of attack is an immunological one and is exerted by the endotoxin as an antigen on the naturally sensitized cells.

Primary toxic target of endotoxin. Minute doses of endotoxin can produce toxic changes similar to those produced by the stimulation of the adrenergic (sympathetic) nerves. All the primary toxic actions of endotoxin can be explained through its action on the adrenergic nerves.

Therefore, some structure in the autonomic nervous system is assumed to be the primary toxic target of endotoxin.

I. ENDOTOXIN SUSCEPTIBILITY

(or primary toxicity)

Summary

The primary toxic action of endotoxin seems to be manifested by the autonomic, mainly the adrenergic, nervous system. The consequence to the host is the state of: „endotoxin susceptibility".

1. The primary toxic effect of endotoxin is nonspecific.

2. Small doses of endotoxin — especially if given directly into the central or peripheral nervous areas — can evoke adrenergic-like symptoms, e. g. corticotrophic hormone release, fever, hyperglycaemia.

3. The effect of endotoxin on reticular formation is evident by changes of behaviour and by the fact that endotoxin effects can be reduced by drugs acting through the reticular formation.

4. At the beginning of endotoxin action, the vasoconstriction of small vessels is considered to be an epinephrine-like effect.

5. The primary toxic targets of endotoxin action are the various areas of the central and peripheral autonomic nervous system. Endotoxin may exert its toxicity both in the presence or absence of the central nervous system; after destruction of the spinal cord; after cutting some nervous pathways; or even in isolated organs.

6. Some effects similar to those of endotoxin can be produced by the mediator of the adrenergic system, norepinephrine, and can be inhibited by its antagonists (especially if given locally). Endotoxin-like effects can also be produced by epinephrine (liberated from the adrenal medulla and also from some adrenergic areas of the brain).

7. The distinction of the pure primary toxic effect of endotoxin is mainly theoretical, because the toxic effects of endotoxin run parallel with endotoxin hypersensitivity.

Effects on the autonomic nervous system in general

Both central and peripheral parts of the autonomic nervous system seem to be primarily affected by endotoxin. Since the effects produced by exotoxins can also be produced by other agents they may be regarded as nonspecific.

Because of the anatomical and physiological unity of the autonomic nervous sysetem these effects (epinephrine-like effects) cannot be separated in the intact animal. Of course, in isolated organs or after the transection of some nervous pathways these effects of endotoxin

may also be observed on some peripheral nervous areas. The participation of the cerebrospinal nervous pathways in the endotoxin effects is not proved.

Experimental observations show that the autonomic areas (centers) of the central nervous system have a particularly marked sensitivity to endotoxin because the symptoms occurring after endotoxin injection are of autonomic nervous character and can be produced by minute amounts of endotoxin given directly into the CNS. Hence, ia may be concluded that one of the main targets of endotoxin is the central nervous system, especially the brain. In this relation, most authors describe an affect on the central nervous system without emphasizing the autonomic areas as a particular target.

Concerning the participation of the central nervous system in the effect of endotoxin ECKMAN, KING and BRUNSON (1958) have carried out interesting experiments. They have demonstrated that endotoxin enhances the permeability of the cerebral vessels in contrast to that of other vessels and renders them permeable to colloidal dyes and even endotoxin.

BENNETT, PETERSDORF and KEENE (1957) have shown the onset of the toxic effect (fever) after intrathecal administration of endotoxin, and stated that a 1000 to 4000 fold greater amount of endotoxin is required to produce the same effect if given intravenously (see also the concluding remarks).

WEIL, MACLEAN, SPINK and VISSCHER (1956) found a prompt arterial hypotension after endotoxin injection also in decapitated animals or in animal (dog) whose spinal cord was destroyed. Thus, the above authors claim that endotoxin has no primary effect on the central nervous system and the action of endotoxin on it is only secondary following the onset of the shock.

EGDAHL's experiments (1959) showed the role of the central nervous system and the peripheral autonomous nervous system, too, in the effects of endotoxin. EGDAHL found that small doses of coli endotoxin (10 μg) given to dogs caused a striking adrenal-cortical stimulation (17—corticosteroid release) and fever, probably by affecting the pituitary-adrenal axis and the thermoregulatory areas in the brain. Larger doses of endotoxin (200 μg) caused both adrenal-cortical and adrenal-medullary stimulation (catecholamine release). (The adrenal medulla may be regarded as a peripheral part of the sympathetic nervous system.) Transection of the spinal cord at C—7 abolished the adrenal-medullary response, but fever and hypotensive and adreno-cortical responses remained unimpaired. (These responses are dependent on adrenergic nerves.) Thus, it may be assumed that the stimulation of the adrenal medulla by the endotoxin is not a direct effect upon the adrenal medulla, but is dependent on the descending nervous pathways in the spinal cord. However, the febrile, hypotensive and adrenal-cortical stimulating effects of endotoxin — the two latter may derive from its action on peripheral targets, too — may also derive from a direct action on the central nervous system.

According to the investigations of PLANELLES (1959) bacterial toxins exert their effect both through the central and peripheral parts of the

nervous system. PLANELLES (1959) suggests that toxins act by a local histamine release. Histamine exerts its effect partly at the periphery of the sympathetic nervous system and partly through the central nervous system. The result is a corticotrophic hormone release from the pituitary that finally stimulates the adrenal cortex to produce cortisone.

PORTER and KASS (1965) examined the role of the central nervous system in the effect of endotoxin. In stereotactic ablation experiments in rats they found that lesions in the anterior hypothalamus caused enhancement of the sensitivity to endotoxin, i. e. hyperthermia and reduced resistance to the lethal action of endotoxin. However, similar lesions in the posterior hypothalamus caused no significant changes in temperature, but resulted in a marked reduction of lethality to endotoxin. Therefore, they assume that the posterior hypothalamus has a prominent role in the endotoxin effect. (The possible role of the autonomic nervous aystem in relation to the thermoregulation will be discussed in the chapter I/b.)

The role of the nervous system in the (primary) toxic effect of endotoxin was suggested by the experiment of RASKOVÁ (1958) and her collaborators. (According to the symptoms these effects must be considered to be autonomic nerve effects!) They carried out experiments in rabbits and cats in which various areas i. e. carotid sinus, mesenteric area and rabbit ear were humorally isolated from other parts of the body but the nervous connections were left intact. They injected $0{,}5 \times 10^{-2}$ ml Boivin extraction of typhoid endotoxin into the peripheral artery of the area and observed immediate reflex-like respiratory and blood pressure changes with sudden death. „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." Pressor responses following the sciatic nerve-stimulation in rabbits were inverted or ceased after intravenous endotoxin administration. Analogous pressor reflexes induced by distension of the urinary bladder or the rectum, were not affected.

During *Salmonella* and *Shigella* infections the plasma cholinesterase activity was diminished in the early phases, especially in severe cases (ADO, 1952; KUMATE, BENAVIDES, PÉREZ, CRIOLLOS and CARRILO, 1956). According to RASKOVÁ and VANECEK (1964) the increase in acetylcholine response observed after repeated toxic reactions and also in the pressor response of intact rabbits can be explained on the basis of the experiments cited above.

KOVÁTS, BÁLINT and VÉGH (1964) showed that the acetylcholine reactivity of dogs was strongly reduced 10—15 minutes after 2 mg/kg purified typhoid endotoxin given intravenously. Therefore, it was concluded that blockade of the cholinergic mechanism also plays a role in the effect of endotoxin (causing sympathetic prevalence) at least in the first hour.

The peripheral effect of endotoxin on the autonomic nervous system is suggested by the investigations of REILLY (cited by THOMAS, 1954). This author has shown that injection of small doses of endotoxin in the immediate vicinity of the splanchnic nerve resulted in haemorrhage and necrosis in the stomach and intestines followed by shock and death. Endotoxin given even in larger amounts was ineffective if injected by

the intracardiac or by other routes. This experiment points to the direct action of endotoxin upon the splanchnic nerve. (Unfortunately this important result has not been confirmed and/or was not further investigated.)

According to GOURZIS, HOLLENBERG and NICKERSON (1961) endotoxin intensely potentiates the *in vitro* reaction of the isolated aorta strip to epinephrine.

According to THOMAS (1959): „It might be postulated, that the systemic effect of endotoxin is either to increase the reactivity of terminal vessels to epinephrine or to increase the amount of epinephrine released by adrenal medulla or liberated in the peripheral tissues."

HINSHAW, BRAKE, EMERSON, JORDAN and MASUCCI (1964) have claimed that splanchnic denervation and adrenalectomy did not inhibit the symptoms of the most severe endotoxin effect; the endotoxin shock in dogs. „No evidence was obtained assigning detrimental roles to the sympatho-adrenal system in this form of shock". On the contrary FINE (1964) stated that the denervation of splanchnic viscera of dogs and rabbits prevented the damage of these tissues after the injection of massive endotoxin doses. (The possible explanation of the different results and the criticism of the above two experiments will be dealt with in the concluding remarks and also in chapter II/3.) FISHEL, SZENTIVÁNYI and TALMAGE (1964) suggest that endotoxin acts through beta-receptor blockade, thus promoting the action of epinephrine sensitive alpha receptors of the autonomic nerves (see also below!).

French authors (e. g. DELAUNAY, LEBRUN, DELAUNAY and FOUQUIER, 1949) reported that endotoxin injection causes intense and prolonged vasoconstriction in the first 5—15 minutes. This constriction changes to vasodilatation within several minutes. The authors suggested. that because of the resemblance to epinephrine-caused vasoconstriction, ischaemia and anoxia, this endotoxin effect is mediated by the adrenergic nerves. (This problem will be dealt with in detail in the vascular effects of endotoxin, see chapter II/I.)

We shall see later that many authors suggest the vascular effect of endotoxin to be a pure adrenergic nerve-dependent effect. Considering that the vascular effect of endotoxin involves features of hypersensitivity simultaneously with an adrenergic effect, the vascular effects of endotoxin wil be discussed in a separate chapter.

Within the central nervous system there are some anatomically and physiologically circumscribed areas on which the effect of endotoxin is striking. The effect on the reticular formation, the thermoregulatory areas, the pituitary, and the hyperglycaemic effect will therefore be discussed separately. These effects seem also to be of autonomic nervous character. Thus, their separate classification is rather for the purpose of simplification and does not imply essential differences in the mode of action of endotoxin on the autonomic nervous areas. The reticular formation contains many autonomic areas. The known connection of thermoregulatory areas, the pituitary and also hyperglycaemia with the autonomic nervous mechanism will also be discussed.

1. Effect on the reticular formation

The participation of the reticular formation in the effects of endotoxin may be deduced from the experiment of STERC (Cited by RASKOVÁ, 1958) who found that larger doses of endotoxin caused general behavioural changes but inhibited conditioned reflexes. This effect of endotoxin is nonspecific, besause the same effects were produced by exotoxins.

The correlation between the reticular formation, behaviour and conditioned reflexes is well known. Injuries to the reticular formation cause mental disorders. In relation to the endotoxin effect in man WEIL (1961) may be quoted: „frequently the onset of shock is first recognised by nursing staff because mental confusion sets in and the patient becomes irrational."

There is indirect evidence for the role of reticular formation in the endotoxin effect. These experiments were carried out with chlorpromazine, which acts mainly through the reticular formation. Endotoxin seems to cause an adrenergic prevalence (see below, too).

ABERNATHY, HALBERG and SPINK (1957) have shown protection of mice against endotoxin by chlorpromazine. These authors discuss the possibility that the target of both endotoxin and chlorpromazine is the reticular formation in the central nervous system, although the drug exerts effects on other areas, and on the periphery, too. „It was shown that protection against endotoxin with chlorpromazine is not critically dependent on the adrenals or sedative, adrenolytic, antihistaminic and hypothermic effects of the drug."

NOYES, SANFORD and NELSON (1956), in their experiments were able to inhibit the toxic effects of endotoxin with chlorpromazine.

LILLEHEI and MACLEAN (1959) could modify the course of haemodynamic changes caused by endotoxin with chlorpromazine treatment.

KOVÁTS, BÁLINT and VÉGH (unpublished results) repeated the above two experiments and concluded that chlorpromazine — although to a lesser extent than stated by the above authors — inhibited the toxic effects in endotoxin shock.

2. Effect on the thermoregulatory areas

The complex mechanism of thermoregulation should first be outlined. Regulation of body temperature is maintained by two centers. The cooling center is located in the anterior hypothalamus. It is heat-susceptible, its adequate stimulus is the temperature of the circulating blood. This center protects the body against hyperpyrexia, because if the blood is overheated this center is excited and sends impulses *via* efferent nervous pathways. These impulses 1) cause vasodilatation in the skin, 2) initiate the secretion of the sweat glands, 3) reduce the metabolism. Thus, by the enhancement of heat loss and the inhibition of heat production the body is cooled by the action of this center. (The above mechanisms seem to be cholinergically mediated.)

The heating center in the posterior hypothalamus protects the body against cooling. Not being heat-susceptible it is not excited by decreased temperature of the blood, but by impulses from the cold receptors of the skin and by pyrogenic materials such as endotoxin. Excitation of this center causes via sympathetic pathways 1) vasoconstriction of the skin; 2) inhibition of sweat gland secretion, 3) enhancement of heat production by shivering, 4) enhancement of metabolism.

There is no doubt about the participation of the adrenergic nervous system in the above four mechanisms. In relation to endotoxin-fever the adrenergic mechanism was investigated by FAVORITE and MORGAN (1942); WELLS and RALL (1948); BOQUET and IZARD (1950). Endotoxin fever may be inhibited by adrenergic blocking agents.

Let us now consider the course of endotoxin fever. Endotoxin fever was mostly investigated in rabbits and dogs. A biphasic fever response begins 10—15 minutes after endotoxin injection (e. g. PETERSDORF and BENNETT, 1957; WOOD, 1958 and 1958/a). The first fever period lasts about one hour. After 30—60 minutes a second febrile period follows which persists about 4—8 hours.

BEESON (1947) demonstrated that repeated injections of small doses of endotoxin result in a partial resistance in rabbits. In view of the fact that this resistance relates always to the second fever period and not to the first, BENNETT and CLUFF (1957) and WOOD (1958) suggested that the first period of fever is based on the direct effect of endotoxin on the thermoregulatory areas. The second fever period begins when endotoxin has practically disappeared from the circulation, and is explained by the fact that endotoxin has a damaging effect on leucocytes, which release endogenous pyrogens (also *in vitro:* DAVIS, MEEKER and MCQUARRIE, 1960). Endogenous pyrogen is thermolabile and acts upon the heating center rapidly, without any incubation period, in contrast to endotoxin (BENNETT and CLUFF, 1957; WOOD, 1958.). Although the biphasic fever response is considered by most authors to be one of the most characteristic effects of endotoxin, this type of fever may be observed in various allergic responses, such as to tuberculin and to viruses (e. g. STETSON, 1955; GÖING, 1960; MOSES and ATKINS, 1961. According to SNELL and ATKINS (1965) endogenous pyrogen is widely distributed in different tissues.

DZEKSEMBAYEV (1961) (cited by PLANELLES and BUDNITZKAYA) explains the pyrogenic effect of endotoxin in rabbits by diminution of heat loss rather than enhancement of heat production. He found that the basal metabolic rate was increased by an endotoxin preparation denoted Pirogenal. After thyroidectomy the fever caused by Pirogenal decreased by 50 per cent. After transection of the splanchnic nerve the fever and the basal metabolic increase were undiminished. He was able to detect endogenous pyrogen more frequently in normal than in thyroidectomized animals.

In this respect, PORTER and KASS's (1965) experiments (see above) should be recalled here. If the heat-susceptible cooling center was destroyed the rats responded to endotoxin with hyperpyrexia, accompanied by vasoconstriction of the skin vessels and enhancement of the basal metabolism. That is to say, after the destruction of the cooling

center, the action of the heating center prevailed. It is interesting that a similar lesion made in the site of the heating center caused no significant changes in body temperature. This would mean that some kind of thermoregulation may also be maintained by the cooling center alone.

3. Effect on the pituitary

It was observed that endotoxin releases hormones from the anterior pituitary. Adrenocorticotrophic hormone (ACTH) — which induces cortisone secretion from the adrenal cortex — was especially examined in this respect. It is known, however, that epinephrine releases ACTH from the pituitary. Various nonspecific stimuli or agents may induce ACTH release and it is not decided whether epinephrine could be the final common pathway. We suggest that an adrenergic-like mechanism triggers the endotoxin-induced adrenocorticotraphic hormone-release. A direct action of endotoxin on the pituitary is also possible. Neither of the two possibilities has been proved experimentally, but the former accords with the autonomic neurotoxicity hypothesis of endotoxin action. Trophic hormone release is considered here as a part of a nonspecific defense mechanism.

It was shown by LONG and FRY (1945) that epinephrine reduced the concentration of ascorbic acid and cholesterol in the adrenal glands, which changes have been attributed to the release of adrenocorticotropin (ACTH) from the pituitary (see e. g. PICKFORD and VOGT, 1951). MCCANN and BROBECK (1954) produced hypothalamic lesions which prevented the pituitary-adrenal stimulation by epinephrine. The corticotropin-releasing factor (CRF) of the hypothalamus (SAFRAN, SCHALLY and BENFEY, 1955; FORTIER, 1961) is the humoral mediator between the nervous system and anterior pituitary.

There are some data that support the above hypothesis. Adrenergic blocking agents diminish the ascorbic acid depleting effect of epinephrine (e. g. RONZONI and REICHLIN, 1950; SIDERIUS and GAARENSTROOM, 1952; and OHLER and SEVY, 1953). Whether this effect is due to a direct or indirect action of epinephrine is still uncertain.

WEXLER, DOLGIN and TRYCZYNSKI (1957) stated that a bacterial polysaccharide 'Piromen' (1—5 μg) stimulates the pituitary, resulting in an enhanced release of ACTH and other trophic hormones, e. g. STH. Simultaneously with this adrenal-cortical stimulation of corticoid secretion there occurs a decrease of Vitamin C and cholesterol. PFEFFER and STAUDINGER (cited by GÖING, 1960) have shown that corticosteroid release is augmented by pyrogens.

The effect of endotoxin upon fhe endocrine system is nonspecific and may be considered as a defence mechanism of the organism (e. g. endotoxin resistance, see chapter IV/4). It is deleterious only in the case of massive endotoxin doses which cause disturbances in the endocrine homeostasis.

The haematologic changes (leucopenia, leucocytosis, thrombocytopenia see chapter III/1) may be partly explained by the adrenal-cortical

stimulation caused by endotoxin. These changes are produced not only by endotoxin, but, for example, by glycogen administration and various allergic reactions, Because of the connection of haematologic changes with hypersensitivity this problem will be discussed in chapter III/1.

4. Hyperglycaemic effect of endotoxin

Blood sugar increases 1—2 hours after the endotoxin injection and hyperglycaemia persists for several hours. If large endotoxin doses are injected the hyperglycaemia is followed by an intensive hypoglycaemia. Liver glycogen decreases simultaneously with hyperglycaemia.

Hyperglycaemia is the consequence primarily of the epinephrine output. Cortisone also potentiates epinephrine hyperglycaemia, as stated by WINTERNITZ and LONG (1952).

According to FÓRIS and KERTAI (1965) hyperglycaemia due to endotoxin results from the activation of glucagon.

More detailed data concerning the endotoxin-dependent changes in carbohydrate metabolism may be found in the review of BURROWS (1951) or THOMAS (1954) and BENNETT and CLUFF (1957).

Concluding remarks

Our first question is: what is the target of the primary toxic action of endotoxin?

Since minute amounts of endotoxin induce toxic features if administred directly into the autonomic nervous structures, we may suppose that endotoxin exerts its toxic effects upon this system. (There are also cellular targets of the action of endotoxin, see chapter IV/4.)

Our second question is: which part of the complex autonomic nervous system is involved in this action of endotoxin?

We may approach the question better by taking into consideration the different routes of administration of endotoxin.

1. After intravenous, intramuscular, subcutaneous or intracutaneous administration, a great portion of endotoxin is cleared by cells and humoral agents and only a small amount remains to reach the central or peripheral parts of the autonomic nervous system. Using the above routes of administration, relatively large doses of endotoxin are required to evoke characteristic toxic symptoms.

2. By intrathecal or intracerebral administration the central nervous system (the brain) must be reached and affected first. Similarly, the peripheral part of the adrenergic nervous system is first affected if the endotoxin is administered directly into it. Using the direct nervous route of administration, minute amounts of endotoxin are required to evoke symptoms. Since the symptoms are of autonomic nervous character we suggest that, independently of the route of administration, the primary targets of the toxic effect of endotoxin are the autonomic areas of the central and peripheral nervous system.

Because of the many connections between the different nervous areas, an overall action of endotoxin will easily spread throughout the whole autonomic nervous system. Therefore, any discussion of an effect of endotoxin in the intact animal must take into account the fact that such effects cannot be isolated either anatomically, or physiologically. Nevertheless by cutting some nervous pathways, destroying some nervous branches and areas, or using isolated organs the direct effect of endotoxin upon some isolated nerves can also be demonstrated.

In the light of the above, some of the experiments should be rediscussed.

In Reilly's experiment it is difficult to see the (direct) effect of endotoxin upon the splanchnic nerve. Reilly injected the endotoxin in the immediate vicinity of this nerve. Most catecholamine secretion occurs at the nerve-endings. There is only a very limited amount of catecholamine in the nerve itself, which cannot be responsible for the endotoxin effect. The effect of endotoxin could only be explained if endotoxin exerted a direct effect on the nerves, or some other mechanism were involved, and further work is needed.

BENNETT, PETERSDORF, and KEENE's (1957) experiments showed that minute amounts of intrathecally administered endotoxin evoked fever. However, the use of this route of administration did not result in any enhancement of susceptibility of the thermoregulatory center; this experiment shows only that this center has an extraordinary susceptibility to endotoxin. It is probable that a similar minute amount of endotoxin reaches this center to excite it if a larger dose is given by other routes.

PORTER and KASS (1965) suggest (see their experiment deseribed above) that the posterior hypothalamus is not the only target of endotoxin activity (peripheral loci may also be targets), but because of its extraordinary sensitivity it heads the list. This high sensitivity of hypothalamus can be explained by its excellent blood-supply and circulation on the one hand, and the lack of a blood-brain barrier on the other. On the basis of Porter and Kass's experiment it seems worthwhile to search for a connection between the thermoregulatory areas and the primary toxicity of endotoxin. Although the toxic effects do not always work *via* this mechanism, there is a possibility that it may play a leading part in initiating the symptoms when the thermoregulatory areas are reached first by endotoxin.

SERAFIMOV (1962) has examined the urinary exeretion of epinephrine and norepinephrine during the treatment of rabbits with 1,5—4 μg Abortus equi endotoxin (Pyrexal). The output of epinephirine was greatly increased compared with the control period. Norepinephrine excretion was only slightly increased. Splanchnectomy prevented the increase, but not the rise in temperature following the endotoxin administration. Therefore, it is concluded that epinephrine release is due to the stimulation of the adrenal medulla *via* the splanchnic nerve, but the increase of body temperature is independent of the adrenal medulla and is probably due to stimulation of the sympathetic part of the central thermostatic regulation.

On the above basis one may assume that the adrenergic effect of endotoxin may also be exerted by the thermoregulatory centers. In any

case this possibility affords further evidence that there is a connection between the central nervous system and the peripheral part of the autonomic nervous system in the endotoxin effect, and that an epinephrine-like agent may initiate the complicated sequence of events comprising toxicity of endotoxin. Furthermore, this concept implies that the thermoregulatory areas are some kind of autonomic centres.

WEIL, MACLEAN, SPINK and VISSCHER (1956) have shown that endotoxin also exerts its action in dogs deprived of the central nervous system. Contrary to their opinion, their experiment does not exclude the role of the central nervous system in the endotoxin action in the intact animal. But from their experiment with decapitated animals whose spinal cord was destroyed it may be concluded that the toxic effect of endotoxin (arterial hypotension) can also be evoked by the action of endotoxin on the peripheral part of the autonomic nervous system without the participation of the central nervous system.

In EGDAHL's (1959) experiments after section of the spinal cord the hypotensive response remained unimpaired. This implies that in hypotension a mechanism is involved in which the adrenal medulla plays no special part.

Egdahl refers to some experiments in which it was found that there are centers (autonomic areas) in the cerebral cortex and hypothalamus the stimulation of which increases catecholamine secretion by the adrenals of the cat.

Considering the role of the reticular formation in the endotoxin effect, direct evidence for its involvement comes from the changes caused by endotoxin in behaviour and in conditioned reflexes. Indirect evidence is provided by the protective effect of chlorpromazine (which mainly acts through the reticular formation.)

To explain the action of endotoxin on the reticular formation and to advance a hypothesis for endotoxin action let us consider the mode of action of chlorpromazine. This drug acts mainly through depression of the activity of the reticular formation. The role of some mediators (neurohumors) is also suggested by recent work on the mode of action of chlorpromazine and other tranquilizers (see e. g. PROCTOR, RIDLON, FUDEMA and PRABHU, 1964). The tranquilizer activity may be at least in part due to depression of the adrenergic-serotonergic activity, i. e. either by blockade, or by reduction of the neurohumor level of the central nervous system with a cholinergic prevalence. (The serotonergic activity of endotoxin will be discussed in the following chapter.) It may well be that endotoxin stimulates the adrenergic-serotonergic areas and blocks the cholinergic activity in the central nervous system. This possibility is not incompatible with the endotoxin effect in other areas, e. g. the peripheral part of the organism. It must be noted that chlorpromazine acts peripherally as a cholinergolytic agent. Endotoxin by contrast causes cholinergic stimulation at some peripheral sites, e. g. salivation, vomiting and increased peristalsis (the latter is partly due to histamine). Chlorpromazine also has some antihistaminic activity.

Probably the role of the reticular formation in the effect of endotoxin could be examined more adequately by EEG examinations and by more precise determinations of catecholamines and acethylcholine

in this region. Larger doses of endotoxin arrest the bioelectric activity of the brain (KOVÁCH, 1965). This effect precedes the development of general haemodynamic changes. The same effect could be produced by intracerebral, intracarotic and intravenous administration of endotoxin.

It is known that small doses of endotoxin may cause abortion in pregnant animals. In the experiment of PARANT and CHEDID (1964) protection against this type of abortion was provided by chlorpromazine in mice. The authors could not demonstrate any labelled endotoxin crossing the placental barrier, and suggested that endotoxin acts by the release of serotonin which causes uterine contraction *in vitro* and *in vivo* and also causes abortion. Histamine plays no role in the above effect. Chlorpromazine protects against abortion by its antiserotonin property. Abortion could not be inhibited by administration of cortisone.

The effect of endotoxin on the central or peripheral part of the autonomic nervous system is usually denoted in the literature as „central nervous effect" or „adrenergic effect". Of course, the adrenergic (sympathetic) features are dominant. However, on the basis of pharmacodynamic principles these effects cannot be separated from the cholinergic nervous mechanism which must be reduced or blocked, as was shown by KOVÁTS, BÁLINT and VÉGH (1964) (at least in the first hour). Thus, endotoxin seems to upset the adrenergic-cholinergic balance.

The role of the autonomic nervous system in the action of endotoxin is supported by the observations showing the participation in it of catecholamines (epinephrine-like substances). Blood sugar increases and liver glycogen falls due to the action both of epinephrine and of endotoxin, and both can be prevented by dihydroergotamine, as shown by BOQUET and IZARD (cited by THOMAS, 1954). The levels of lactic and pyruvic acid similarly increase after injection of endotoxin or epinephrine. A further resemblance is found in the leucocytosis and the interference with diapedesis of leucocytes in inflamed tissues. Both substances can cause petechial haemorrhages and necrosis in the stomach and gastrointestinal tract, mesenteric lymph nodes and liver (PENNER and BERNHEIM, 1942; BOQET and LEHOULT, 1948). Because such changes are connected with vascular effects and hypersensitivity these alterations will be discussed in more details in the following chapters.

Some experiments denying or supporting the role of the autonomic (sympathetic) nerves in the endotoxin effect will be discussed below.

HINSHAW, BRAKE, EMERSON, JORDAN and MASUCCI (1964) denied the role of the sympathoadrenal system in the endotoxin effect because these effects also appeared after splanchnic denervation and adrenalectomy in their experiment carried out in dogs. FINE (1964), however, has obtained opposite results. Splanchnic denervation prevented the toxic manifestations of endotoxin. The explanation of these contrary results is difficult. Perhaps the marked seasonal sensitivity changes of animals (see chapter IV/2) may be responsible.

It may be concluded from EGDAHL's (1959) experiment that endotoxin may exert a dual effect on the autonomic nervous system 1. adrenalcortical stimulation of endotoxin induced directly by the humoral mediators of the centers which are more sensitive to endotoxin 2. adrenal medullary (i. e. peripheral) stimulation to release catecholamines by

descending nerve pathways, which can be elicited only with 20 fold greater quantity of endotoxin

To be able to identify the primary targets of endotoxin action we have to summarize the kinds of catecholamines and the loci where they are synthetized and released. This problem was recently reviewed by WURTMAN (1965). Catecholamines include 1. norepinephrine, 2. epinephrine and 3. dopamine. Norepinephrine is primarily a neurohumor (mediator) and also a hormone. It is synthetized in the sympathetic nerve-endings, brain, and chromaffin tissue (mostly in the adrenal medulla) from its immediate precursor dopamine. Epinephrine is primarily a hormone. It is synthetized and released mainly from the adrenal medulla, which contains much more epinephrine than norepinephrine. The chromaffin cells of the peripheral tissues also secrete epinephrine

The catecholamines influence a variety of targets, such as the vascular smooth muscle, adipose tissue, liver, and myocardium. (All these targets are influenced also by endotoxin.) Although there is a continous release of catecholamines from the adrenal medulla there is evidence that a large quantity is secreted in „spurts", or on the action of insulin and histamine. Only norepinephrine is secreted on the stimulation of sympathetic-nerve endings. (Reflex stimulation of the adrenal medulla results in both norepinephrine and epinephrine output. Insulin selectively releases epinephrine.)

Most of the norepinephrine in the blood originates in sympathetic-nerve endings, most of the epinephrine is released by the adrenal medulla as a result of sympathoadrenal activation. The circulating levels of both vary strikingly after certain stimuli (e. g. hypotension and hypoglycaemia).

In the light of the physiological role of catecholamines and of sympathoadrenal (adrenergic) activation it is easy to indicate the mode and site of action of endotoxin. We recall again that the major part of epinephrine derives from the adrenal medulla, but that endotoxin sensitivity does not decrease after adrenalectomy. This means that the most important catecholamine playing a role in the endotoxin effect is norepinephrine, secreted mostly from the sympathetic-nerve endings. The third catecholamine, the dopamine has not been examined in relation to endotoxin effect.

II. VASCULAR EFFECTS OF ENDOTOXIN

Summary

The vasomotor disturbances and haemorrhagic necrotic effects mainly involve the adrenergic actions and damaging effects of endotoxin on vascular endothelium and the hypersensitivity mechanism seems to be of minor importance. However, in the endotoxin caused haemodynamic changes all three mechanisms may have equal importance. Because of the particular characteristics of these effects they are discussed in this separate chapter.

The complex picture of vascular effects of endotoxin — according to our present knowledge — may be outlined as follows:

1) Two types of vascular effect should be distinguished, a) the direct effect on small peripheral vessels and b) general haemodynamic changes involving large vessels. The causes of these alterations are listed below:

2) It is probable that both primary toxicity, hypersensitivity and damaging action on the vascular endothelium play a part in the vascular effect of endotoxin.

3) The primary toxic and vascular effect of endotoxin is probably mediated through several pharmacologically active agents (e. g. chatecholamines, 5—HT, histamine, vasoactive polypeptides, etc.).

4) These agents may be released at different rates in different species. Thus, the mechanism varies from species to species.

5) The different rates of release, the duration of action, and species differences influence the sites and course of events rather quantitatively.

6) Several agents may be released almost simultaneously. The initiating role of catecholamines is probable since minute amounts of endotoxin are able to produce epinephrine-like effects (toxic effects).

7) A histamine-like effect also may be seen after larger doses of endotoxin and this effect seems to be the most prolonged one, probably because of the slow destruction of this agent, and/or because of the production of induced histamine during the endotoxin action (hypersensitivity effects).

8) There is an interrelationship between the different agents due to their antagonistic or synergistic effect and their ability to influence each other's secretion. Apart from these possibilities they certainly trigger off counter-mechanisms which complicate the symptoms even more.

9) Death in endotoxin shock may derive either from the impairment of the nervous system, or because of the haemodynamic collapse. Considering the close connection between the nervous system and haemo-

dynamic collapse, both of them must participate in the final outcome of endotoxin shock.

10) Intravascular thrombus formation caused by endotoxin may also play a role in the haemodynamic changes.

1. Vasomotor disturbances

The action of endotoxin upon arterioles represents a characteristic effect involving the mechanisms described below. This is the most characteristic action of endotoxin. According to DELAUNAY, LEBRUN and COTEREAU (1947), DELAUNAY, BOQET, LEBRUN, LEHOULT and DELAUNAY (1948), DELAUNAY, LEBRUN, DELAUNAY and FOUQUIER (1949) the earliest effect of endotoxin is constriction of the small vessels. Arteriolar constriction and dilatation, which change within several minutes, take place 5—15 minutes after the endotoxin injection. Alternation of vascular tone continues for several hours. If lethal doses of endotoxin are given constriction is changed to an extremely strong and persisting dilatation. The initial vasoconstriction can be blocked by pretreatment with an antiepinephrine agent: dibenamine, but not by sympathectomy.

THOMAS (1959) states that the vascuar effect of endotoxin is independent of the hypersensitivity mechanism. He claims, especially on the basis of the lethal effect of endotoxin on chick embryos, that these effects are direct toxic effects on the blood vessels. He writes „Nevertheless, it is possible that the final common pathway leading to tissue damage may involve analogous physiologic processes in both circumstances, in view of the similarities that do exist". „It might be postulated, that the systemic effect of endotoxin is either to increase the reactivity of terminal vessels to epinephrine or to increase the amount of epinephrine released by the adrenal medulla or liberated in peripheral tissues". A discussion of the above concept may be found in the concluding remarks.

The possibility of the participation of hypersensitivity mechanism in the vascular effects of endotoxin will be given in the following subchapters. There is, of course, no doubt about the participation of the autonomic nervous mechanism in the vascular effect of endotoxin, There are experiments which show that endotoxin causes changes in peripheral vascular reactivity to epinephrine and norepinephrine. However, there are no data that epinephrine and serotonin *per se* can produce vascular effects identical with those produced by endotoxin.

KATZ (cited by THOMAS, 1959) measured the resistance in the perfused and isolated rabbit ear. Small amount of endotoxin (0,5 μg) perfused in the rabbit ear resulted in 200—850 fold potentiation of the normal epinephrine reaction. The opposite effect, a marked vasodilatation instead of constriction, was obtained with 50—80 fold greater quantity of endotoxin.

ZWEIFACH (1948) has reported similar results in vivo on the rat mesoappendix. Endotoxin potentiated a thousandfold the constrictor response of epinephrine. After a short period of vasoconstriction a large dose of endotoxin caused complete unresponsiveness to massive doses of

epinephrine. The above two results were not, however, confirmed in the experiments of MEYER and BALLIN (1959) and GÖING and MIKA (1960).

There is no evidence that histamine plays any part.

2. Necrotic and haemorrhagic effects

As stated earlier by SHWARTZMAN (1937), later by PENNER and BERNHEIM (1942), and BOQUET, DELAUNAY, LEHOULT and LEBRUN (1948) very large doses of endotoxin injected into animals can produce haemorrhages in the mucosa and submucosa of the stomach and upper gastrointestinal tract, in the abdominal lymph nodes, adrenal cortex and sometimes in the thymus, spleen and liver.

The above authors have claimed that the ability of endotoxin to cause haemorrhages is connected with its toxic effect upon small vessels, i. e. with adrenergic vasoconstriction, ischaemia and anoxia.

Because of the resemblance of this type of haemorrhage to epinephrine-induced haemorrhages and because of the prevention of both types by the same drugs (dibenamine, chlorpromazine, cortisone) it is possible that they are due mainly to the adrenergic mechanism (acting together with the direct vascular-endothel damaging mechanism). Thus, such haemorrhage would be a result of the primary toxicity of endotoxin, and endotoxin hypersensitivity may be of minor importance. (But in other types of endotoxin-caused haemorrhages, i. e. in the Shwartzman reaction, the hypersensitivity mechanism seems rather to be involved as will be shown below.) Furthermore, the experiments of DELAUNAY and his collaborators (1948) show that haemorrhagic lesions resembling those caused by endotoxin cannot only be produced by large endotoxin doses but also by large doses of epinephrine, and that they can be blocked by adrenergic blocking agents.

There are some experiments showing the mutual potentiating effect of epinephrine and endotoxin on vessels which may not be considered as pure adrenergic effects.

THOMAS (1956) has shown that 10 μg epinephrine or norepinephrine given intradermally causes a massive haemorrhagic necrosis in the skin of rabbit which received an intravenous endotoxin dose during the preceding four hours. A similar lesion was produced by intradermal injection of a mixture of endotoxin and epinephrine. THOMAS (1959) explains this phenomenon on the basis of KATZ's and ZWEIFACH's experiment discussed above: „As the arterioles lost their capacity to respond to epinephrine, they gradually became widely dilated, but the venules remained tightly constricted after each application of epinephrine, with the result that large amounts of sluggishly moving blood were entrapped in the engorged capillary bed. At this time, each application of epinephrine was followed by showers of petechiae that appeared within a few minutes throughout the site of application." These events lead to the pooling of blood in dilated capillaries, to anoxia and finally to the rupture of the capillaries and haemorrhagic necrosis. THOMAS supposes that apart from epinephrine, local serotonin release is also involved in the above endotoxin reaction.

FISHEL, SZENTIVÁNYI and TALMAGE (1964) have shown that haemorrhagic lesions, similar to those described by Thomas, can be elicited by the intradermal injection of DCI (dichlorisoproterenol) and intravenous administration of endotoxin. DCI exerts a marked vasoconstriction, since it blocks the beta adrenergic receptors and thus the alpha receptors do not have an antagonizing effect.

It is easy to understand that in the neighbourhood of the haemorrhages necrosis may occur. But necrosis may also occur afer endotoxin injection without haemorrhage, e. g. in the spleen, liver and lymph nodes (MORGAN, 1943 and BUENO, 1947).

The close connection between haemorrhagic necrosis and adrenergic nerves may be assumed from the experiments of REILLY mentioned above. He has stated that the injection of minute doses of endotoxin in the immediate vicinity of the splanchnic nerve resulted in haemorrhage and necrosis in the stomach and intestines, followed by shock and death. If injected by other routes endotoxin, even in larger amounts, was without effect.

In relation to the vascular effect of endotoxin it is interesting to mention the experiment of DIETRICH (1941) who has observed desquamation of endothelial cells together with mixed thrombus formation after giving bacterial filtrate twice within 24 hours. TANAKA, NISHIMURA and YOSHIYUKI (1959) have shown by immunofluorescense technique that the endothelial cells of the arterioles mostly contain endotoxin. Together with the adrenergic mechanism this effect may contribute to the vascular damaging effect of endotoxin. It is rather interesting that the brain and the kidney, the two organs most sensitive to endotoxin, retain the smallest amount of the endotoxin in the arteriolar endothelium.

RUBENSTEIN, LOONS and FINE obtained similar results in 1962 (cited by FINE, 1964) and came to similar conclusions. They identified the intravenously injected endotoxin in the intima, the media, the macrophages, as well as the adventitia of the venules and the arterioles throughout the body, including the central nervous system. They suggest that endotoxin produces a circulatory deficiency by a direct action upon the vascular muscle destroying its reactivity.

Because of the connection of the endothelium damaging effect with hypersensitivity this effect of endotoxin will be discussed in detail in the chapter on hypersensitivity.

Another type of haemorrhagic necrosis caused by endotoxin has already been shown by SHWARTZMAN (1937) (pp. 225) who found that haemorrhagic necrosis may be induced by endotoxin in some tumour-tissues. This effect was extensively investigated by SHEAR (1943) and SHEAR and TURNER (1943), but its mechanism is not yet clear. It may be connected with the primary toxicity of endotoxin.

The very extensive haemorrhagic necrotic lesion of the local and generalized Shwartzman reaction will be discussed in chapter III.

3. Haemodynamic effect and endotoxin shock

The haemodynamic effect of endotoxin may be distinguished from endotoxin shock. Haemodynamic changes may be produced by sublethal doses of endotoxin. *Endotoxin shock* is produced by lethal doses of endotoxin. Some effects may be inverted, e. g. hypothermia instead of fever, hypoglycaemia instead of hyperglycaemia and vasodilatation instead of vasoconstriction. Endotoxin shock, including all the primary toxic and hypersensitivity effects of endotoxin, implies more extensive changes than a purely haemodynamic effect. However, the terms haemodynamic effect and endotoxin shock are commonly used in a similar sense. Most authors consider the haemodynamic effect responsible for the lethal outcome.

Because of the similarity between endotoxin and anaphylactic shock, the possible role of hypersensitivity in endotoxin shock will be discussed separately in chapter III.

Haemodynamic changes are particularly characteristic of sublethal or lethal doses of endotoxin. The experiments of French investigators have initiated a development in this respect (see e. g. DELAUNAY, BOQUET, LEBRUN, LEHOULT and DELAUNAY, 1948). The whole problem is excellently discussed in the review of GILBERT (1960.) Only some characteristic points of this problem will be dealt with here.

In spite of some species differences (GILBERT, 1960) a sudden fall of arterial pressure develops in the first or second minute after injecting endotoxin into all species. The hypotension persists about 2 hours after the injection of sublethal endotoxin doses.

Two types of the haemodynamic effect of endotoxin should be distinguished: 1) The effect on small peripheral vessels and 2) The effect on the large vessels.

In the haemodynamic effect of endotoxin on small vessels it is usually possible to distinguish an early phase — vasoconstriction — and a late phase — vasodilatation in the peripheral arterioles, using medium doses there is a biphasic aortic hypotension. The early profound hypotension may be due to a decreased venous return and cardiac output caused by portal constriction in the dog (the species examined mostly). This is associated with portal hypertension, pooling of blood in the liver and splanchnic areas.

The initial drop in blood pressure tends to return towards normal levels. (This is assumed to be due to the release of pooled blood and catecholamines.) There follows the late phase in which arterial tension falls irreversibly.

It is not entirely correct to denote arterial hypotension as „systemic". There is a rise in pressure in the renal (GILLENWATER, DOOLEY and FROHLICH, 1963) and gastric arteries (JACOBSON, DOOLEY, SCOTT and FROHLICH, 1963) and, initially, in the peripheral arterioles.

There are differences according to species in the haemodynamic changes caused by endotoxin, e.g. in cat, rabbit and sheep (see later) the hypertension is marked in the pulmonary artery, with pooling of blood in the lung, and not in the splanchnic areas (GILBERT, 1960).

There is no increased pressure in the peripheral veins. It should be noted that in spite of arterial hypotension peripheral resistance is not reduced, but rather increased intially in all species, suggesting constriction of peripheral arterioles. Thus, this type of shock is not due to generalized vasodilatation (e. g. GILBERT, 1960, WEIL, 1961).

According to WEIL (1961) some observations in man indicate that „pooling may take place in the splanchnic bed with a marked reduction of cardiac output in the presence of low pressure. However, demonstration of the site of this presumed pooling in man must await further studies". In many cases the fatal outcome in man may be due to renal failure.

WAISBREN (1964) denotes the endotoxin shock occurring in man as a „gram-negative shock" and distinguishes it from that occurring in animals. This differentiation is probably correct because bacteriaemia occurs in man together with the release of endotoxin circulating in the blood.

Let us turn now to the role of the chemical mediators.

Catecholamines are the first vasoactive agents to be discussed because they probably initiate the primary toxic events in the endotoxin reaction. Catecholamines may participate in various nonspecific damaging stimuli and different types of shock (but not especially in anaphylactic shock).

After small doses of endotoxin the initial reaction is a vasoconstriction in the peripheral vessels of tissues (e. g. skin), due to norepinephrine — probably liberated from the peripheral nerve-endings or peripheral stores and to epinephrine liberated from peripheral stores. Administration of larger doses results in a rapid fall of blood pressure (within 1 minute). The initial fall is followed by a secondary rise. This may be due to the liberation of catecholamines from the adrenal medulla (see below) and to the release of pooled blood.

The typical haemodynamic changes: portal hypertension, liver pooling, reduced venous return, etc. in dogs; arterial hypotension and pulmonary artery hypertension in rabbits and other animals, cannot be elicited by the injection of epinephrine or norepinephrine, but can be prevented by some adrenolytic agents.

The release and the suggested role of catecholamines in the haemodynamic effects of endotoxin is supported by the experiments described below.

HEIFER, MUNDY and MEHLMAN (1960) have stated that the adrenal medulla of rabbits injected with endotoxin releases catecholamines, followed by an increased plasma level during the next few hours.

ROSENBERG, LILLEHEY, MORAN and ZIMMERMAN (1959), ROSENBERG, LILLEHEY, LONGERBEAM and ZIMMERMAN (1961) have observed that 5 minutes after the intravenous injection of lethal doses of endotoxin catecholamine concentration rose strikingly in the plasma of dogs (5 minutes was the earliest period that catecholamines were assayed after endotoxin injection). During the succeeding 30 minutes concentrations approached control levels and remained unchanged until prior to death, when extremely high values were observed, especially of epinephrine.

GILLENWATER, DOOLEY and FROHLICH (1963) found an initial transient (18 sec) and a secondary intense vasoconstriction in renal arteries (5—10

min.) after lethal doses of typhoid endotoxin were injected into dogs. Renal arterial pressure remained elevated for 30 minutes. Since phentolamine blocked the second vasoconstriction, this effect is supposed to be due to catecholamine release.

NOLAN and O'CONELL (1965) have given a small dose (50 μg) of coli endotoxin which caused a sudden and direct slowing of blood flow in the isolated perfused rat liver lasting 5—15 minutes. Since phentolamine (Regitine) and methysergide (both in doses of 1,0 mg) did not inhibit this effect, they deny the role of catecholamines or serotonin as key mediators in the primary vascular effect of endotoxin. However, they could inhibit this effect by a simultaneous large dose, or previously applied small dose of hydrocortisone. (Since the primary role of hydrocortisone to modify the effects of vasoactive mediators is not known, it is difficult to explain the above results. The dose of catecholamine and serotonin antagonists seems to be small. Perhaps the variation of time-, and dose-relationship of the catecholamine and serotonin-antagonist used would approach the solution.).

EGDAHL (1959) found that small doses of endotoxin (10 μg) caused adreno-cortical stimulation in rabbits. Larger doses of endotoxin (200 μg) caused both adreno-cortical (hydrocortisone release) and adrenomedullary stimulation (catecholamine secretion). Transection of the spinal cord at C-7 abolished the catecholamine secretion, but the adrenocortical-, febrile- and hypotensive-responses remained unimpaired. It is assumed that adrenomedullary stimulation by endotoxin reaching the brain centres is dependent on the descending nerve pathways in the spinal cord. Epinephrine release (*via* descending nerve pathways from the adrenal medulla) is not necessary for the febrile and adrenocortical stimulating effect of endotoxin. It is known from the experiment of DELAUNAY et al (1948) already mentioned that some effects, e. g. the haemorrhagic-necrotic effect of endotoxin (an effect on small vessels) can be mimicked by large doses of epinephrine. However, epinephrine cannot produce the haemodynamic effect of endotoxin.

SHIMAMOTO, INOUE, KOIZUMI, IWAHARA and KONISHI (1958) succeeded in potentiating the toxicity of endotoxin by MAO- (mono-aminooxydase) inhibitors. This experiment also shows the participation of epinephrine in the effect of endotoxin.

Apart from the release of catecholamines there are some indirect proofs of the role of these agents. Various adrenolytic drugs are able to decrease the haemodynamic changes caused by endotoxin, probably by improving venous return and reducing pooling.

LILLEHEY and MACLEAN (1958, 1959) have observed that the two vasoconstrictors: norepinephrine and Aramine (metaraminol) aggravate endotoxin shock and that pretreatment with Dibenzylamine or chlorpromazine ameliorated the shock state and increased survival.

IAMPIETRO, HINSHAW and BRAKE (1963) have studied the effects of pretreatment with phenoxybenzamine on the haemodynamic changes in dog induced by endotoxin shock. Pretreatment with phenoxybenzamine augmented venous return by reducing the pooling in the liver and probably other areas. The striking fall in systemic arterial pressure which is a common result of endotoxin administration was absent in the

treated dogs; arterial tension, however, dropped to the level of non-treated dogs within 30 minutes. These authors stated that phenoxybenzamine (Dibenzyline) had a beneficial effect on survival in dogs. Previously, WEIL and MILLER (1961) had obtained similar results.

MASUCCI and HINSHAW (1964) observed some beneficial effect of phenoxybenzamine (Dibenzyline) on the haemodynamic changes caused by endotoxin, but they could not observe such beneficial effects on the survival. The differences between the above experiments in respect of survival is unexplained.

WEIL (cited by LANDY and BRAUN, 1964) states: „In the mouse treated with phentolamine, which is commercially available as Regitine, there is increased survival, but in the case of the same animal treated with phenoxybenzamine (Dibenzyline), the mortality is actually higher than that of the controls. After chlorpromazine (Thorazine), the situation is even more complex. In the first 24 hours there is a marked increase in survival but these animals died between the second and seventh day so that no persistent benefit was obtained. I would suggest that the mechanism of action of phenoxybenzamine, and chlorpromazine, is in itself dissimilar and it is, indeed, very difficult to be certain that these agents produce their effect by sympathetic blockade".

HINSHAW, BRAKE, EMERSON, JORDAN and MASUCCI (1964) deny the detrimental role of the sympathoadrenal system in endotoxin shock in dog, cat or monkey. These authors observed that endotoxin-induced hepatosplanchnic pooling is not dependent on an intact nerve supply to the abdominal viscera or the presence of adrenals in dogs since splanchnic denervation and adrenalectomy was ineffective. They state that the release of catecholamines does not hasten the development of irreversible endotoxin shock. Thus, the adrenergic system is not involved in the death of the animals.

(In the above experiment the adrenergic system is probably involved in the initiation of endotoxin shock *via* the nerve-endings or tissue stores, and not *via* the adrenal medulla.) (Author's remark.)

On the other hand, FINE (1964) stated that denervation of the splanchnic viscera of dogs and rabbits prevented the damage of these tissues following a lethal dose of endotoxin. The survival of denervated animals was also enhanced. Fine suggested that the injury in the intact animal was caused by norepinephrine, and that the sympathetic nerve pathways supplying the splanchnic area (especially liver and intestines) were implicated in the death caused by endotoxin.

It was suggested in the previous chapter that the discrepancies between these experiments were due to seasonal variations in the sensitivity of animals.

The investigation of SPINK, REDDIN, ZAK, PETERSON, STARECKI and SELJESKOG (1966) showed that in canine endotoxin shock only the epinephrine and not the norepinephrine level rises significantly with the onset of hypotension. The epinephrine level rose sharply after 90 seconds and gradually returned to normal after 4 hours. They state that the plasma epinephrine level rose similarly in resistant animals in spite of the lack of hypotension. The plasma epinephrine increase was abolished

by cervical cord section or adrenalectomy in the above experiment. Under these two conditions small doses of endotoxin caused marked shock resulting in rapid death.

The fact that resistant animals similarly produce epinephrine (in plasma) on endotoxin challenge indicates the possibility that epinephrine does not participate in the onset of endotoxin shock. However, resistant animals may develop a counter mechanism against epinephrine and on the other hand, their animals may not be considered as definitely resistant. They induced tolerance by 0,5—0,75 mg/kg endotoxin. If the animals survived they were considered tolerant after the elapse of 3 weeks. At this time (see resistance, IV/3 and IV/8 chapter, p. 57 and p. 62) the animals begin to lose tolerance.

WEIL, HINSHAW, VISSCHER, SPINK and MACLEAN (1956) have shown the improving effect of Aramine (metaraminol) on venous return after endotoxin injection in dog, especially using delayed administration. The authors explained the effect of Aramine by its specific dilatating effect on hepatic veins.

Two of the adrenergic receptors should be noted: 1. the alpha receptor-stimulating effect of norepinephrine and epinephrine resulting in vasoconstriction. This effect is antagonized by Dibenzyline (phenoxybenzamine). 2. The beta receptor stimulation (by epinephrine and isopropylnoradrenaline) resulting in vasodilatation. DCI (dichlorisoproterenol) is a beta receptor antagonist.

According to MCLEAN and BERRY (1961) a beta receptorantagonist: DCI protects mice against the lethal effect of endotoxin.

HALMAGYI, STARECKI and HORNER (1963) found in sheep a beneficial effect of isoproterenol on the haemodynamic effect elicited by endotoxin. These authors found that the lungs are the main target organ for the endotoxin effect in this species. They observed a marked rise in pulmonary arterial and pulmonary arterial wedge pressure (with left atrial pressure unchanged) and a fall in cardiac output and in systemic arterial pressure which closely followed the onset of pulmonary hypertension and was one of the first in the sequence of pathological changes. Pretreatment with antihistaminics, antiserotonin and adrenolytic agents, or norepinephrine was without effect. However, treatment with isoproterenol reduced the pulmonary arterial hypertension, increased the cardiac output and promptly raised the systemic arterial pressure.

According to VICK (1964) the vasodilatator phenoxybenzamine can counteract the haemodynamic changes caused by endotoxin and increases survival in primates. In his opinion progressive hypotension, reduced renal function, hyperpnea, and reduced peripheral vascular flow are the factors responsible for the irreversibility of endotoxin shock in this species. The cause of the reduced peripheral vascular flow may be the combined result of catecholamine-induced arteriolar constriction and histamine-induced venospasm.

Serotonin (5—Hydroxytryptamine), another vasoactive agent also varies sharply in concentration after endotoxin injection.

The effect of serotonin on small blood vessels is vasodilatation, i. e. the opposite effect to that of epinephrine. According to JANCSÓ (1961)

serotonin and/or histamine play an important role in increasing vascular permeability. OYVIN and SHEGEL (1965) found that serotonin injected locally increases the permeability of small blood vessels. Serotonin reduced the rate of removal of radiophosphate by causing dilatation of small vessels with concomitant hyperaemia and stasis.

SHIMAMOTO, YAMAZAKY, SHAGAWA, IWAHARA, KONISHI and MAEZAWA (1958 and SHIMAMOTO et al 1958/a) have found an increased plasma serotonin level in rabbits after administration of bacterial endotoxin.

In the experiments of DAVIS, MEEKER and MCQUARRIE (1960) a fall in serum serotonin (and a small rise in plasma serotonin) ensued in some animals within one minute after intravenous endotoxin injection.

DES PREZ, HOROWITZ and HOOK (1961) have shown *in vitro* release of serotonin from platelets. (Histamine may also be released from platelets, see below). Incubation of platelet-rich rabbit plasma with *E. coli* endotoxin caused platelet aggregation and transfer of serotonin to plasma.

In view of this, high levels of serotonin in the whole blood of endotoxin-treated animals might be expected. Instead of a high 5—HT level there is in fact only a small and very transient increase in the plasma with a concomitant decrease in the whole blood. The authors explain this decrease by „transfer of 5—HT from platelets to plasma with subsequent rapid enzymatic degradation, and by removal of platelet bound 5—HT as the sequestration of platelets develops". ARMIN and GRANT (1957) have observed that an acetone extract of the plasma from rabbits injected with endotoxin was similar to 5—HT in its action on the rat uterus.

HINSHAW, KUIDA, GILBERT and VISSCHER (1957), in examining the effects of endotoxin on the isolated perfused lung of dog observed that the rise in organ weight and vascular resistance did not occur when the perfusate containing endotoxin was cell-poor plasma, dextran, or gelatine. Considering that 5—HT itself induces similar changes in pulmonary vascular resistance and lung weight (MCGAFF and MILNOR, 1960; KABINS, MOLINA and KATZ, 1959) it is probable that the substance in cell-rich plasma necessary for the above effects is 5—HT derived from platelets.

THOMAS (see previous subchapter) is of the opinion that serotonin released *locally* damages vessels in concert with epinephrine.

It is interesting that GORDON and LIPTON (1960) could reduce the endotoxin mortality in mice by pretreatment with serotonin. Considering that this effect was potentiated by cortisol, it is difficult to explain how DES PREZ, FALLON and HOOK (1961/a) were able to protect against the lethal effect of endotoxin even with an antiserotonin agent. These problems will be discussed in the concluding remarks.

Histamine is also an important mediator which is released in blood of animals treated with endotoxin.

It should be noted that the experiments described below were carried out with large doses of endotoxin and the histamine was estimated in whole blood. Smaller doses were not tested for their effect on the histamine concentrations. HINSHAW (1964) explains the toxicity of endotoxin by its histamine-releasing ability.

HINSHAW, JORDAN and VICK (1961, 1961/a) have reported significantly raised blood histamine levels during endotoxin treatment in dogs and primates. Considering the role of histamine in the haemodynamic effect of endotoxin HINSHAW, EMERSON, IAMPIETRO and BRAKE (1962) have also carried out experiments to discover a relationship between the effects of histamine and endotoxin. Their results indicate definite similarities between the effects of histamine, a histamine liberator 48/80, and endotoxin in the haemodynamic changes in dogs: a sudden increase in portal vein pressure, accompanied by a decrease in the systemic arterial pressure. The early reactions to endotoxin were greatly reduced when 48/80 was given prior to endotoxin.

SPINK, DAVIS, PORTER and CHARTRAND (1964) found that the plasma histamine level rose on an average to 8 fold 30 to 60 seconds after injection of a lethal dose of endotoxin. 5 minutes later the value was still significantly raised, but tended to decline toward the normal histamine level until the 4th hour. These authors could diminish the response by administering epsilon-amino-caproic acid (EACA) and cortisol. Histamine infusion (see also SPINK, CHARTRAND and DAVIS, 1963) before endotoxin injection modifies the haemodynamic changes and increases survival in dogs.

SCHAYER (1960) explains the histamine release during endotoxin treatment by an increased activity of histidine decarboxylase. There is a liberation of preformed histamine after the onset of the shock and continued synthesis of histamine by histidine-decarboxylase which may be even more important. According to Schayer's observation endotoxin and large doses of epinephrine significantly augment the activity of histidine decarboxylase.

BRAKE, HINSHAW and EMERSON (1964) state that phenoxybenzamine apart from its antiadrenergic effect, has antihistaminic properties and prevents the portal pressure rise and hepatic-splanchnic pooling in dog. The beneficial effect of this antiadrenergic, antihistaminic and antiserotonin drug may show that epinephrine, histamine and serotonin all play a role in the deleterious effect of endotoxin. In this respect the action of phenoxybenzamine resembles that of chlorpromazine.

TRANK and VISSCHER (1962) explained the persistent hypotension due to endotoxin by its effect on baroreceptors in the carotid sinus. They conclude that in the later phase of endotoxin shock the reflex alteration may play a predominant role in maintaining hypotension, and that the compensatory mechanism is impaired by endotoxin.

Coagulation alterations caused by endotoxin also may play some role in endotoxin shock. According to HARDAWAY and JOHNSON (1963) endogenous heparin is also released during endotoxin shock. The liberation of endogenous heparin was observed (KOVÁTS, REÖK and KARÁDY, 1958) also after provocation of the Shwartzman reaction by endotoxin.

From the point of view of endotoxin shock the experiments performed by HARDAWAY and JOHNSON (1963) on the clotting mechanism are particularly interesting. In their opinion coagulation factors play a definite role in the final outcome of endotoxin shock. They explain the aortic hypotension by obstruction of the blood flow in the liver and lungs, due to temporary occlusion by thrombi, causing portal and caval

hypotension and pulmonary hypertension. This diminishes the venous return and cardiac output, and so causes aortic hypotension. In Hardaway and Johnson's experiment injection of *E. coli* endotoxin to dog resulted in the gradual decrease of fibrinogen, factor V and factor VII, and in sudden disappearance of the platelets. Reduction in these factors was probably due to their consumption by an intravascular clotting process. Later the intravascular clotting capacity was enhanced and conversely, endogenous heparin was raised for some hours. It is noteworthy that preheparinization prevented the fall in fibrinogen and the drop in blood pressure. Thus, there seems to be a connection between endotoxin shock and intravascular coagulation.

There is another experiment carried out with Phlogodym (neodymium pyrochatechol disulphonate) in the endotoxin shock. This compound of JANCSÓ (1961) is an anticoagulant binding the prothrombin and factor VII. It does not cause shock-like symptoms in rats and is well tolerated by rabbits too. One of its properties is to counteract inflammatory reactions — due to bee venom, cobra venom or dextrane — in rats. According to LÁZÁR and KARÁDY (1965) Phlogodym markedly aggravates endotoxin shock in rats by potentiating the effect of endotoxin in reducing the fibrinogen level and platelet count. This reduction derives from the intravascular coagulation which is primary in the above described effects. These authors state, moreover, that the labile fibrinogen of Lyons formed on the effect of Phlogodym creates the predisposition for intravascular coagulation.

Concluding remarks

The vascular effects are very characteristic of endotoxin.

The vasomotor disturbances and haemorrhagic necrotic effects mainly involve 1. the autonomic (adrenergic) mechanism and 2. the endotoxin storing capacity of vascular- (arteriolar) endothelium *plus* endothelium damaging capacity of endotoxin and 3. a hypersensitivity mechanism which may be of minor importance for the above.

In addition to the adrenergic and vascular endothelium damaging mechanism the haemodynamic changes show the features of hypersensitivity.

Though they occur together, some symptoms may be assigned to adrenergic (neurotoxic) and others to hypersensitivity mechanism.

THOMAS stated that the adrenergic (toxic) effect of endotoxin lacks hypersensitivity features even in its vascular effects; in his terminology this means vasomotor disturbances and haemorrhages. However, so far, it has not yet been determined whether or not histamine and acetylcholine (characterizing hypersensitivity reactions) play a role in the above vascular effects.

However, in the haemodynamic effect of endotoxin the hypersensitivity features are more pronounced (see chapter III/2.) The question arises whether these hypersensitivity features are only the result of a final common pathway manifested in both hypersensitivity and nonspecific toxicity. This problem can be approached from etiological conside-

rations, namely whether the primary toxic or hypersensitizing activity of endotoxin leads to the final sequence of events. It is possible that both mechanisms are involved in inducing the characteristic symptoms and the final outcome. Endotoxin acts both as a primary toxin and an allergen.

Although the complex mechanism of endotoxin shock and haemodynamics has not yet been fully elucidated some conclusions may be drawn from the pertinent experiments.

In spite of the fact that some authors overstress the role of vasoactive agents examined by them, several vasoactive agents or chemical mediators are probably released as a result of endotoxin and are jointly responsible for its complex effects. It must be noted that neither the sequence of the release of these agents, nor their quantitative relations are exactly known. Even the effect of the known mediators or vasoactive agents is not yet entirely detected in all vascular areas. The vasoactive agents and perhaps other unidentified substances released in the early phase may trigger off different counter-mechanisms which may complicate the picture.

The role of vasoactive agents in endotoxin-caused haemodynamic alterations may be summarized as follows:

In the initial period (within 30 seconds after the intravenous injection of endotoxin) there is an increased blood-level of catecholamine and histamine associated with a small and transient increase in plasma 5—HT and a somewhat persistent fall in whole blood 5—HT.

Catecholamines are released by nonspecific stimulation of the adrenergic nerves. The typical mediator of hypersensitivity reactions, however, is histamine and its release may be regarded as a consequence of a hypersensitivity reaction to endotoxin. Histamine may, of course, be released by nonspecific stimuli also.

The threshold quantity of endotoxin required to release these agents varies. The epinephrine-like effect (vasoconstriction in small vessels) may be seen after giving very small doses of endotoxin, and endotoxin and epinephrine can potentiate each other's action. Histamine release and histamine-like effects are elicited only by large doses of endotoxin (see, e. g. SCHAYER, 1964).

It may be concluded that at least in the initial phase catecholamines are responsible for the vascular changes involved in the primary toxic effect of endotoxin.

However, it must be noted that in some species the haemodynamic changes of endotoxin (arterial hypotension, liver pooling, increased pressure in the pulmonal artery) cannot be produced by epinephrine or norepinephrine injection. Yet these changes can be antagonized by some epinephrine- and norepinephrine blocking agents.

It is interesting that haemodynamic changes similar to those caused by endotoxin can be induced by intravenous injection of histamine, or 48/80, but these haemodynamic changes cannot be inhibited by the usual antihistaminics like metapyriline and diphenylhydrazine. However, alpha-receptor antagonists — e. g. phenoxybenzamine possessing antihistaminic and also antiserotonin properties — can prevent endotoxin-caused haemodynamic changes (HINSHAW, 1964).

In connection with the role of histamine and epinephrine in the action of endotoxin the attractive hypothesis of SCHAYER (1964) should be mentioned. He explains the endotoxin-caused microcirculatory changes by alterations of the level of histidine decarboxylase, which has inducible or adaptive characteristics. Accordingly „the actions of induced histamine must be differentiated from those of histamine released from a preformed, bound state. The latter, since it is released rapidly in high local concentrations, is associated mainly with short term pathological effects. Induced histamine is produced by continuous new synthesis in relative minute quantities and seems to be involved in the physiological process of microcirculatory regulation". „Injection of a small amount of endotoxin leads to a rapid release of catecholamines and glucocorticoids and to vasoconstriction. In response to the resulting ischaemia, there is a compensatory increase in induced histamine synthesis and a gradual allevation of the vasoconstriction. After larger doses of endotoxin, induced histamine synthesis may become prominent; thus, there is a biphasic reaction in which the early vasoconstriction is succeeded by a period of dilatation and reduced responsiveness of microcirculatory smooth muscle to topical epinephrine." „Glucocorticoids released rapidly by endotoxin, antagonize actions of induced histamine on the microcirculation, as a consequence of reduced effectiveness of its natural antagonist epinephrine exerts an abnormally powerful vasoconstrictor action." „In dogs hyperreactivity to epinephrine after endotoxin injection is not observed. However, this species is atypical, in that endotoxin causes a rapid release of preformed histamine from liver, an event which could prevent development of the hyperreactive phase." This theory explains the change of vascular tone discussed above.

The role of induced histamine suggested by Schayer, may, however, be limited to rodents, since there is no reliable or confirmed evidence for its occurrence in the tissues of man, cat and dog according to WATON (1963). The most probable source of histamine in the latter species is the intestinal flora. (Perhaps the slow disintegration of platelets, tissue mast cells and leucocytes may lead to the persistent release of histamine, even in the absence of histidine decarboxylase activity.)

The species-differences may be partially explained by the Mauthner-Pick effect, i. e. histamine caused constriction of hepatic veins, which occurs in dogs.

Histamine is also released by treatment with 48/80. The benefical effect of 48/80 pretreatment on endotoxin shock and the haemodynamic changes may be explained by depletion of the stored histamine; so that endotoxin cannot release histamine. Of course, 48/80 causes the liberation of other vasoactive substances (e. g. 5—HT) and their level may also be diminished by pretreatment.

In spite of the inhibition of haemodynamic changes by histamine or 48/80 pretreatment, the animal does not usually survive. Any beneficial effects of histamine infusion, or 5—HT pretreatment may be due to the counterregulation mechanism which they induce and which may protect the organism at the time when the endotoxin is injected.

The favourable effect of chlorpromazine on endotoxin shock may be due to its combined antiadrenergic, antiserotonin and weak antihis-

taminic activity. (The mode of action of chlorpromazine in this relation is discussed in one of the previous subchapters dealing with the action of endotoxin on the reticular formation.) Phenoxybenzamine has similar characteristics in its mode of action.

Phenoxybenzamine dilates the vessels and renders them more sensitive to every kind of hypotonia (or haemodynamic reactions, e. g. orthostatic collapse) and the animal easily succumbs if the vessels are not filled. Nevertheless, phenoxybenzamine effectively prevents shock (KOVÁCH, 1965) if the vessels are kept filled with dextran or blood.

The role of serotonin in the endotoxin effect is not yet clear. The increase of the plasma serotonin level after endotoxin injection is very slight and confined to the first minutes. The serotonin level in the whole blood or serum tends to fall during the first hour. This may be due to rapid enzymatic destruction, or the removal of platelet-bound 5—HT by sequestration, as proposed by Des Prez and his collaborators. Since some experiments point to the injurious role of 5—HT in the endotoxin effect, it is difficult to explain the alleged benefical action both of serotonin or antiserotonin in endotoxin shock.

Some experiments suggest that the known vasoactive agents induce different effects on different vascular areas. Jacobson and his collaborators state that while norepinephrine acts as a vasodilatator, histamine causes constriction of the stomach vessels. A similar effect was observed in the peripheral arterioles and veins (see e. g. GILBERT, 1960 and VICK, 1964).

The interrelations between histamine and epinephrine effects and haemodynamics may also be of interest. „In the presence of vasoconstrictor amount of histamine, epinephrine is a vasodilatating agent. In the presence of high levels of epinephrine, histamine has its typical vasodilating effects. It is altogether possible that for certain types of vascular smooth muscle the histamine-epinephrine relations are such as to produce contraction at the same time that other types are being relaxed." (cited by GILBERT, 1960).

The different mediators also influence the release of one another. SPINK, DAVIS, PORTER and CHARTRAND (1964) state: „It is known that histamine stimulates the secretion of epinephrine from the adrenal medulla." In contrast BAUR and STAUB (1949) had earlier found increased blood histamine levels following the administration of epinephrine. Norepinephrine was 5-times less effective in this respect.

It is not clear how histamine and epinephrine influence each other's secretion. Is it *via* the peripheral or the central nervous system? GREEN (1964) states that histamine and perhaps methylhistamine may prove to be physiological neurotransmitters, or neuromodulators. Injected histamine can potentiate (in small doses) and stimulate (in large doses) synaptic transmission in the peripheral sympathetic centers. Perhaps these mechanisms provide common pathways by which histamine and epinephrine can influence the release of each other.

The resemblance between the haemorrhagic effect of epinephrine and of small doses of endotoxin does not necessarily mean that endotoxin induces this effect through epinephrine alone. Although large doses of histamine may enhance secretion of epinephrine, the injection

of histamine does not induce the extensive local haemorrhages which are caused by epinephrine during endotoxin administration. This is unexplained.

It is an important question whether or not histamine is released in the blood when minute doses of endotoxin sufficient to cause shock are administered directly to the central nervous structures. It would be interesting to measure histamine in such circumstances.

Let us now return to the mechanism of haemodynamic changes caused by endotoxin. How far are purely mechanical factors involved in the rapid fall of aortic pressure? Portal vein or pulmonary artery constriction itself may arrest the blood flow to the heart and diminish cardiac output; when the quantity of blood in the large arteries is reduced, how far do the vasoactive mediators contribute to the persistent fall of blood pressure? Alternatively, if thrombi are later formed in the small vessels, to what extent is the blood-flow arrested by this? If any mediators are to act at this time they would be epinephrine and other vasoconstrictors involved in counterregulation.

In the eviscerated dog MacLean and Weil (cited by Weil, 1964) could not observe the immediate fall of pressure usually induced by endotoxin. It may be that in this case the thrombi formed in the small vessels also contributed to the arrest of blood flow. It should be noted, that thrombi formation does not occur in the first minute, but only after several minutes (Hardaway and Johnson, 1963).

Munoz (1964) stated that in mice pertussis-induced hypersensitivity to histamine and to anaphylaxis may be connected with beta-adrenergic blockade. Beta-adrenergic blocking agents, e. g. DCI can produce pharmacological changes (histamine sensitivity) similar to pertussis treatment. According to Fishel, Szentiványi and Talmage (1964) this may also occur in the endotoxin effect. Beta adrenergic blockade potentiates the histamine effect because histamine exerts the above action through epinephrine. (Epinephrine — perhaps through cortisone — may antagonize the histamine effect.)

DCI a beta-receptor blocking agent causes vasoconstriction. It is beneficial in endotoxin shock of sheep and protects mice against death. Phenoxybenzamine which is also beneficial has an alpha-receptor antagonizing effect, but acts also as an antihistaminic and antiserotonin agent (see e.g. Zweifach, 1964). Therefore, this drug may block vasoconstriction.

These data point to the various mechanisms of endotoxin shock in different species.

III. ENDOTOXIN HYPERSENSITIVITY

Summary

The possibility of a specific endotoxin hypersensitivity will be considered in this chapter.

The O-antigen is fully antigenic or allergenic and can produce a state of hypersensitivity. The purified lipopolysaccharide lacks the polypeptide and may rather be considered to be an allergen.

The possibility that the endotoxin molecule has two distinct properties (1. primary toxicity, 2. antigenicity) has already been discussed. The problem is whether the intense reactions elicited by endotoxin are only consequences of its nonspecific neurotoxicity or antigenicity also plays a part. To decide this we have to find 1. similarities between endotoxin reactions and allergic reactions and 2. to demonstrate immunospecificity in eliciting such reactions.

If an animal is immunized (sensitized) by endotoxin the resulting reaction is not wholly identical with any classical hypersensitivity reactions. Owing to the primary toxicity of endotoxin the reaction is more severe and more complex.

Endotoxin reaction can be evoked without — except for special purposes — artificially sensitizing an animal to endotoxin. We suppose that the vertebrates (due to their symbiosis with endotoxin-producing flora and infections) have a „natural" endotoxin sensitivity. This means that the animal reacts to the first endotoxin injection as if it had been previously sensitized with it. The local or systemic reactions of endotoxin which may be seen in normal animals are similar in some respect to the recognized anaphylactic, early and delayed type hypersensitivity reactions. This natural sensitivity changes during life and it may be influenced artificially.

The similarities between the known allergic hypersensitivies and endotoxin hypersensitivity are as follows:

1. Histamine release
2. Endogenous heparin release
3. Reduction of the complement level
4. Activation of proteolytic enzymes
5. Common histological features
6. Leucopenia and thrombocytopenia, followed by leucocytosis
7. Clumping and damage of platelets and damage to mastocytes (release of histamine, serotonin and heparin)
8. Damage to vascular endothelium
9. Damage to the membranes of lysosomes

10. Similarity of the shock syndromes
11. Anamnestic reactions and a state resembling immunoparalysis can be elicited with endotoxin
12. Acceleration of endotoxin skin reactions by immune serum
13. The rapid appearance of opsonins and bacteriocidin (natural antibodies) after endotoxin injection (result of an anamnestic reaction)
14. Immunospecificity in inducing resistance to endotoxin
15. Rabbits made resistant with one type of endotoxin can respond with Shwartzman reactions to heterologous type of endotoxin
16. Cytolytic effect of endotoxin *in vitro* needs the presence of specific antibodies.

1. The role of humoral and cellular agents of hypersensitivity states, in the effect of endotoxin

Both kind of agents may play a role in the systemic and local hypersensitivity effects of endotoxin.

Rapid alterations in histamine and serotonin blood levels were discussed in the previous chapter. Similar changes are found both in endotoxin shock and anaphylaxis. Increased endogenous heparin levels also were observed, and HARDAWAY and JOHNSON (1963) found endogenous activation of heparin and fibrinolysis after endotoxin treatment. They assume that these alterations are the body's defence mechanism against intravascular coagulation.

The sharp drop in complement level after endotoxin injection is also similar to that found in allergic reactions.

The striking changes caused by catecholamines released after endotoxin injections are not characteristic of anaphylaxis or early hypersensitivity. It may be assumed that the epinephrine-like effect initiates the symptoms due to endotoxin, but that afterwards other agents found in anaphylaxis early hypersensitivity come into operation. Epinephrine-like action alters the picture in endotoxin hypersensitivity. Epinephrine and/or other agents influence the vessels in such a way that they do not become more permeable to dyes. Consequenly, PCA induced by endotoxin cannot be detected (see chapter III/11).

MILES and MILES (1952) have stated: „High local concentration of histamine inhibit blueing by inducing local vasoconstriction during the period of increased permeability, so that by the time the constriction is relaxed the vessel walls have recovered their normal low permeability." — The failure of dyes to leak into the tissues may perhaps be explained on this basis. However, the increased amount of epinephrine and other unknown agents, released locally, may also contribute.

Endotoxin causes marked cellular changes. Leucocytosis following leucopenia and damage of leucocytes ensues after endotoxin injection as with antigens in sensitized animals. There is a clumping and damage of platelets (e. g. DES PREZ et al., 1961) and damage of mast cells similar to that occurring in allergic reactions (HUMPHREY and JAQUES, 1955). Vascular endothelium stores endotoxin, and is damaged as in allergic reactions (e. g. DIETRICH, 1941 see p. 35).

KESSEL BRAUN and PLESCIA (1966) state: „Cytotoxic effects of endotoxins *in vitro* seem to require the presence of endotoxin-specific antibody at the surface of susceptible cells. This conclusion is based on the finding that exposure of guinea pig macrophages to anti-guinea pig gamma globulin interferes with the cytotoxic effects of subsequent exposure to endotoxins from wide variety of Gram-negative bacteria. The conclusion is also supported by the finding that endotoxin from *Br. abortus*, a nonubiquitous Gram-negative organism, is not cytotoxic *in vitro* unless macrophage donors have first been sensitized by injection with live *Br. abortus*."

According to MORGAN and BENNETT (1947) injection of endotoxin into the joints of rabbits causes inflammation, synovial proliferation, degeneration of cartilage and articular fixation. It was demonstrated in the experiments of STUART (1950,, 1951, 1952) that endotoxin causes dispersion and later disappearance of the cytoplasmic granules of mast cells (predominantly in the vascular system) followed by pyknosis and cellular disintegration. Similar alterations on mast cells were found in anaphylaxis by HUMPHREY and MOTA (1959).

2. Endotoxin shock and its relation to anaphylaxis

The problems of endotoxin shock were discussed in the chapter of haemodynamic changes. Although this type of shock is caused by large doses of endotoxin compared to those of protein antigens causing anaphylaxis, there are common pharmacological and histological features. Some mediators and vascular changes are involved in both.

The first event in endotoxin shock in an epinephrine-like effect, i. e. the constriction of the small vessels with concomittant decrease of permeability. After some minutes a histamine-like effect — vasodilatation — will prevail. The waves of arteriolar spasm and dilatation were explained previously as due to a change in the amount of epinephrine and histamine. This in not apparent in anaphylaxis. In both type of shock, the drop in blood pressure within 1—2 minutes, is probably due to histamine.

The main reaction of the anaphylactic shock is smooth muscle-contraction (histamine, acetylcholine and SRS-A effect) and the increase of capillary permeability. There is no doubt that in anaphylaxis the histamine-effect prevails and the epinephrine effect — if it exists — is of minor importance.

The common chemical pathway and common final outcome does not necessarily imply a common etiology of hypersensitivity. But, let us first examine the common features in endotoxin and anaphylactic shock.

As we have seen in dog, within the first minute after endotoxin injection there is a fall in systemic blood pressure, rise in portal vein pressure and decrease in venous return and renal blood flow. Nearly the same events and time relation occur in dog anaphylaxis (MCMASTER, 1959).

WEIL and SPINK (1957) pointed out the similarity between these two types of shock, though they noted that similar changes have also

been observed after injection of several substances including glycogen. DAVIS, MEEKER and MCQUARRIE (1960) have reported a rapid fall in serum serotonin level and platelet count within one minute in dogs given endotoxin intravenously.

SPINK, DAVIS, PORTER and CHARTRAND (1964) supported the concept that the initial haemodynamic changes in endotoxin shock may be related to an anaphylactic type of immune-mechanism. Plasma histamine level rose on average to 8 times the original level between 30—60 seconds after i. v. injection of endotoxin. Similarly as in anaphylaxis there was a drop of the complement titer to a minimum after 10 minutes.

GILBERT and BRAUDE (1962) concluded in their immunological studies on endotoxin shock in rabbit that endotoxin has an anaphylactic effect. In their experiments the injection of lethal doses of endotoxin into normal rabbits regularly caused a quick and lasting fall of the complement level and lowered the titers of natural antibody to endotoxin as shown by haemagglutination technique. The antibody-level was similarly decreased in animals immunized artificially by endotoxin.

The rapid fall of complement, platelet and serotonin levels and a rapid rise in histamine level are typical of the reaction when an antigen is injected into a previously immunized animal. Incoagulability of blood, heparin release and histamine-like agents were shown both in anaphylactic and endotoxin shocks.

These changes after endotoxin injection may be due to the „natural" immunization of the rabbits by intestinal symbiotic bacteria (SCHAEDLER and DUBOS, 1961).

SPINK and VICK (1961) have demonstrated that the action of endotoxin on the blood vessels in lethal endotoxin shock was mediated through a thermo-labile factor which was suggested by them to be a complement component. It has been also suggested by PEARLMAN, SAUERS and TALMAGE (1962) that complement may be bound by antibody forming cells after injection of endotoxin.

From the experiment of KOSTKA and STERZL (1962) it may be concluded that the inactivation of complement by endotoxin is due to interaction with antibody. They have shown that the serum of colostrum-deprived piglets shortly after birth contained complement but no *E. coli* antibody. Endotoxin caused no inactivation of complement in such piglet serum. However, endotoxin added to the serum of adult swine, containing *E. coli* antibody, caused a significant reduction of complement.

3. Similarities between the Arthus-type, tuberculin-type and endotoxin-mediated reactions

Endotoxin injected into the skin of rabbits causes a dermal lesion not unlike that seen in early and delayed hypersensitivity reactions. All the reactions have some similar histological features, though the macroscopic reaction to endotoxin becomes visible more slowly. This may be because after endotoxin the early development of increased vascular permeability and oedema formation is inhibited by the effect of released catecholamines or by other agents.

URBACH and GOLDBURGH (1942) first pointed out the similarity between the Shwartzman- and tuberculin types of reactions. STETSON (1951) also suggested a similarity between the Arthus and local Shwartzman reactions. However, the Shwartzman phenomenon is more complex than the effects of a single intradermal endotoxin injection. It is more correct to compare cutaneous allergic reactions with the reaction caused by a single injection of endotoxin, i. e. the first stage of the Shwartzman-phenomenon.

STETSON (1955) has also examined the similarity between the cutaneous reaction to endotoxin and the dermal tuberculin reaction. He found that intradermal injection of endotoxin caused a delayed reaction, appearing only after several hours and maximal after about 24 hours. He describes mild oedema, erythema, and induration appearing within 6—12 hours, maximal after about 24 hours. When a highly potent endotoxin preparation was used haemorrhage and necrosis were also seen about at this time.

With large doses of endotoxin Stetson observed polymorphonuclear leucocyte infiltration within 18 hours, with degenerative changes in the central portion of the lesions. With smaller doses of endotoxin there was massive mononuclear cell infiltration especially around the periphery of the lesion. Such mononuclear infiltration is also characteristic of the tuberculin reaction. Stetson produced a (tuberculin type) delayed inflammatory reaction by injecting endotoxin into the avascular cornea.

KOVÁTS, LÁZÁR and VÉGH (1963) have examined the skin reaction of rabbits after the injection of 25—50 μg potent typhoid endotoxin. 14—18 hours after endotoxin injection there was increasing induration. Between 24—48 hours necrosis developed and the induration — in most cases — diminished. The lesion healed in the course of a few days.

Histological studies revealed perivascular infiltration of polymorphonuclears after 2—3 hours ,at which time mononuclears could rarely be detected. By 6—8 hours the number of mononuclear cells increased, so that the polymorphonuclear — mononuclear ratio was 9:1. The number of mononuclears reached its peak between 24—48 hours, when the polymorphonuclear — mononuclear ratio changed to 6:4. The number of eosinophil leucocytes also increased significantly during 24—28 hours. Polymorphonuclear cells predominated for 48 hours in every case, but had declined considerably by the fourth day. Histologically recovery had occurred on the tenth day. This skin reaction is probably a mixture of the Arthus and tuberculin types. Macroscopically, the Arthus type of reaction develops slowly. It corresponds to the first part of the Shwartzman reaction.

LEE and STETSON (1960) observed that rabbits injected intravenously with endotoxin exhibited from 1 day to 1 month accelerated skin reactions to endotoxin similar to the Arthus phenomenon. Endotoxins derived from Gram-negative intestinal strains showed cross-reactivity in this respect. The accelerated reactivity could be transferred with serum, which the authors assume to be due to the presence of non-precipitating cross-reactive antibodies rather than of specific precipitating antibodies. They suggest that „crossreacting activity of the early sera may be directed against that portion of the endotoxin molecule

which determined its toxicity and which may be similar or identical from one endotoxin to another, with the specific precipitating antibody which appears later being directed against the somatic polysaccharide." This reaction seems to be similar to the Arthus type of reactions caused by antigen in sensitive animals.

The experiments of SELL and BRAUDE (1961) supply further proof to suppose that the above endotoxin reactions may be a specific hypersensitivity reaction to endotoxin. These authors have shown that human subjects injected intradermally with their own erythrocytes coated with old tuberculin gave a delayed hypersensitivity response, provided that they were tuberculin positive. Erythrocytes coated with *E. coli* endotoxin induced an immediate inflammatory response with a delayed component *in all human subjects*. From this they infer the general existence of endotoxin hypersensitivity in man.

Another experiment made by LARSON, RIBI, MILNER and LIEBERMAN (1960) supports the concept of specific endotoxin hypersensitivity. These authors have described an „inflammatory lesion" used by them for the titration of endotoxin on the skin of rabbits. This type of lesion could be elicited only by endotoxin. Polysaccharide haptens, purified *Vi* antigen and bacterial protoplasm components of *Salmonella* strains devoid of endotoxin were inert. Because of this specificity a specific endotoxin hypersensitivity may be assumed to underlie this lesion.

STETSON (1959) states that the similarity between the systemic reaction to tuberculin and that to endotoxin may be due to the contamination of tuberculin preparations by endotoxin, because relatively enormous doses of tuberculin are required to produce systemic changes. Endotoxin shock also requires relatively large quantities of material compared with the amounts of protein antigens which evoke anaphylaxis.

It may be concluded that both in the systemic and local reactions of endotoxin the features of immediate, early, and delayed hypersensitivity reactions can be found. Some of the features common to specific hypersensitivty reactions and endotoxin effects are not reproduced by a variety of non-specific insults or damage. Hence it is unlikely that this similarity is due only to the existence of a final common pathway for the processes leading to tissue damage, as stated by THOMAS (1959). If we assume the existence of a natural hypersensitivity it is easier to understand the reactions caused by endotoxin. The normal animal reacts to endotoxin both locally and systematically as if it were an allergen (antigen) to which the animal was previously sensitized.

4. The problem of desensitization of the delayed component of endotoxin hypersensitivity

Resistance both against endotoxin and tuberculin can be induced by repeated injections of the appropriate materials. In both cases resistance develops rapidly but disappears some weeks after the last injection. This is not necessarily due to a specific immunological desensitization. The mechanism of desensitization in delayed sensitivity presents a difficult problem PAPPENHEIMER and FREUND (1959) state: „Very little is known about desensitization to tuberculin-type sensitivity. At-

tempts to desensitize guinea pigs by repeated doses of tuberculin have met with questionable success. In those instances where desensitization seems to have been accomplished, many doses and very large amounts of tuberculin were required. The desensitized animals suffered severe weight loss. They were apparently in a poor condition at the end of the experiments and it is difficult to rule out the possibility that they had been rendered anergic. It is known that only minute doses of antigen are required to induce the delayed hypersensitive state and only very minute doses suffice to elicit skin reactions in sensitized animals." It is easier to accomplish desensitization in delayed hypersensitivity against a single protein antigen. However, the duration of the desensitization state is short in both instances (about 3—7 days: UHR and PAPPENHEIMER, 1958).

The production of tolerance to endotoxin is not analogous with the process of desensitization in delayed hypersensitivity as STETSON (1959) states, nor to desensitization to anaphylaxis. Tolerance to endotoxin can be reversed by reticuloendothelial blockade but the desensitized state in anaphylaxis cannot be reversed by this blockade (VÉGH and KOVÁTS, 1963).

5. The rôle of the vascular endothelium in the endotoxin hypersensitivity

DIETRICH (1941) injected rabbits intravenously with *E. coli* suspension followed 24 hr later by coli culture filtrate intravenously, as for elicitation of the generalized Shwartzman phenomenon. 24 hr after the injection of *E. coli* filtrate he found proliferation and desquamation of vascular endothelium. There was separation of the endothelial cells of the large and small vessels with subendothelial leucocyte migration and subendothelial deposition of platelets and homogenous hyaline-fibrinoid-like materials. Mixed thrombus formation followed. Bacteria and bacterial constituents (endotoxin) are meanwhile retained in the endothelial cells. Dietrich discussed his earlier experiments showing that similar endothelial and intimal reactions could also be produced by an allergic mechanism, e. g. by horse serum in sensitized rabbits. Since a single intravenous injection of *E. coli* suspensions could not produce such a lesion the mechanism of the Shwartzman-Sanarelli reaction (generalized Shwartzman reaction) was assumed to be involved.

TANAKA, NISHIMURA and YOSHIYUKI (1959) have examined the distribution of intraperitoneally injected *Salm. enteritidis* endotoxin after 24 hours in mouse tissues. Using an immunofluorescence technique they found that the most prominent localization of endotoxin occurred in the cells of the reticulo-endothelial system, especially the Kupffer cells of the liver and the large round cells of the splenic red pulp. They detected endotoxin also in the capillary and large vessel endothelium of various organs. Endotoxin was detectable for about 12 weeks in the endothelial cells of the vessels. These authors assumed that: „Invasion of endotoxin into the vessel endothelium system may be concerned with biological activity of the endotoxin, especially Shwartzman phenomenon and tumour-damaging effects".

RUBENSTEIN, LOONS and FINE in 1962 (cited by FINE, 1964) had similar results and drew similar conclusions. They also detected endo-

toxin in the macrophages of the adventitia and suggested that the endotoxin caused circulatory failure by direct action on the vascular muscle. GRAY (1964, cited by LANDY and BRAUN, 1964) could also demonstrate lipopolysaccharides in the vascular endothelium, 4 minutes after the intravenous injection of 5 μg. There was endothelial vacuolation and swelling and lipid droplets in the damaged arterial wall after about 8 hr. He found that the smooth muscles of the small arteries of the lung are extremely susceptible to injury. Within 4 minutes fragmentation and duplication of the internal elastic lamina with endothelial damage could be seen.

These endothelial alterations seem to be non-specific, yet the similarity between the allergic and endotoxin reactions points also to the possibility of a hypersensitivity mechanism. Of course, damage to the endothelial cells must be also an important factor in the vascular effect of endotoxin. The injury of the endothelial cells is rapidly followed by the adherence and invasion of platelets, leucocytes and fibrinoid material and by mixed thrombus formation.

The endothelial damage caused by endotoxin may play a role only during the first minutes after the injection. This effect deserves more intensive investigations. The role of lysosomes in the above vascular processes should also be investigated.

6. The possible course of the development of natural and acquired endotoxin hypersensitivity

The experiments of SCHAEDLER and DUBOS (1961) (discussed in the chapter on resistance) demonstrate that pathogen free mice were very resistant to endotoxin. JENSEN, MERGENHAGEN, FITZGERALD and JORDAN (1963) showed that germfree mice were similarly resistant. Establishment of the usual symbiotic flora into such mice resulted in an enhanced endotoxin susceptibility, and these authors infer that there is a connection between the symbiotic flora and endotoxin sensitivity. The development of a 1000-fold increase in sensitivity with the age (described in SMITH and THOMAS's (1954) experiment) may be explained on a similar basis.

The normal symbiotic flora of the human and animal body (WILSON and MILES, 1957) is composed of several species previously considered to be non-pathogenic (saprophytic) or facultative pathogenic strains. Later it became clear that these strains may under certain conditions act as pathogens.

Many species of the normal flora can produce endotoxin. Most of them are Gram-negative. Pneumococcus, meningococcus, influenza bacillus and haemolytic streptococcus in the nasopharyngeal and respiratory tract flora and *Veillonellae* in the oral cavity produce endotoxin.

The normal flora of the intestines is of special interest. The most common of endotoxin producers are the *E. coli* strains, but proteus and pyocyanea strains may also occur, soon after birth. These strains may be found also on the skin and in the urethra.

The most obvious way of aquiring endotoxin hypersensitivity is infection by endotoxin producing microrganisms. Under these circumstances, the membranes of intestines and other body cavities are more permeable, and endotoxins are easily absorbed. But there in a normal slow, but continous absorption of endotoxin from the bowels as shown by RAVIN and his collaborators (see below). We suggest that this slow absorption of endotoxin may be a physiologic stimulator of the non-specific defense mechanism of the organism. But even this slow endotoxin absorption may be harmful to the organism if its resistance is lowered by non-specific damage. If the endotoxin absorbed cannot be eliminated and detoxified by the cells or humoral agents it may sensitize the organism immunologically. Apart from infection this may be a mechanism for acquiring hypersensitivity to endotoxin.

Another problem is: whether endotoxin hypersensitivity can be innate. Since endotoxin cannot cross the placental barrier (PARANT and CHEDID, 1964) innate hypersensitivity can occur only through the antibodies of the mother. ADAMSON, LÖFGREN and MALMNAS (1951) have shown H and O antibodies to *E. coli* in the umbilical cord blood of newborn infants. These are precipitating types of antibodies. Delayed hypersensitivity is generally not thought to be transferred from mother to offspring, but this has not been examined in the particular case of endotoxin hypersensitivity. Antibodies which cross the placenta might play a partial role in endotoxin hypersensitivity.

KAPLAN, CATSOULIS and FRANKLIN (1965) demonstrated that human 7S and 19S globulins and its fragments readily enter the foetal circulation of rabbits. 19S type of immunoglobulin cannot cross the human placenta.

STETSON (1955) discussed the cause of the elicitation of the Shwartzman phenomenon in newborn rabbits. If its cause is hypersensitivity, it must be acquired from the mother. „... if bacterial hypersensitivity is involved in the reactions of rabbits to endotoxins, the hypersensitivity is probably not acquired. It is possible that there exists a „natural hypersensitivity", analogous to the „natural antibodies" to Gram-negative somatic antigens".

In contrast to adults, newborn guinea pigs did not respond by delayed skin hypersensitivity to endotoxin (UHR, 1962). Previous studies of UHR (1960) have indicated that active sensitization of pregnant guinea pig to protein antigens did not confer delayed skin reactivity on the progeny, although embryos could be sensitized *in utero*. The differences betveen the two species remain to be explained.

7. The type of antibody playing a possible role in the endotoxin hypersensitivity

LANDY (1962) and his collaborators elicited antibodies (opsonins-bacteriocidins) to unrelated Gramnegatives by injecting small doses of endotoxin.

LEE and STETSON (1960) observed acceleration of the endotoxin skin reaction probably by the nonprecipitating, cross-reactive antibodies.

Inasmuch as endotoxin hypensensitivity includes the features of anaphylactic-, early-, and delayed types of hypersensitivity reactions, precipitating and non-precipitating antibodies may be involved in the different endotoxin reactions.

KOVÁTS and his collaborators have shown the possible role of precipitating and non-precipitating antibodies in the elicitation of PCA reactions (see chapter III/11.).

KIM and WATSON (1965 ,see in chapter IV.) showed 19S immunoglobulin to be responsible for endotoxin tolerance in rabbits. However, its relation to endotoxin hypersensitivity is not yet known.

In the experiments of ROWLEY and TURNER (1964) a single intraperitoneal injection of *S. typhimurium* endotoxin into pig induced a rapid increase in the opsonin level and $beta_2$ macroglobulin (19S) level in the serum for some days. The macroglobulins were indistinguishable from the natural antibodies present in the pig serum.

The problem whether lower vertebrates may have (natural) antibodies and some degree of endotoxin hypersensitivity should be considered. JENKIN (1964) detected antibodies in blood of lizard and shark by opsonic tests. Jenkin quoted Good's finding that various fish species responded to antigenic stimulation like the higher vertebrates. MUSCHEL (1964) was unable to show complement or any effective bactericidal system in the lamprey, but demonstrated that the carp kills many rough species of Gram-negative organisms, apparently by an antibody-complement system.

According to our hypothesis all vertebrates possess a natural endotoxin hypersensitivity varying in extent, i. e. there are no animals non-sensitive to endotoxin.

8. Hyperreactivity to endotoxin in the course of infections and after vaccine treatments

In order to avoid confusions in terminology, the term „endotoxin hyperreactivity" is proposed to denote the simultaneous expressions of endotoxin susceptibility and hypersensitivity. In the survey of the literature, however, the original expressions of the authors will be used.

Hyperreactivity to endotoxin is especially enhanced in the course of infections and vaccine treatment.

SUTER, ULLMAN and HOFFMAN (1958), HOWARD, BIOZZI, HALPERN, STIFFEL and MOUTON (1959), SUTER and KIRSANOW (1961) have shown that intravenous injections of BCG, or H37 RV strain, or the cord factor of mycobacteria markedly increase the reactivity of mice to endotoxin. The use of dead bacteria or intraperitoneal administration are less effective in this respect. The hyperreactivity reaches a maximum at 7—9 days and persists for at least 3 weeks or occasionally more.

A similar increase in reactivity to endotoxin was found by BENACERRAF, THORBECKE and JACOBY (1959) after injecting zymosan intravenously or infecting mice with BCG. They could inhibit the enhanced endotoxin reactivity by cortisone. Therefore, they state that the impairment of the adrenal cortex is the responsible factor. The effect

of pretreatment with zymosan was dependent (also in FREEDMAN and SULTZER's (1961) experiment) upon the zymosan employed and the route of administration. On varying the kinds of zimosan the results ranged from increased reactivity to increased resistance.

PIRSCH, MIKA and VAN DER MAATEN (1957) claim that the rapid death of *Coxiella burnetti* infected guinea pigs is adequately explained by a hypersensitivity developed to endotoxin.

ABERNATHY, BRADLEY and SPINK (1958) have observed increased reactivity (susceptibility) to brucella endotoxin in brucella infected mice. ABERNATHY and SPINK (1958) suggested that dermal and systemic hypersensitivity to endotoxin in patients with brucellosis are probably due to a specific immunoreactivity to the somatic antigen.

BRAUDE and SIEMIENSKI (1961) have suggested that this type of hypersensitivity to endotoxin can be produced not only by infection, but also by immunization with endotoxin. Mice and rats were rendered more susceptible to lethal doses of homologous endotoxin by intraperitoneal injections of either *E. coli* or *Proteus mirabilis* endotoxin. The hypersensitivity to homologous endotoxin was readily transferred to normal mice by the peritoneal injection of the whole blood, but not by the serum, of previously sensitized mice.

Since whole blood was needed for the transfer of sensitivity it is probable that cellular elements (perhaps with serum factors) are the carriers of this sensitivity (see below). From this experiment it may be concluded that immunological specificity also plays some part in endotoxin hypersensitivity ,since the two strains from which the endotoxin was derived are immunologically unrelated. These authors suggest that specific hypersensitivity to somatic antigen (endotoxin) may be one of the factors responsible for the enhanced lethality but „In addition, a non-specific form of hypersensitivity to endotoxin seems to operate in those infections that induce increased susceptibility to endotoxins possessing no demonstrable immunological relationship to the infection agent."

BOX and BRIGGS (1961) have noted increased endotoxin susceptibility of mice in experimental histoplasmosis. PIRSCH, MIKA and VAN DER MAATEN (1957) have found an increased endotoxin susceptibility in *Coxiella burnetti* infection of guinea pigs. This increased susceptibility could be reduced by treating the animals with homologous antiserum or tetracycline. Box and Briggs state that a delayed sensitivity and increased susceptibility to endotoxin are separate phenomena as judged by their temporal relationship to the course of a sublethal *Histoplasma* infection. Although the susceptibility to endotoxin decreased, hypersensitvitiy to the homologous challenge persisted when most of the organisms were eliminated.

TRAKATELLIS, STINEBRING and AXELROD (1963) state that the development of systemic and cellular reactivity to PPD differs from that of hyperreactivity to endotoxin in BCG immunized guinea pigs. „Hyperreactivity appears not to be related to altered cellular reactivity in contrast to PPD sensitivity or delayed sensitivity to well characterized proteins." As already stated it is known that hypersensitivity to endotoxin occurs after some days of BCG administration but this does not

exclude the possibility that the two reactions have a different mechanism. Hypersensitivity to endotoxin appears after 6—12 days, but the maximal sensitivity to PPD develops after 21 days.

SUTER (1964) listed 16 infectious agents which cause hyperreactivity to endotoxin. He assumed that this state is not primarily due to antibody but rather to alteration at a cellular level.

9. The Shwartzman phenomenon

The phenomenon of haemorrhagic tissue reaction first observed by HANGER (1927), later by Shwartzman in 1928, now denoted as Shwartzman phenomenon is a local cutaneous haemorrhage and necrosis which develops in rabbits some hours after the second of two injections of bacterial endotoxin. The first, or preparatory injection is made intradermally, the second, or provocative injection is given intravenously about 20 hours later. Within 2—5 hours after the intravenous provoking injection purple areas of haemorrhage and necrosis appear at the site of the preparatory skin-injection (SHWARTZMAN, 1937).

This phenomenon is considered to be the consequence of the toxic properties of endotoxin and it is stated that it has no relationship with any of the immune or specific hypersensitivity mechanisms (see e. g. BOYD, 1956).

Neither the preparatory nor the provoking injection given alone can cause the reaction. The provoking injection must follow the preparatory injection within a limited period (generally 8—36 hours). It is not necessary to use endotoxin of the same strain for preparation and provocation. Shwartzman suggests that only substances (bacterial filtrates) which can cause inflammatory swelling for about 30 hours are able to prepare the skin of rabbits for the provocation of the reaction. Shwartzman stresses that the skin preparation proper does not need a toxic damage of the tissues, but causes a functional disturbance of tissue-cells, bringing about a transitory state of tissue vulnerability. This reversible state of vulnerability predisposes the tissues to striking pathological alterations following the second (intravenous) provoking endotoxin injection. The provoking injection need not be endotoxin. The Shwartzman reaction can be provoked by starch, glycogen or kaoline (STETSON, 1951/a) or agar. When the animal is anaphylactically sensitive to an antigen, the Shwartzman reaction can be provoked by the antigen given intravenously at a skin site prepared by endotoxin.

SEEGAL states (cited by BOYD, 1956) that the Schwartzman reaction differs from a local hypersensitive reaction of either the immediate or the delayed type in several ways: „1. Reinjection of material into the same site as that employed for the preparatory dose is not effective. 2. The time range between the injections differs from that of any known immunological response. 3. There is no specificity".

The Shwartzman reactivity cannot be transferred by the serum or by the tissue extracts of normal animals.

There are experiments to elucidate the tissue and cellular alterations in the reaction.

BECKER (1948) reported that the Shwartzman reaction could not be induced in rabbits treated with nitrogenmustard for a few days. He believed that this unresponsiveness was due to the action of nitrogen-mustard on the reticuloendothelial system, the vascular endothelium and, in addition, to its leucopenic effect. SCHLANG (1950) confirmed Becker's result.

The preparatory reaction of the skin includes a polymorphonuclear reaction in the tissue around the venules (APITZ, 1933; STETSON and GOOD, 1951).

STETSON and GOOD (1951) and STETSON (1951) have extended the investigations to the role of the granulocytes and showed that inhibition of local and generalized Shwartzman phenomenon following nitrogen-mustard is due to a marked decrease of granulocytes. The authors correlated the role of the granulocytes with the release of lactic acid in consequence of the enhanced aerobic glycolysis, and with the production of proteolytic enzymes by these cells.

According to the experiments of JOHNSTONE and HOWLAND (1958) in rabbits treated with nitrogen-mustard and X-ray irradiation, circulating polymorphonuclears are not an absolute prerequisite for the elicitation of the Shmartzman reaction. If the phenomenon could be induced in spite of the leucopenia caused by X-rays, a great number of leucocytes were found locally in the areas damaged by endotoxin. In rabbits treated with nitrogen-mustard together with the leucopenia a local lack of leucocytes could be observed, and in these circumstances the phenomenon could not be evoked. These authors suggest that lymphocytes, as well as polymorphs, are involved in the phenomenon.

The role of the reticuloendothelial cells in the Shwartzman reaction was first emphasized bi BEESON (1947/a) who found that rabbits made tolerant to endotoxin were unresponsive to the elicitation of the local Shwartzman reaction. However, reactivity returns after the reticulo-endothelial system is blocked by thoriumdioxide or trypan blue.

The experiments of GOOD and THOMAS (1952) also point to the role of the reticuloendothelial system in the Shwartzman reaction. Administration of thoriumdioxide or trypan blue increased sensitivity to such an extent that a generalized Shwartzman reaction could be elicited by a single intravenous injection of endotoxin, and the local Shwartzman phenomenon by a single intradermal endotoxin dose.

THOMAS and GOOD (1952 and 1953) have also succeeded in provoking local and generalized Shwartzman phenomena with a single injection of endotoxin in rabbits treated with cortisone. In their opinion this potentiating effect of cortisone is due to lympholysis which interferes with the protective function of the reticuloendothelial system.

However, this effect of cortisone on the Shwartzman reaction may perhaps be better explained by the very high polymorph count after the cortisone treatment, which favours the elicitation of the reaction. If the polymorphs were suppressed by nitrogen mustard in their experiment the reaction could not be elicited.

Considering the role of proteolytic enzymes in the Shwartzman reaction ANTOPOL and CHRYSSANTHOU (1960 and CHRYSSANTHOU and ANTOPOL, 1961) found that trypsin potentiates and trypsin inhibitors

inhibit this reaction. HALPERN (1964) could inhibit the reaction by an antiprotease, kallikrein inhibitor. ANTOPOL and CHRYSSANTHOU suggest that the activation of proteolytic enzymes by endotoxin results in action upon plasma globulin to release bradykinin and/or other vasoactive polypeptides. CHRYSSANTHOU and ANTOPOL (1964) observed that bradykinin potentiates the Shwartzman reaction, and bradykinin antagonists inhibit it (ANTOPOL and CHRYSSANTHOU, 1963). These authors found that bradykinin and serotonin antagonists together inhibited the development of the Shwartzman reaction more efficaciously than if they were administered separately. In their microscopic observations they found that haemorrhage, congestion and oedema were abolished or ameliorated but leucocytic infiltration and thrombosis were not influenced. The authors state that vasodilatation, increased permeability (increased permeability in endotoxin effect is questionable except in the brain!) and migration of leucocytes caused by bradykinin could account at least in part for the pathological findings seen in the local Shwartzman phenomenon.

In the experiments of THOMAS (1964) the active factors in leucocytes causing the Shwartzman reactivity were lysosomal acid hydrolases. THOMAS could prepare the skin for a haemorrhagic reaction by purified lysosomes, if an intravenous endotoxin dose was injected later.

Evidence was presented by STETSON (1951/a) that marked thrombocytopenia and granulocytopenia occur after the intravenous provoking injection before the onset of haemorrhage in the prepared skin site. Platelets and granulocytes were accumulated by this time in the capillary bed of the lungs and perhaps other viscera, and this may facilitate the intravascular formation of leucocyte — platelet thrombi as the reaction developed. The capillaries and small veins became occluded by these thrombi; necrosis and rupture of these vessels resulted in the extravasation of blood. Metabolic changes with hypoxia and anoxia were also thought to be involved in the development of the lesion.

The importance of the coagulation factors is also stressed in the development of the Shwartzman reaction. GOOD and THOMAS (1953) could inhibit the Shwartzman reaction by heparin. It was inhibited also by dicumarol (SPANOUDIS, EICHBAUM and ROSENFELD, 1954). VOLK and LOSNER (1955) found a prolongation of the Lee-White clotting time after the provocation of the Shwartzman reaction. This is probably due to heparin or heparinoid substances. KOVÁTS, REÖK and KARÁDY (1958), REÖK, LÁZÁR and KOVÁTS (1960) have shown the release of endogenous heparin after elicitation of the Shwartzman phenomenon, and this may protect the organism against intravascular coagulation. A similar beneficial effect of endogenous heparin was suggested by HARDAWAY and JOHNSON (1963) in endotoxin shock (see above).

THOMAS, SMITH and KORFF (1955) suggest that the intravascular deposition of fibrinoid (probably derived from fibrinogen since the plasma fibrinogen level decreases) has an important role in the development of generalized Shwartzman reaction.

KESZTYŰS, SZABÓ, BOT and JÓKAI (1958) concluded that disturbance in the carbohydrate metabolism plays an important role in the pathogenesis of the Shwartzman phenomenon. After the preparatory endotoxin dose they found hypoglycaemia, but following the intravenous

provocation they observed a significant hyperglycaemia. The activity of phosphohexoisomerase was extremely increased. This increase is probably due to muscle damage. The authors succeeded in potentiating the Shwartzman phenomenon by treatment with sympatholytic agents, especially with ergotamine. Therefore, they concluded (in contrast to many authors) that an adrenergic mechanism is not involved in the effect of endotoxin.

SZILÁGYI and DAMJANOVICH (1964) have found that local Shwartzman reaction could be inhibited or diminished by TEAB and hexamethonium if given simultaneously with the provocation. It seems probable that the above effect is exerted by the blocking of catecholamines. They have seen no effect if the above ganglion-blocking agents were given simultaneously with the preparing dose.

Nevertheless an antiadrenergic agent (Dibenamine) given simultaneously with the provoking injection into the skin site of the previous endotoxin preparation reduced the skin haemorrhage (KOVÁTS, BÁLINT and VÉGH, 1964).

SZILÁGYI, KISS and CSABA (1962) have observed that the development of the Shwartzman reaction was markedly inhibitel by alloxan diabetes and also by hyperglycaemia induced by glucose administration. In contrast, insulin treatment promoted the development of the Shwartzman phenomenon. They assume the role of the disturbance of carbohydrate metabolism in the mechanism of the Shwartzman reaction.

From the above data it may be concluded that 1. intensive leucocyte infiltration, 2. damaged reticuloendothelial function, 3. adrenergic prevalence, 4. coagulation defects and 5. metabolic (enzyme) disturbances underly the development of the Shwartzman phenomenon. The first two changes occur during the preparatory phase, whereas the third may operate in both phases, and the last two take place only after provocation of the reaction.

By counteracting all the five changes as outlined below the Shwartzman reaction can be inhibited: 1. by nitrogen-mustard, 2. by the stimulation of RES-function (by small doses of endotoxin), 3. by the adrenolytic dibenamine, 4. by anticoagulants, 5. by antiproteases. The reaction can be inhibited also by cortisone and salycilate pretreatment, as well as by serotonin and bradykinin antagonists.

10. Some data on the generalized Shwartzman phenomenon

SANARELLI (1924) has observed that rabbits given an intravenous injection of living cholera vibrios and injected intravenously 24 hours later with a sterile filtrate of *E. coli* died after the second injection. Autopsy revealed gastrointestinal and kidney haemorrhages. APITZ (1934 and 1935) produced the reaction by two intravenous injections of *E. coli* culture filtrate 24 hours apart and studied the lesions histologically. This reaction was later denoted „generalized Shwartzman reaction". The most important finding is bilateral cortical haemorrhagic necrosis in the kidneys. THOMAS (1959) stresses the role of intravascular

fibrinoid in this lesion. Areas of cortical necrosis and haemorrhage may be seen between and 24 hr after provocation.

Although there are some differences between the local and generalized Shwartzman phenomena it may be assumed that a common or very similar mechanism underlies both reactions (THOMAS and GOOD, 1952 and 1952/a). Both reactions can be prevented by nitrogen-mustard or heparin. The dose of endotoxin and the most suitable time between preparation and provocation are similar in both. The leucocyte-platelet thrombi are not characteristic of the generalized reaction, but homogenous fibrinoid material may be found within the glomerular capillaries. The provoking injection in the generalized reaction must also be endotoxin, and glycogen, starch or colloidal materials which can provoke the local reaction are ineffective. Following administration of cortisone either reaction can be provoked by a single administration of endotoxin.

GOOD and THOMAS (1952) could provoke generalized Shwartzman phenomenon in rabbits by a single intravenous endotoxin dose if the animals were prepared previously with thorotrast or trypan blue. KOVÁTS, LÁZÁR and VÉGH (1963) could also elicit a reaction resembling a generalized Shwartzman phenomenon in guinea pigs by a minute endotoxin dose accompanied by colloidal silver treatment. A RES-blockade was assumed to play a part in the above reaction.

BRUNSON (1964) was able to prevent this reaction by rendering the rabbits epinephrine-tolerant. Therefore, adrenal medulla is supposed to play a role in it.

Recently, it became clear that endotoxin is not the *conditio sine qua non* for eliciting the generalized Shwartzman reaction, since LEE (1962, 1964) was able to reproduce it in rabbits by interaction of BSA and antibody under suitable conditions. Furthermore, he stated that the blockade of the reticuloendothelial system by the first endotoxin dose is a prime condition for the elicitation of this reaction. The malfunctioning RES cannot efficiently remove the circulating fibrin polymers that lead to intravascular thrombus formation and deposition in glomerular capillaries.

11. The possible role of an immunological mechanism in the development of the Shwartzman phenomenon

The classical Shwartzman phenomenon can be elicited only in rabbits (see e. g. SHWARTZMAN, 1937; BOYD, 1956). Rapidly developing haemorrhage and tissue damage are associated with the non-specific primary toxicity; and the skin-preparing and intravenous provoking injections may employ endotoxins of unrelated bacterial strains (e. g. BEESON, 1947; BOYD, 1956; JOHNSTONE, MICHAELSON, TUTTLE and HOWLAND, 1958). Therefore, the authors suggest that the Shwartzman reaction is non-specific and is not correlated with hypersensitivity phenomena or with any specific immune-mechanism.

JOHNSTONE, MICHAELSON, TUTTLE and HOWLAND (1958) have attempted to elicit the Shwartzman phenomenon by the usual intradermal and

intravenous method in 7 different species, and were successful only in rabbits.

STONE and FREUND (1956) prepared with endotoxin the lip of rats sensitized against foreign protein. 19—21 hours later they injected the homologous antigen into the prepared lip and a haemorrhagic lesion resembling the Shwartzman reaction appeared within 4 hours. The reaction only appeared if both injections had been given into the same area. KELLY, SMITH, WODINSKY and RALL (1957) could evoke a Shwartzman-like reaction in suitable mouse strains with a single intradermal endotoxin injection. Some increase of the lesion could be observed if an intravenous provoking injection was given 24 hours later. They could inhibit the haemorrhagic reaction by dicumarol and by an adrenergic blocking agent. ARNDT and SCHNEIDER (1958) induced haemorrhagic skin necrosis in the inbred BSVS mouse strain from the Rockefeller Institute stock by means of an intraperitoneal challenge after intradermal preparation with endotoxin. In these experiments in mice or in rats, the usual sequence of intradermal preparation and intravenous provocation were not employed.

However, it has been claimed that the first phase of the Shwartzman phenomenon, i. e. preparation of the skin by endotoxin, is an immuno-specific reaction, namely local endotoxin hypersensitivity, and that under appropriate circumstances it may be elicitel by endotoxin on the skin of guinea pigs and rats also (KOVÁTS, 1961; KOVÁTS, LÁZÁR and VÉGH, 1963).

The participation of a hypersensitivity mechanism in the Shwartzman-reaction is based on four hypotheses:

1. The preparatory intradermal endotoxin injection into the skin of rabbits was suggested (previous chapter) to be partly a specific hypersensitivity reaction with anaphylactic, early and delayed components (local endotoxin hypersensitivity), and partly caused by the nonspecific primary toxicity of endotoxin.

2. Endotoxin hypersensitivity is probably present in all vertebrates coexisting with endotoxin producing microorganisms.

3. The fact that active cutaneous hypersensitivity is not sufficient to evoke the Shwartzman phenomenon in most animals, can be explained by supposing that they have a lower endotoxin sensitivity, or that they eliminate endotoxin better than rabbits.

4. The second part of the Shwartzman reaction, i. e. the intravenous provocation is a non-specific reaction, it can be elicited not only by endotoxin and acts only at a cellular level (see below).

The following experiments made in our laboratory seem to probe the above hypotheses. Guinea pigs and rats were sensitized by weekly subcutaneous injections of increasing doses of bacilli or endotoxin. (Typhoid and coli endotoxin prepared by the Boivin method were reprecipitated by ethanol. Their Shwartzman reactive unit was 3 μgm.) Animals sensitized 3—4 weeks previously with *S. typhosa* or *E. coli*, display haemorrhagic necrotic skin reactions at the sites prepared 24 hour beforehand with 25—50 μg endotoxin, after intravenous injection of 50—100 μg endotoxin. Animals not pretreated with the bacteria do

not produce haemorrhagic skin necrosis. These experiments show that the endotoxin sensitivity can be increased in these species.

Although reactivity cannot be transferred by the normal rabbit serum it can be transferred with hyperimmune antiendotoxic sera.

When rabbit antiserum, obtained by hyperimmunization with endotoxin, was injected into the skin of guinea pigs and rats, followed after 24 hours by intravenous endotoxin, skin haemorrhages resulted. The histology of the skin lesion resembled that of the Shwartzman phenomenon, but leucocyte — platelet thrombi were only occasionally seen.

Intradermal preparation with a mixture of endotoxin with colloidal silver followed by intravenous provocation with minute amounts of endotoxin mixed with colloidal silver results in skin haemorrhage in guinea pigs (KOVÁTS, LÁZÁR and VÉGH, 1963). A similar reaction was elicited also in rats if an intraperitoneal zymosan injection was also given in addition to the employment of the above method of preparation. In other experiments we succeeded in eliciting a renal lesion resembling that in the generalized Shwartzman phenomenon by a single endotoxin dose (0,007 μg/100 g) mixed with colloidal silver in guinea pigs. — The above experiments show that lesions resembling the local Shwartzman phenomenon can be elicited by a combination of intradermal and intravenous injections in guinea pigs, by blocking the reticuloendothelial system, and in rats, by blocking this system and probably inactivating the properdin system. Of course, colloidal metals and zymosan may have an effect more extensive than only to block the RES and properdin system. (LANDY and WEIDANZ (1964) put forward the possibility of identity of properdin and natural antibody.) The elicitation of the reaction under the above circumstances may be explained by localization of endotoxin in relatively high concentration in the skin by the antibody or by the effects of colloidal silver.

Normal rabbit, guinea pig or rat peritoneal cells ($2—10\times10^6$) injected intradermally into rabbits, guinea pigs, and followed by intravenous endotoxin provocation 24 hours later, result in skin haemorrhage. This reaction was previously supposed to be a passive cellular transfer of delayed endotoxin hypersensitivity, and it was assumed that the combined result of the delayed type of reaction and intravenous endotoxin brings about a Shwartzman phenomenon. Relatively low numbers of mononuclears were used to produce the above reaction. The reaction was elicited only occasionally with polymorphonuclears, injected even in five times larger amounts. Later THOMAS (1964) succeeded in eliciting a similar reaction in rabbits with lysosomes made from peritoneal polymorphonuclears. Further experiments are needed to prove the role of leucocytes and their granules in the Shwartzman reaction.

It is certainly important to prove immunospecificity in the preparation of the Shwartzman phenomenon with endotoxin. It is difficult to demonstrate immunospecificity in endotoxin hypersensitivity elicited by a single intradermal endotoxin dose (or by eliciting the Shwartzman phenomenon) when many different kinds of endotoxin can be used in rabbits. The fact that the reaction can be induced with various endotoxins may be because the rabbits have a natural hypersensitivity

against all of them. Quantitative differences would therefore be hard to detect.

We tried to demonstrate immunospecificity on the basis of resistance to endotoxin. That is to say if we induce resistance to one kind of endotoxin the Shwartzman phenomenon cannot be induced by the same (homologous) endotoxin. However, if there is any immunospecificity in endotoxin hypersensitivity or resistance it should be possible in these circumstances to induce the phenomenon by skin preparation with heterologous types of endotoxin. This proved to be the case.

The Shwartzman phenomenon was elicited in one group of rabbits (KOVÁTS and VÉGH, 1966) with *E. coli* 0,55 endotoxin used both for skin preparation and intravenous provocation. In a second group of rabbits the phenomenon was induced by *S. marcescens* endotoxin. 20, 10, 5 and 2,5 μg endotoxin was employed for preparation and 10 μg/kg for intravenous provocation in both cases. 90 per cent of the rabbits had clear haemorrhagic skin reactions with the 2,5 μg preparing dose of endotoxin. 2 days later resistance was induced by coli endotoxin in the first group and serratia endotoxin in the second group of rabbits (by 2,5, 2,5, 5, 5, 5 μg intravenous endotoxin doses given during five days). On the 6 th day we prepared the skin of all rabbits with 20, 10, 5, 2,5 μg coli and serratia endotoxins (and by *S. typhi* 0,901 endotoxin in three coli endotoxin resistant animals).

One day after the last resistance-inducing injection we tried to provoke the Shwartzman reaction by coli or serratia endotoxin.

The reaction could not be evoked in rabbits resistant to coli endotoxin (1-st group) at the sites prepared with the homologous coli endotoxin. However, at all sites prepared with the heterologous serratia or typhoid endotoxins a marked haemorrhagic reaction appeared. Intravenous provocation could be carried out either by coli or serratia endotoxin.

No Shwartzman phenomenon could be elicited in the serratia endotoxin-resistant rabbits where the skin was prepared with the homologous serratia endotoxin, but it was elicited at skin sites prepared with the heterologous coli endotoxin. Once again it could be provoked either by homologous or heterologous endotoxin.

The above experiments showed that there was immunospecificity in the *preparation* of rabbit skin for the Shwartzman reaction, but not in the intravenous provocation.

Further experiment revealed that the immunospecificity is only partial. If a more intensive resistance was induced with 7—9 endotoxin doses the phenomenon could not be prepared or provoked by any type of endotoxin employed. The reasons may be: 1. after longer treatment the non-specific elements of resistance suppress immunospecificity, 2. cross-reacting antibody to common antigens may confuse the picture.

The immunospecificity in resistance or in sensitivity is dose-dependent. By raising the preparing or provoking dose, the Shwartzman reaction can be induced by heterologous or homologous endotoxins in rabbits having a higher degree of resistance (KOVÁTS and VÉGH, 1967).

The above experiments were made at the beginning of June.

However, when the experiment was repeated in the first week of October under identical experimental conditions, the differences between the experimental groups were not so marked. The animals proved to be naturally more resistant to endotoxin. Complete resistance could readily be induced by 2—3 doses of endotoxin and the resistant rabbits required 20—50 μg intravenous serratia endotoxin to elicit Shwartzman reactions, and these were usually weak. This probably occurred because of seasonal variations in endotoxin sensitivity and the problem will be discussed in chapter IV/2. See also VÉGH and KOVÁTS, 1967.

12. The role of endotoxin in modifying the reaction of tissue proteins

There is another way in which endotoxin can modify the hypersensitive state of the organism.

EINBINDER, NELSON and FOX (1962) observed that endotoxin mixed with the antigen can enhance the development of anaphylactic sensitivity in mice. CONDIE, ZAK and GOOD (1955) have shown that endotoxin specificially stimulates the antibody-forming apparatus to produce a greater amount of antibodies. (See also JOHNSON, 1964.)

KOVÁTS (1961/a) has assumed that endotoxin could modify homologous proteins by rendering them antigenic. By the use of a homologous tissue extract + endotoxin he was able to produce tissue alterations resembling autoimmune processes. The author has observed that sensitization of rabbits with homologous myocardium homogenate + endotoxin brought about chronic connective tissue proliferation, sometimes with Ashoff-like nodules. If the animals so treated were injected with an intravenous endotoxin dose, 4 weeks after the sensitizing course haemorrhages and leucocyte infiltration also occurred at the sites of the chronic lesion. Sensitization of rabbits by homologous liver homogenate + endotoxin evoked a chronic liver injury with necrosis. Intravenous endotoxin injection in some of these animals resulted in acute focal liver necrosis. Although the lesions produced were chiefly localized in the tissues to which the animals were sensitized, other organs were also involved. Neither injection of homologous tissue homogenate, nor the endotoxin alone (in the quantity used in the experiments) produced tissue damage. The author considers that in addition to the effect of endotoxin in altering homologous proteins, the role of some drug, viruses, fungi etc. may not be excluded. „However, the prominent role of endotoxin in modifying self proteins to become antigenic may be presumed to be the consequence of lifelong symbiosis of animals with the endotoxin producing microorganism."

The above experiments are supported to some extent by DAVIES, GERY, ROSENMANN and LAUFER (1963) who found that endotoxin administered together with homologous heart tissue homogenate led to the production of anti-heart antibodies in rats. They observed focal infiltrative myocarditis which was attributed both to the endotoxin and to the administered antigen. These authors have drawn the same conclusions as KOVÁTS (1961/a). They stated: „It is suggested that the

autoimmune process can be partially explained on the basis of modification of tissue antigen by linkage to endotoxin".

Another explanation may be that the myocardium contains an autoantigen and that its weak antigenicity is enhanced by the strong adjuvant effect of endotoxin. (See also concluding remarks.)

Concluding remarks

It is not easy to prove the existence of the specific endotoxin hypersensitivity. First, endotoxin hypersensitivity is not analogous with any single type of hypersensitivity reaction because it is probably a mixture of anaphylactic, early and delayed types of reactions complicated by the non-specific reaction (susceptibility) caused by the primary toxicity of endotoxin (KOVÁTS, 1962). Furthermore, it is difficult to demonstrate the hypersensitivity character of this reaction because of the lack of animal species insensitive to endotoxin. It may be assumed that all vertebrates coexisting with endotoxin-producing microorganisms have a natural endotoxin hypersensitivity. Because of this sensitivity cannot be demonstrated in the way as other acquired hypersensitivites. Hypersensitivity can be enhanced or diminished by related or unrelated endotoxins which induce hyperreactivity or resistance. Animal species in normal conditions may have an enhanced, diminished or „latent" sensitivity to endotoxin. Experiments to be carried out in germfree animals should supply better understanding of endotoxin hypersensitivity.

There are some earlier experimental data suggesting the existence of natural endotoxin hypersensitivity.

STETSON (1955) stated that many effects of endotoxin in normal rabbits are similar to those of tuberculin in specifically hypersensitive rabbits. „The intradermal injection of both substances results in a delayed local inflammatory reaction. The reactions of normal rabbits to endotoxin were generally so like those of the hypersensitive animals to injections of the specific antigen, that the hypothesis is advanced that some mechanism may be common to both experimental models. If this latter interpretation be correct, then the possibility must be seriously considered that ‚normal' rabbits possess tuberculin type hypersensitivity to Gram-negative-bacterial somatic antigens, or endotoxins." Stetson states that there are some arguments against this hypothesis. One is that it was impossible to elicit a generalized Shwartzman reaction by tuberculin in hypersensitive rabbits.

However, LEE (1964) was able to elicit a generalized Shwartzman reaction in RES-blocked rabbits immunized with BSA if the animals were provoked intravenously by the same antigen (see chapter III/10.).

We suppose that the failure may be explained by the fact that 1. tuberculin reaction is only a delayed reaction and the generalized Shwartzman reaction involves in addition an anaphylactic and perhaps an early hypersensitivity mechanism, 2. tuberculin has a distinct kind of primary toxicity as compared with that of endotoxin.

Stetson states further: „It is difficult to conceive bacterial hypersensitivity as being transmitted across the placenta as would require to account for the susceptibility of newborn rabbits to endotoxins. It is

likewise difficult to account for the observed lethal effect of endotoxin on chick embryos."

The marked sensitivity of chick embryos to endotoxin at the age of 9—12 days was explained on an immunological basis by STETSON (1964). He stated that on about the 10—11th days of incubation the chick embryo displays for the first time circulating antibodies for some Gram-negative bacteria. Such antibodies may be responsible for the sensitivity to endotoxin which is also manifest at the same time. The subsequent loss of sensitivity could be explained — as Stetson stated — by the sequential transfer of sensitizing and blocking, or incomplete types of antibody.

Considering that antigen-antibody reactiors lead to biphasic fever STETSON (1964) assumed that this may be the case also in endotoxin reaction. Furthermore, Stetson summarized his latest opinion concerning an immunological basis in the endotoxin action: „... it now appears that all the major effects of endotoxin — fever, shock and death, skin and corneal reactions, generalized Shwartzman phenomenon as well — can be reproduced by antigen-antibody interactions in defined systems. It may be that the endotoxin also has some intrinsic pharmacological activity, perhaps manifest in its depressant effect upon RES and perhaps in its adjuvant effect on antibody production, but it must be said that all the formal demonstrations to date point to an immunological basis for endotoxin action. Earlier suggestions of pathogenic similarities between the Shwartzman and Arthus phenomena and of the existence of some form of „natural" hypersensitivity, ... and the observations of Dubos and Schaedler certainly support the view reactivity to endotoxin is strongly dependent on „natural" exposure to this antigen. It is to be expected that further qualitative and quantitative studies of the „natural" antibodies to endotoxin will provide a basis for more complete understanding of these reactions."

The Shwartzman phenomenon is an excellent model for demonstrating the local early component and the susceptibility factor in endotoxin hypersensitivity, because it shows up as massive haemorrhages. Therefore, we will now discuss some problems of the Shwartzman phenomenon in its relation to specific endotoxin hypersensitivity.

It may be assumed from the experiments mentioned in this chapter (KOVÁTS and his collaborators) that the Shwartzman phenomenon has a hypersensitivity mechanism, i. e. the first part of the reaction is an immunospecific early (and delayed) hypersensitivity reaction and the second part of the reaction, which can be evoked not only by endotoxin, but by other substances, too (e. g. starch, glycogen, kaoline) (STETSON, 1951/a), is only a nonspecific cellular reaction, perhaps due to their leucocytosis-promoting activity.

We will deal here with the immunospecific aspects of the Shwartzman phenomenon. The relative importance of the early and delayed component of the local endotoxin hypersensitivity is not yet clear. On histological grounds local hypersensitivity and primary toxicity seem to be involved and their immunospecificity is not proved. Perhaps because *enterobacteriaceae* have a common antigen component (KUNIN,

BEARD and HALMAGYI, 1962) or a common toxophor group, different endotoxins behave alike in this respect.

In the work of RADVANY, NEALE and NOWOTNY (1966) different fractions of endotoxin were obtained by chromatographic fractionation. All toxic fractions were also serologically reactive. Thus, they assume that antibody receptors and toxic properties may be present on the same macromolecule.

We tried to demonstrate the existence of immunological specificity in the preparation of the Shwartzman reaction in the following way. If we produced resistance in rabbits to *E. coli* 0,55 endotoxin it should be impossible to prepare skin sites on these animals by the homologous endotoxin, but it should be possible with heterologous, e. g. serratia and typhoid endotoxins, if immunospecificity is important in the preparation stage. This was found to be the case in our experiments. At the same time we may conclude that the chemical groups associated with primary toxicity are similar in unrelated endotoxins, but they are attached to molecules with different antigens or haptens. — The relation of immunospecificity and the resistance will be discussed in detail in chapter IV., concluding remarks, see also KOVÁTS and VÉGH 1967.

Sensitivity to some endotoxins can also be enhanced, affording direct evidence that there is a state of specific endotoxin hypersensitivity. BRAUDE and SIEMIENSKI (1961) could specifically enhance the sensitivity to the homologous endotoxin by use of coli or proteus endotoxins. SCHAEDLER and DUBOS (1961) (see chapter IV/2.) also observed specificity in inducing endotoxin sensitivity in pathogen-free mice.

Let us discuss now again the mechanism of the Shwartzman phenomenon from another point of view. The delayed hypersensitivity to endotoxin was previously considered as an important factor in the elicitation of the phenomenon (KOVÁTS, 1961). In the view of recent experiments this opinion must be altered. 2—3, or more repeated endotoxin doses (or repeatel elicitation of the phenomenon) do not diminish the delayed sensitivity but on the contrary even enhance it (unpublished observation); yet such treatment will render animals insusceptible to elicitation of the Shwartzman reaction. Thus although the basis of the Shwartzman phenomenon may be a mixed hypersensitivity, the phenomenon can be abolished by diminishing the early sensitivity and susceptibility alone.

Let us now examine the mechanism of the Shwartzman phenomenon from histological and biochemical point of view. If endotoxin is injected into the skin of rabbits, neutrophil leucocytes appear at the site of injection within 2—3 hours and their number increases for 24—48 hours. After 6—8 hours mononuclears begin to appear around the small vessels. This is the earliest period when Shwartzman phenomenon can be elicited. Although neutrophils predominate, the greatest number of mononuclears can always be seen within 24—48 hours at the time when the phenomenon is easiest to provoke. Shortly after the intravenous provocation of the phenomenon there is an apparent leucopenia and thrombocytopenia, preceding the leucocytosis which follows later. A complex chain of events will take place in this period. The leucocytes and their lyso-

somal granules are damaged (THOMAS, 1964) and acid hydrolases cathepsins are released from them, so as to injure the tissues. Injury of endothelial cells, and their separation from the vessel's walls also takes place at this time (DIETRICH, 1941; TANAKA et al, 1959). The damage caused by the intravascular and sometimes ruptured leucocytes and platelets creates conditions suitable for the formation of special thrombi which aggravate the reaction and lead to destruction of the vessels proper.

Of course, similar events, e. g. the damage of the endothelium and release of lysosomal enzymes may also occur as the final pathway of other non-specific damaging agents.

The transfer of Shwartzman reactivity by leucocytes (KOVÁTS, 1961) or by the lysosomes of leucocytes (THOMAS, 1964) points to the importance of leucocytes in the elicitation of the phenomenon. We were able to transfer such reactivity either with large numbers of neutrophils or with a smaller number of mononuclears. Of course, the role of mononuclears in carrying the sensitivity must be further examined. Although, mononuclears were supposed to carry the actual endotoxin sensitivity it may be that in both types of cells the lysosomal enzymes play a role in the elicitation of the reaction.

Thomas explains his results as follows: the granules of leucocytes and their contained enzymes render vulnerable the neighbouring tissue cells and when endotoxin is given later it causes rupture of these granules. Hypersensitivity mechanism as well as a non-specific toxic effect may lead to such labilization of lysosomes as a final common pathway. Although, rupture of lysosomes may be caused by allergic mechanisms, non-specific toxic damage or inflammation, in the case of endotoxin it must be emphasized that a specific hypersensitivity mechanism triggers probably the process. Rupture of lysosomes may also underlie endotoxin-induced endothelial injury.

The difference in endotoxin sensitivity between different species, and the failure of most species to demonstrate the Shwartzman phenomenon, depends on the effectiveness of their defense mechanism in eliminating endotoxin. We have shown that by the simultaneous administration of endotoxin and colloidal silver or zymosan a haemorrhagic reaction akin to the Shwartzman phenomenon can be elicited also in guinea pigs and rats.

The hyperreactivity to endotoxin occurring in some infections and following non-specific vaccine treatment has been used by some authors as an argument against the existence of endotoxin hypersensitivity. Our explanation of this type of hyperreactivity of endotoxin is as follows: in cases of hyperreactivity to endotoxin the infections and vaccine treatment cause damage severe enough to injure the RES, and possibly other defense mechanisms. In these conditions the organism may be anergic to endotoxin. In the cases of BCG infections, rather than desensitizing the mycobacteria may act as powerful adjuvants for development of hypersensitivity states. Since tuberculin, PPD, cord factor, or other components of mycobacteria are antigenically unrelated to endotoxin, no decrease in endotoxin reactivity would be expected. Endotoxin sensitivity is enhanced also in histoplasmosis, and after in-

jection of certain zymosan preparations. In these circumstances despite the increase of the RES-function there is increased sensitivity to endotoxin. Zymosan, it must be noted, binds many proteins e. g. bacteriocidin, the phagocytosis promoting factor, serum phosphatase and various natural antibodies. (Cited by SHILO, 1959.) It is worth considering that mycobacteria, vaccines and infections may act in a similar way: by inactivating such materials and so rendering the organism more reactive to endotoxin. The recent experiments of SAITO and SUTER (1965 and 1965/a) afford a very adequate explanation for the hyperreactivity of endotoxin in infections. These authors demonstrated that BCG infection induced a significant increase of lysosomal acid hydrolases in macrophages. If mice infected with BCG were also treated with endotoxin, they displayed not only hyperreactivity to endotoxin but a rapid and marked increase of acid hydrolases in the plasma.

The experiments of HALL, BROOM and BRUNSON (1964), and BRUNSON (1964) seem to prove that catecholamines are involved when endotoxin causes Shwartzman reactions. No generalized Shwartzman reaction can be elicited in epinephrine tolerant rabbits, and the norepinephrine tolerant rabbit fails to respond with a local Shwartzman reaction. Local reactions could occur in epinephrine tolerant rabbits even if they had been pretreated with nitrogen-mustard, so as to produce almost complete absence of leucocytes. It was therefore concluded that leucocytes might not be necessary for the production of the lesion, at least in animals made tolerant to epinephrine. This would mean that a reaction resembling the local Shwartzman phenomenon (massive haemorrhages and thrombosis) can be elicited by some different means, independently of the presence of leucocytes, i. e. by a primary toxic mechanism. It should be also born in mind that the long catecholamine pretreatment may disturb the endocrine homeostasis in some manner, and the factor responsible for the effect might not be the epinephrine itself. Furthermore, it may be that peripheral adrenergic nerves (norepinephrine) are involved in the local- and the adrenal medulla (epinephrine) in the generalized Shwartzman reaction.

The problem of endotoxin hypersensitivity should be discussed also from the point of view of symbiosis and infections with endotoxin-producing microorganism.

Endotoxin hypersensitivity may be the consequence on the one hand of infections and on the other symbiosis with endotoxin producing microorganisms. The appearance of hypersensitivity during life may actually cause or modify infections. Diseases like haemorrhagic tonsillitis, appendicitis, enteritis, colitis, are the most striking examples of such diseases. Endotoxin hypersensitivity due to the continuously absorbed endotoxin of symbiotic flora does not cause acute haemorrhagic disorders. Its manifestations are probably chronic (and sometimes secondary) alterations involving the defense mechanism specifically or nonspecifically injured so as not to eliminate or detoxify endotoxin. In this condition endotoxin may modify self proteins and render them antigenic, so causing autoimmune-like diseases. Apart from endotoxin other bacterial and non-bacterial constituents absorbed in the circulation also may modify the proteins. Viruses, fungi and drugs

probably act in a similar way in modifying the antigenicity of protein. It may be that they are more effective protein-modifying agents than endotoxin, but the importance of endotoxin lies in the constant exposure of the body to it. Of course, further experimental evidence should decide whether endotoxin has any actual role in the above processes.

It may be that only an „autoantigen-adjuvant" effect is induced in the above action of endotoxin.

Apart from endotoxin the organism may naturally be sensitive also to other constituents (e. g. polysaccharides) of symbiotic strains.

IV. ENDOTOXIN RESISTANCE AND ENDOTOXIN DETOXIFICATION

Summary

1. After repeated small doses of endotoxin there is a diminished susceptibility and diminished anaphylactic and early hypersensitivity to endotoxin.

2. The delayed component of endotoxin hypersensitivity is not affected.

3. Resistance involves two different mechanisms:

a) components of the non-specific defense mechanism: e. g. the RES, properdin, hormones, lysozyme,

b) immunological mechanisms are also involved in endotoxin resistance, sometimes including cross-resistance between different kinds of endotoxins, though under certain circumstances immunospecificity may also be observed.

4. Immunospecificity in endotoxin resistance can be demonstrated only after a short course of endotoxin injections. In response to longer endotoxin treatment the immunospecific resistance is dominated by non-specific cross-resistance to unrelated endotoxins.

5. Probably because of the long standing symbiosis with endotoxin-producing bacteria there seems to be a balance under normal conditions between endotoxin susceptibility, hypersensitivity and resistance.

6. Considering that small doses of endotoxin stimulate the defense mechanism it is suggested that the slow, continuous absorption of endotoxin from the symbiotic strains is the normal stimulant of non-specific defense mechanisms.

7. However, endotoxin in large quantities impairs the defense mechanism and smaller amounts of endotoxin may also be harmful if the defense mechanism is injured by various noxa (locus minoris resistentiae) since it cannot be eliminated or detoxified.

8. The state of resistance may be the result of two mechanisms: a) some cells and humoral agents of the organism may reduce the toxicity of endotoxin, b) the reactivity of the organism to endotoxin itself may change.

9. Repeated small doses of endotoxin may improve some diseases in a non-specific way.

10. No relationship could be detected between the phosphorous content of endotoxin and its toxicity. However, a correlation was found between fatty acid content, the antigenic structure and biological action of endotoxin in chemical detoxification experiments.

1. The absorption of endotoxin, its clearance from the blood and its distribution in the organs

The experiments of RAVIN, ROWLEY, JENKINS and FINE (1960) suggest that there is a continuous and slow absorption of endotoxin from the intestines.

After intravenous injection endotoxin is rapidly taken up by granulocytes. From the blood of normal animals radiochromium-labelled coli endotoxin disappears completely within two hours. The clearance of endotoxin from the blood of resistant animals is much quicker, taking only 15 minutes. Simultaneously with clearance from the blood endotoxin accumulates mainly in the Kupffer-cells of the liver, but it may be found also in cells of the lung and spleen (see BRAUDE, CAREY and ZALESKY (1955 and 1955/a), BRAUDE, CAREY, SUTHERLAND and ZALESKY (1955), DOERING, CLEMENS and FRITZE (1959). A similar distribution of endotoxin was found after the elicitation of the Shwartzman reaction by SZABÓ, CSONGOR, CSABA, KOCSÁR and KESZTYŰS (1961).

TANAKA, NISHIMURA and YOSHIYUKI (1959) demonstrated endotoxin in the RES-cells, particularly in the Kupffer-cells and in the vascular endothelium of all organs by means of fluorescence method. Although brain and kidney were most susceptible to endotoxin, limited amounts could be detected in the vascular endothelium of the above organs after absorption. (See also FINE, 1964.)

The significance of the absorption of endotoxin and its storage in cells, especially in the vascular endothelium, was discussed in relation to hypersensitivity.

2. Changes in endotoxin reactivity

Young rabbits are far less (about 100 fold) sensitive to endotoxin than older ones (SMITH and THOMAS, 1954). This finding may be related to the progressive increase of both endotoxin hypersensitivity and susceptibility with increasing age and can be explained by the symbiosis and infections with endotoxin-producing microorganisms.

SCHAEDLER and DUBOS (1961) have shown that a pathogen-free (*E. coli*- and *proteus*-free) strain of mice exhibited a marked resistance to the lethal effect of endotoxin. The establishment of *E. coli*- and *proteus* strains in the above mice made them sensitive to endotoxin. This sensitivity displayed some serological specificity. The authors conclude that during life hypersensitivity to endotoxin develops due to symbiosis with microorganisms. Irrespective of endotoxin-resistance pathogen-free animals were more susceptible to (staphylococcus) infections than conventional ones. Jensen and his collaborators (see also previous chapter) have shown that germfree mice were highly resistart to endotoxin.

In contrast to the above UHR (1962) found newborn guinea pigs about 7 fold more susceptible than adults to the lethal effect of bacterial endotoxin. The cause of this increased susceptibility is not adrenal deficiency or immaturity of the reticuloendothelial system.

Neither the delayed inflammatory reaction seen in adults after the intradermal injection of endotoxin, nor granulocytopenia or sustained fever after intravenous challenges, were observed in newborn guinea pigs. The difference between rabbits and guinea pigs in unexplained.

HALBERG, JOHNSON, BROWN and BITTNER (1960) have demonstrated diurnal changes in the lethal effect of endotoxin. Mice perish following a far smaller dose of endotoxin in the early afternoon than after midnight. Apart from diurnal changes there is a marked seasonal variation in endotoxin reactivity. In our experience rabbits in the autumn season were the most resistant to endotoxin. The experiments were carried out with animals of identical strains though not kept under airconditioning. According to FUKUDA (1963) rabbits were unresponsive to endotoxin in the hot sumer season. BERRY (1964) states that the experimental results obtained in mice with endotoxin in summer are not always confirmable in the winter months.

The problem of seasonal variations in endotoxin sensitivity deserves much more attention.

3. Endotoxin resistance

Endotoxin resistance (or tolerance) is the opposite of endotoxin susceptibility (primary toxicity) and anaphylactic, early type of endotoxin hypersensitivity. There is a reduction or absence of the central nervous and autonomic nervous effects, of vasomotor disturbances, haemorrhage, haemodynamic changes and endotoxin shock following the repeated injections of small doses of endotoxin.

In studies concerning the reduction of the toxic manifestations of endotoxin during the resistant state the following experiments may be cited: fever: TENNENT and OTT (1953), lethal effect: ZAHL and HUNTER (1944), CREECH, HANKWITZ Jr. and WHARTON (1949), leucopenic-: BEESON (1947), hyperglycaemic-: DELFIELD (1934), leucocyte inhibiting-: BERTHRONG and CLUFF (1953), hypotensive-: TAYLOR, CORCORAN and PAGE (1949), Shwartzman phenomenon-inhibiting-: BEESON (1947 and 1946/a), tumour necrotizing-: ZAHL, HUNTER and COOPER (1943) antibody titer enhancing effects: CONDIE, ZAK and GOOD (1955).

The state of endotoxin resistance may be the result of two mechanisms, 1. some cells and humoral agents of the organism may diminish the toxicity of endotoxin, 2. the reactivity of the organism to endotoxin itself may change.

The change in the reactions of the macroorganism indicates first an enhancement of the cellular and humoral non-specific defense mechanism. Considering that several cellular and humoral agents may eliminate or detoxify endotoxin, it is difficult to separate detoxification of endotoxin from changes in the reactivity of the macroorganism in most cases. Stimulation of the release of hormones for example, alters only the reactivity of the organism.

Apart from non-specific factors some specific changes in immunity or hypersensitivity may affect endotoxin resistance.

Let us first consider some experiments on endotoxin resistance in general. According to PETERSDORF and BENNET (1957) excessive amounts

of endotoxin cannot induce resistance for many days. The development of resistance also depends on the frequency of the endotoxin administration. Rabbits given endotoxin three times weekly react with higher fever than rabbits given the same doses daily (BEESON, 1947/a). Maximal resistance may be achieved by daily doses of endotoxin for a fortnight.

Although repeated small doses of endotoxin induce resistance to somewhat larger doses of endotoxin — the resistance is not absolute. A 3—4 fold larger dose of endotoxin is toxic to an animal although it may be resistant to smaller doses of endotoxin (BENNETT and CLUFF, 1957). It is known that rabbits may be made unresponsive to the Shwartzman phenomenon when the same provoking dose is repeated 2—4 times (BEESON, 1947/a). However, if the provoking dose was repeatedly stepped up 2- or 3-fold we found that the Shwartzman reaction could be elicited repeatedly. (VÉGH and KOVÁTS unpublished observation.). In spite of the disappearance of Shwartzman reactivity the delayed skin reaction increased after such serial endotoxin doses.

Resistance is only a transitory state. If endotoxin injections are stopped resistance will diminish or cease within 2—4 weeks (BEESON, 1947 and 1947/a; BENNETT and CLUFF, 1957; WATSON and KIM, 1963).

The non-specificity of resistance was previously considered to be one of its most characteristic features. Animals made resistant to one kind of endotoxin would be resistant to endotoxins derived from other bacterial species. Later, resistance proved to be far more marked for the homologous endotoxin, indicating that serological specificity may also be involved in resistance (SCHAEDLER and DUBOS (1961); MERGENHAGEN and JENSEN (1962); WATSON and KIM (1963); KOVÁTS and VÉGH (1966 and 1967), see previous chapter).

4. The role of normal humoral and cellular factors in resistance

Normal plasma or serum can diminish the toxicity of endotoxin. HEGEMANN (1957 and 1959) has shown that fresh human serum is antipyrogenic. According to RALL, GASKINS and KELLY (1957) fresh rabbit serum can neutralize the pyrogenicity of endotoxin. LANDY, SKARNES, ROSEN, TRAPANI and SHEAR (1957), SKARNES, ROSEN, SHEAR and LANDY (1958) described an agent in fresh blood of man and various animals which neutralized the tumour-necrotizing ability of endotoxin. The endotoxin detoxifying component (EDC) of plasma proved to be heat labile and to act only on incubation. According to the above authors this substance is neither complement, properdin, nor specific antibody.

It has been shown by LANDY and PILLEMER (1956 and 1956/a) and by PILLEMER, SCHOENBERG, BLUM and WURZ (1955), that the properdin level rises after administering endotoxin. The properdin system can bind endotoxin, and properdin is probably identical with the natural antibody to endotoxins (see below).

GREISMAN, CAROZZA and HILLS (1963) performed experiments to show that a humoral mediator (an opsonin with high endotoxin specificity) operates in the development of resistance.

Small doses of endotoxin stimulate RES-activity (BENACERRAF and SEBESTYEN (1957), HOWARD (1959). Larger doses first block, and later stimulate the RES. In BEESON's (1947 and 1947/a) experiments reticuloendothelial blockade abolished the resistance to endotoxin. Consequently it was considered that reticuloendothelial cells play a decisive role in the development of resistance and detoxification. Furthermore, since BEESON found that the resistance disappeared when the antibody level began to rise he concluded that resistance is not dependent on an immune mechanism.

Nevertheless since in BEESON's experiment the RES-blockaded normal animals were more sensitive than RES-blockaded tolerant animals, RES-blockade must abolish only one part of tolerance.

It should be noted that the methods used in RES-blockade are damaging and do more than merely block the function of RES-cells (JANCSÓ, 1955).

The key role suggested for the reticuloendothelial system was not supported by the work of BOEHME and DUBOS (1958) who found no evidence of any direct causal relationship between reticuloendothelial system activity and resistance (see chapter IV/9.). Furthermore, FREEDMAN (1960) was able to transfer endotoxin resistance (see chapter IV/5.) by the plasma of RES-blockaded animals.

WEISSMANN and THOMAS (1964) considered that the RES-cells are the primary targets of the action of endotoxin. They suggest that this concept is supported by data showing that RES-blockade abolishes endotoxin tolerance and enhances the susceptibility to endotoxin, and furthermore, that a single intravenous endotoxin challenge under RES-blockade elicits the generalized Shwartzman reaction. These authors state that endotoxin (after „sham phagocytosis" and metabolic changes) damages the membranes of the lysosomes of the RES-cells inducing the release of lytic enzymes.

In our opinion in these examples given by Weissmann and Thomas, the RES-cells were already damaged by Thorotrast (the RES-blocking agent) and the further effect of endotoxin upon these cells is questionable. But, in the absence of a well-functioning RES, endotoxin can act on, for example, the adrenergic nerve-structures (primary toxic targets) more effectively than on already damaged RES.

However, apart from the primary toxic targets there are undoubtedly also cellular targets of endotoxin attack, and the RES in one such target. It includes vascular endothelial cells which play a special role in haemorrhages and in thrombus formation; leucocytes which may be a main source of endogenous pyrogen; macrophages and mastocytes may also be damaged by endotoxin (perhaps with the release of toxic material). — The RES is certainly a part of the defense mechanism against endotoxin and can even be stimulated by small quantities of endotoxin. With moderate doses of endotoxin the RES is damaged, but this damage is only transitory and is followed by increased function. Larger doses of endotoxin injure all cells severely, and the RES is then only one of the cellular targets of endotoxin.

Apart from RES-cells, other cells also may suffer direct damage from endotoxin, but this is not strictly proved. Platelets are destroyed

within seconds after endotoxin-exposure *in vivo* and *in vitro*. The vascular endothelium is damaged within 4 minutes. Injury of the leucocytes and macrophages was observed after some minutes.

How can the action of endotoxin upon these cells be explained? It may be assumed that the mechanism is immunological, i. e. that the cells are already naturally sensitized and are injured by the antigenic action of endotoxin, with the consequences already described.

It should be noted that larger doses of endotoxin are necessary for this cellular damage than for the primary toxic action. The immunological (hypersensitivity) manifestations always require large doses of endotoxin.

Nevertheless, reticuloendothelial cells may play a certain, but not exclusive part in the detoxification of endotoxin. RUTENBURG, SCHWEINBURG and FINE (1960) observed that macrophages detoxify endotoxin *in vitro* and this detoxification runs parallel with the splitting off of phosphorous.

KERBY (1952) claimed that polymorph leucocytes can also inactivate endotoxin. Kerby's experiment demonstrated that the above action may be due to the transport function of leucocytes on the one hand and to lysozyme on the other.

Since adrenalectomy enhances the reactivity of animals to endotoxin it was suggested that adrenal hormones play a role in the defense of host against endotoxin. Adrenalectomized animals could be made resistant despite the absence of corticoid secretion (CHEDID, PARANT, BOYER and SKARNES, 1964). This proves that adrenals or adrenal cortical hormones are not indispensable for the development of endotoxin resistance. Since neonatally thymectomized mice can be made as tolerant to endotoxin as can control mice and, furthermore, cortisone overdosage rendered mice highly susceptible to endotoxin, the above authors assume that no immune mechanism is involved in tolerance.

RUTENBURG, RUTENBURG, SMITH and FINE (1965) state that an endotoxin detoxifying protein-principle is present only in a considerable quantity in the spleen, whilst in liver and lung it is present only in a minute amount. Since the degree of tolerance varied with the potency of the detoxifying protein and not with antibody titer, they consider that an adaptive enzyme response is involved in tolerance or detoxification. The author's interpretation does not deny the role of immune substance, which does not essentially contribute to detoxification or the defense mechanism. They namely found high antibody titer in shock in which state the animal lost its detoxifying ability.

(However, this antibody may be anti „O" which is not related to defense [protection] against endotoxin) (see elsewhere).

The experiment of CHEDID, PARANT and BOYER (1966) strongly incriminates the liver as a major site of endotoxin detoxification.

5. The passive transfer of endotoxin resistance

FREEDMAN (1959 and 1960) has shown that endotoxin resistance can be transferred passively by the plasma or serum of resistant animals. FREEDMAN and SULTZER (1964) injected rabbits with small doses of plasma from endotoxin-resistant donors insufficient to modify the course of fever produced by the first test dose of endotoxin. These small doses resulted in a state of resistance when the animals were retested 24 hours later even with a larger dose of endotoxin. The authors conclude that the induction of tolerance to endotoxin requires both endotoxin and a blood factor (mediator) appearing in the endotoxin-treated animals. On the basis of these experiments they deny the possibility that the humoral mediator of resistance acts directly upon the endotoxin by inhibition, detoxification, or opsonization, so effectively diminishing the dose of endotoxin to the animal. (Since Freedman and Sultzer gave the plasma and endotoxin about at the same time (within 5 minutes) an antibody-antigen complex might have been formed in the organism which stimulated the antibody forming-, and perhaps the nonspecific defense-apparatus more intensively.)

FREEDMAN (1964) later recognised the possibility that the humoral mediator of tolerance may be an antibody. He added that: „If it is an antibody, our results would suggest that tolerance is a host response initiated by an antigen-antibody interaction."

6. Non-specific stimulators of endotoxin-resistance

From what has been discussed previously it is not surprising that non-specific stimuli or substances can also induce resistance to endotoxin. ZWEIFACH (1961) demonstrated that repeated shock-treatment increased the resistance to endotoxin. RASKOVÁ and VANECEK (1961/a) induced resistance to *Shigella shigae* toxin by repeated drum shock, RASKOVÁ and VANECEK 1961) could also elicit a long-lasting resistance to endotoxin and to different toxins by repeated phenol injections. However, such resistance was less marked than that resulting from endotoxin pretreatment. Other authors have elicited resistance with various irritating substances (cited by RASKOVÁ and VANECEK, 1964).

The above experiments definitely show that nonspecific mechanisms play a part in the induction of endotoxin resistance.

Since endotoxin releases histamine the development of endotoxin resistance was explained by ZWEIFACH (1960) on the grounds not that histamine release stimulates the reticuloendothelial system, but that it increases the resistance of the organism to shock, endotoxin and irritating substances.

7. Biochemial, morphological and metabolic factors in resistance

The experiments of JANOFF and ZWEIFACH (1963) suggest a new explanation for endotoxin resistance on a morphological and biochemical basis. They suggest: „... that stabilization of hepatic lysosomal membra-

nes in endotoxin-tolerant animals represents a selective response of these particles to repeated small doses of the toxin and plays an important role in the development of tolerance to this agent." „Carbon tetrachloride induced leakage of 2 acid hydrolases from mouse liver lysosomes, in vitro, was inhibited by chronic pretreatment of mice with endotoxin. Cortisone pretreatment inhibited one of the enzymes. Pretreatment of the mice by Thorotrast ,a reticuloendothelial-blocking agent which neutralized tolerant states, had the opposite effect and caused accelerated release of acid hydrolases from liver lysosomes of tolerant as well as normal animals." The release of these enzymes may be responsible for different injuries of the cells.

FUKUDA and AKIYAMA (1963) also suggested that there is a connection between endotoxin-tolerance and liver metabolism. They state: „... one of the basic mechanisms of the tolerance seemed to be the stabilization of liver metabolism which prevents the depletion of liver glycogen responsible for the prostration". „That this might be due to the ability of liver metabolism was also suggested by the reduced metabolism response to thyroid in the tolerant state."

One should not forget the possibility that changes in the lysosomes and liver metabolism may be late consequences rather than causes of the evolution of endotoxin resistance!

8. The possible role of an immune mechanism in the development of endotoxin resistance

WHITBY, MICHAEL, WOODS and LANDY (1961), LANDY (1962) have designed experiments to show that increased phagocytic activity of mononuclears and the concomittant action of specific epsonins are the factors responsible for endotoxin resistance. Considering that opsonin represents a specific antibody, endotoxin resistance is connected with some immune mechanism. „Because absence of specificity is the most striking feature of this tolerance to endotoxins it is generally believed that immunity, in the classic sense, plays no part in the development of this resistance".

The role of an immune mechanism in endotoxin resistance is apparent from JAQUES and JAQUET's (1962) interesting experiments. They found that about 10 times greater EDC capacity could be demonstrated in the plasma of rats immunized with endotoxin than in that of normal rats. These authors consider their result as compatible with the idea of the EDC being an antibody.

In our own experiments (VÉGH and KOVÁTS, unpublished observations) we have also found that the serum of rabbits hyperimmunized with endotoxin diminished the toxicity tenfold. The diminution of endotoxin toxicity was assayed by its capacity to elicit the Shwartzman reaction. Since the endotoxin can be recovered by phenol-extraction from the incubated hyperimmune serum-endotoxin mixture in a similar manner as from normal serum *plus* endotoxin mixture control, the above effect may be no more than the neutralization of endotoxin in an antibody-endotoxin complex.

In studies of resistance in rabbits WATSON and KIM 1963) explained tolerance to endotoxin on an immunological basis. The authors observed — as described earlier by BEESON (1947 and 1947/a) — that resistance ceased in rabbits after 35 days. By injection of a single small dose of the original endotoxin resistance could be restored. This mechanism was compared with the immunological anamnestic response.

In these experiments the tolerance was observed 2 months after the injection of 3 weeks old rabbits with large doses of endotoxin. The authors assume this to be similar to Felton's immune paralysis. The rabbits were less susceptible to the pyrogenic, lethal and skin reactive effects of endotoxin. They explain tolerance in the same way as WHITBY et al. (see above): tolerance is based on the presence of specific antibodies which assist the reticuloendothelial system in the clearance and destruction of endotoxin.

They could not induce tolerance to the parent endotoxin by pretreatment of rabbits with Lipid A. Accordingly the endotoxin molecule must contain one component (toxophore group) responsible for the primary toxicity and another component for hypersensitivity. It should be commented that Lipid A and the parent endotoxin may show no cross tolerance because Lipid A has lost most of its chemical and antigenic relationships with the parent endotoxin (RIBI, HASKINS, LANDY and MILNER, 1961).

MOSES and ATKINS 1961) consider that tolerance to tuberculin superficially resembles to that of endotoxin. There is, however, a certain degree of immunospecificity since in both cases the tolerant animals respond normally and produce endogenous pyrogen when unrelated pyrogenic agents are injected.

According to KIM and WATSON (1966): „Colostrum-deprived piglets lacking detectable immunoglobulins were susceptible to the lethal effect of endotoxin. It is concluded that endotoxin has a true primary or intrinsic toxicity not dependent on antigen-antibody reactions. Colostrum-fed piglets showed an increased resistance greater than 10-fold to the lethal effects of endotoxin." According to the authors the antibody appearing in the colostrum-fed sera is thought to be distinct from O-antibody and is comparable to the protective antibody of the 19S class isolated from immunized rabbits.

They state further: „The presence of a protective immunoglobulin in sow colostrum capable of passively protecting these piglets against the primary toxicity gives additional evidence for the involvement of a classical immune mechanism in endotoxin resistance. It also suggests that an antigenically common toxophore group is present in most endotoxins regardless of the source, and that antibodies specific for this group can assist the RES in the destruction of the toxin."

Considering Kim and Watson's experiment the presence of non-detectable antibodies in colostrum-deprived piglets cannot be excluded. Moreover, the presence of delayed sensitivity cannot either by ruled out because it is independent of circulating antibodies. Furthermore, the increase of toxicity to 10-fold may be near to the range of normal variability, and the number of test animals should also be increased to draw a final conclusion. However, if in further experiments these

results will also prove to be valid this contradicts (to some extent) the results obtained with germ-, or pathogen-free mice which tolerated far larger doses than conventional mice. In these cases the increase of immunoglobulin seems to be in correlation with the increase of sensitivity and not with resistance.

KIM and WATSON (1965) have shown that 19S immunoglobulin isolated from tolerant rabbit serum conferred complete tolerance in respect of pyrogenic and lethal effects of endotoxin on normal rabbits. They found no quantitative correlation between the presence of antibodies to ‚O' antigen and the ability to transfer tolerance. „It is concluded that endotoxin tolerance involves a classical immune mechanism which includes both 19S immunoglobulin specific for toxophore groups common to many endotoxins and a normally functioning RES."

It may be that two different types of 19S antibodies develop in response to endotoxins or Gram-negative bacilli, one responsible for endotoxin resistance as suggested by Kim and Watson, the other being anti-O or protective antibody. If two such kinds of 19S macroglobulin exist then both should be implicated in the infection-resistance (see chapter IV/9.). The role of 7S globulins in endotoxin resistance is not proved.

LANDY and WEIDANZ (1964) state that natural antibodies to Gram-negative organisms in the sera of mammals are also macroglobulins. Their specificity was proved, to some extent, by absorption and inhi bition tests. There are, however, also cross-reacting antibodies because Gram-negative bacteria possess a common hapten (KUNIN, BEARD and HALMAGYI, 1962). These also have bactericidal activity. Such antibodies are ubiquitous in adult mammals, and are probably induced by symbiosis with Gram-negative flora. Landy et al found markedly reduced levels of natural antibody in germ-free animals. After the contact of germfree animals with Gram-negative bacteria or with their somatic antigens a prompt rise in antibody titer followed.

Thymectomy or pretreatment of animals with irradiation or immunosuppressive drugs did not influence the level of natural (anti-coli) antibodies. However, a single intravenous injection of bacterial endotoxin raised the level of *heterologous* antibodies Repeated injection of endotoxin caused an increase in *homologous* antibody.

According to Landy natural antibodies are probably identical with properdin. This is indicated by the many similar characteristics of both substances.

BRAUN and KESSEL (1964) assume that cytotoxic effect of endotoxin resulting in DNA breakdown products stimulates an immunological mechanism which contributes to host resistance. In the first phase endotoxin associated with the surface of macrophages and other cells acts either by itself or in combination with preexisting specific antibody. The result is damage to the cell membrane and leakage of DNA breakdown products which stimulate preexisting antibody forming cells (presumably by enhancing DNA synthesis). The consequent elevation of antibody level will increase host resistance. According to these authors

the experimental evidence of this concept is still circumstancial. „However, the picture fits in well with the growing recognition that many of the endotoxin initiated biological effects involve phenomena akin to, but not necessarily identical with, phenomena of delayed hypersensitivity. This is not surprising, since we are dealing in the case of endotoxins with materials containing antigens that are almost ever present in the natural environment of man and animals."

BRAUN and KESSEL discuss the possibility that in higher organisms there has evolved a state of dependence of the antibody forming cell system on materials released from macrophages and other phagocytes. Thus, host defense (resistance) would be activated by cell damage. The prompt rise in specific antibody level occurs only when the animal has experienced more than one exposure to the antigen.

Instead of the above hypothesis we propose the following: Endotoxin damages the membranes of cells since they are naturally sensitized to it. Obviously leakage of DNA products will ensue in this case also and may enhance the formation of any kind of antibody, but in any case specifically primed antibody-forming cells will respond more readily (anamnestic reaction). In addition to antibody synthesis endotoxin as a physiological stimulator stimulates the function and generation of cellular elements (RES).

9. Endotoxin resistance and its relationship to infection-resistance and non-specific disorders

Endotoxin can change the reactivity of the organism in two ways: smaller doses enhance, and larger doses first diminish but later enhance resistance in various infective and non infective diseases. Though endotoxin has a similar effect upon the activity of the RES, the latter probably does not play an exclusive role in enhanced resistance to infections.

Experimental data suggest that infection-resistance depends upon the reticuloendothelial cells and antibody. The mechanism by which endotoxin enhances resistance against infections and other disorders is very complex, more complex than that involved in the resistance to endotoxin itself. The specificity or otherwise such resistance depends on dosage and timing of endotoxin administration and on the activation of immune and non-specific factors.

Now let us examine some examples of the relationship between endotoxin and infection-resistance.

DUBOS, SCHAEDLER and BOEHME (1957) observed increasing resistance to staphylococcal and mycobacterial infections 1—4 weeks after the injection of 5—50 μg endotoxin. Conversely, resistance was depressed and mice succumbed rapidly if endotoxin was injected within a few hours after the challenge injection. In these experiments the resistance lasted longer than the enhancement of activity of the RES, and no direct causal relationship could be found between the two.

The brief period of enhanced susceptibility which preceded the increased resistance after endotoxin administration may be due to the

initial leucopenia (FUKUI, 1964). This susceptibility is also dose-dependent, because smaller doses of endotoxin did not produce such an effect, but enhanced protection at a time when a larger dose depressed it.

The serum opsonin content and the activity of phagocytes was enhanced after injection of endotoxin in mice in the experiments of JENKIN and PALMER (1960). They observed that macrophages are more active in phagocytosis of bacteria, in the presence of serum opsonin, after endotoxin pretreatment. Furthermore, macrophages of endotoxin-treated mice were more efficient than those of normal mice.

The role of natural antibodies and their specificity was described by LANDY (1962) and LANDY and WEIDANZ (1964). They assume that RES cells (macrophages, mononuclears) play a role in endotoxin resistance in concert with specific opsonins (antibody-like agents). As described in the chapter IV/8. they were able to elicit ‚natural' antibodies to unrelated Gram-negative organisms by injection of a single, small dóse of endotoxin. How far such a mechanism is involved in endotoxin and infection-resistance was discussed above.

Endotoxins induce both early and late resistance to Gram-negative organims. The early resistance develops rapidly in mice and lasts for a few days only. LANDY and PILLEMER (1956/a) found such a rapid resistance to 18 endotoxins after *Salm. typhosa* infection of mice. They found, in addition, that typhoid endotoxin rapidly induced a high level of resistance to infections with *E. coli, P. vulgaris, Ps. aeruginosa* and *Klebsiella pneumoniae,* but not to *Staph. aureus, Streptococcus pyogenes,* or *Diplococcus pneumoniae.* — They explained their results by a rise in the level of the serum properdin. The properdin system is only active against certain Gram-negative, and not against Gram-positive organisms, and is probably identical with natural antibodies.

The late resistance to infections was of the classical type of prolonged acquired immunity to systemic infections, but this was not examined very extensively.

ABERNATHY (1957) observed a complete and specific resistance to brucella infections in mice immunized by *Br. melitensis* endotoxin. Yet immunization with this endotoxin does not protect against infection with *Salm. typhi, E. coli,* or *Shigella sonnei.*

SCHAEDLER and DUBOS (1961) reported that a pathogen-free (*E. coli* and *proteus*-free) strain of mice had a marked resistance to the lethal effect of endotoxin. Reestablishment of E. coli and proteus strains in such mice made them sensitive to endotoxin. This sensitivity displayed some serological specificity. The authors conclude that a hypersensitivity to endotoxin develops due to symbiosis during life. In spite of endotoxin-resistance the pathogen-free animals were more susceptible to (staphylococcus) infections than conventional ones. These authors were able to induce resistance to endotoxin by injection of killed Gram-negative bacteria or BCG. They conclude that the pathological effect of endotoxin can be explained by at least two different mechanisms: 1. primary toxicity (which causes a weight loss of the mice and the enhancement of the infection to small doses of endotoxin), 2. immune reaction which is evidenced by the death of the animals sensitized by endotoxin.

SCHAEDLER and DUBOS (1964) state: „The intestinal flora seems to play a major role in the host's ability to respond to endotoxin and infections. The bacterial relationships which were responsible for this phenomenon are not completely defined. However, it appears that the Gram-negative bacilli must have the capacity to „invade" or „infect" the host in order to sensitize.'

JENSEN, MERGENHAGEN, FITZGERALD and JORDAN (1963) reported that germfree animals (as well as pathogen-free animals) are much more resistant to the lethal effect of endotoxin.

LANDY, WHITBY, MICHAEL, WOODS and NEWTON (1962) found that germfree mice have the same susceptibility to *S. enteritidis* and *Sh. flexneri* endotoxin as conventional ones. The cause of this conflicting result may be (according to Jensen and his collaborators) that the above endotoxins were derived from stains not indigenous to the mouse

The difference between germfree and conventional animals is evident in the resistance to endotoxin and infection.

The great resistance of germfree animals to endotoxin is due to the lack of their exposure to endotoxin. Exposure to endotoxin (or to bacteria) activates the RES and the non-specific defense mechanisms as well as the antibody-forming apparatus. It may be seen that the development of resistance to infections is connected with the development of sensitivity to endotoxin in conventional animals. This may be the consequence of symbiosis with endotoxin-producing microorganisms.

If in sensitive animals an endotoxin resistance is induced it will rather enhance the resistance to some infections. Let us see now some examples of endotoxin pretreatment (resistance) in experimental infections.

According to SHILO (1962) lipopolysaccharides given 24 hours prior to challenge, induced an increased resistance towards dermonecrotic staphylococcal lesions for at least seven days.

In the experiment of MICHAEL and MASSEL (1962) endotoxin derived from several species of Gram-negative bacteria induced non-specific resistance to typhoid bacilli, but not to infection with virulent strains of Group A streptococci. Nevertheless if in the latter case a minute amount of type-specific antiserum (which was ineffective by itself) was given after endotoxin a marked degree of protection was obtained.

BERGER and FUKUI (1963) were able to protect against *Ps. aeruginosa* infection in mice with *E. coli* endotoxin.

ABERNATHY, BRADLEY and SPINK (1958) demonstrated that injection of brucella endotoxin into mice was followed by resistance both to endotoxin and to brucella infections. In contrast, infection of mice with *Br. melitensis* increased the susceptibility of the animals to the lethal effect of brucella, typhoid and coli endotoxins.

There are experiments showing the effect of endotoxin resistance in viral diseases. WAGNER, SNYDER, HOOK and LUTTRELL (1958) state that endotoxin administered 24 hours before challenge increased the resistance to intracerebral infection with EEC and EMC viruses. Endotoxin inhibits moderately the multiplication of EMC virus in the brain, but not in tissue culture. The authors assume therefore that the effect is not due to viral interference.

Hook and Wagner (1959, 1959/a) induced resistance to *Salm. typhimurium* infection and to the neurotoxic effects of influenza virus by means of coli endotoxin (10 μg) pretreatment 24 hours previously.

Gledhill (1964) claims that endotoxin treatment of mice results in a dual action on ectromelia infection, namely a large dose lowers, a small dose raises the host resistance. The lowered resistance is explained by the activity of endotoxin in rendering RES cells better able to synthesize virus. The raised resistance is explained by a diffusible sparing substance present in the blood of mice treated with endotoxin.

Infections also may induce resistance to endotoxin:

Mergenhagen and Jensen (1962) injected mice with viable or heat-killed *Veillonella* strains and thus increased the resistance to the lethal effect of *Veillonella* enotoxin. Resistance was best developed to endotoxin from the serologically specific strain used for vaccination.

McCabe (1961) found resistance in man to the pyrogenicity of a heterologous endotoxin following pyelonephritis caused by Gram-negative bacteria.

Greisman, Hornick, Carozza and Woodward (1963) observed that the patients showed resistance to the pyrogenic effect of typhoid and coli endotoxin during the first 12 days after cessation of fever caused by *typhus abdominalis*. The authors conclude that physiologically active quantities of circulating endotoxin contribute to the pathogenesis of the illness.

It should be noted that vaccine pretreatment or infections may easily have the opposite effects, namely: hyperreactivity to endotoxin, as was seen in chapter IV/8.

The capacity of endotoxin to lower resistance is evidenced by the following experiments. Skin infections with *Staphylococcus aureus, Cl. perfringens* and *Ps. aeruginosa* were intensified in guinea pigs by sublethal doses of endotoxin given within 2—3 hours (but not later) intraperitoneally (Miles and Niven, 1950). The most probable explanation of these experiments and those of Braude and Siemienski (1961) is the local inhibition of leucocyte diapedesis.

McKay, Jewett and Reid (1959) examined the causes of materal death and stated that the uterus was the portal of entry of endotoxic materials that caused bilateral cortical necrosis of the kidneys and vascular collapse. It has been demonstrated that a reaction resembling the generalized Shwartzman phenomenon could be provoked by a single dose of endotoxin in pregnant animals.

Zahl and Bjerknes (1943) described decidual haemorrhages caused by relatively small doses of endotoxin in pregnant animals. According to Kass (1960) prematurity that might have been caused by decidual injury was encountered twice as frequently in the infections of the renal tract with bacteriuria as in normal women.

The lethal effect of irradiation (e. g. gamma, X-ray) may be due to overwhelming infection (Fishman and Shechmeister (1955), Miller, Hammond and Anderle (1960).

According to Hook, Carey and Muschel (1960) irradiation injury diminishes the defense mechanism of the organism.

SMITH, ALDERMAN and GILLESPIE (1958) have noted that pretreatment with endotoxin protected mice against lethal doses of irradiation. Susceptibility to irradiation can be restored by RES-blockade.

Endotoxin may play a secondary role in the final outcome of non-specific injuries which do not depend on infections. ZWEIFACH and THOMAS (1957), ZWEIFACH, BENACERRAF and THOMAS (1957) have demonstrated that 87 per cent of the rats made tolerant to endotoxin survived 850 turns in the Noble-Collip drum in contrast to 15 per cent of controls.

FINE (1956), RAVIN, SCHWEINBURG and FINE (1958) have found that endotoxin is responsible for the lethal outcome of irreversible haemorrhagic shock. According to their experiments the source of endotoxin is the flora of the intestinal tract. Endotoxin released from the intestinal tract is not harmful in healthy animals. They found that the resistance of the animals to haemorrhagic shock could be increased by elimination of the gut bacteria with antibiotics. MCCLUSKEY, ZWEIFACH, BENACERRAF and NAGLER (1960); NAGLER and ZWEIFACH (1961); KOVÁCH (1961); however, were unable to confirm these results.

GECSE, KARÁDY and WEST (1965) in their experiment concerning endotoxin shock subjected rats to antibiotic treatment which exterminates the intestinal flora. These authors could not observe any beneficial effect of the ‚endotoxin deficiency' on traumatic shock caused with Noble-Collip drum.

Some experiments have been carried out to examine the relationship between anaphylaxis and endotoxin shock. EINBINDER, NELSON and FOX (1962) stated: „Pretreatment of sensitized mice with small amounts of endotoxin up to 3 hours prior to challenge with antigen enhances the incidence of fatal anaphylaxis. In contrast, administration of endotoxin from 4 to 24 hours, prior to challenge is protective, and the evidence of fatal anaphylaxis is decreased... Mice made tolerant to endotoxin by repeated injections are also tolerant to anaphylaxis. Endotoxin administered at the time of sensitization may act as an adjuvant, but the incidence of fatal anaphylaxis is not increased significantly."

OZEREDZKOVSKIJ (cited by PLANELLES, BUDNITZKAYA, 1961) state that an endotoxin-preparation denoted Pirogenal had no effect in a single dose, but when administered in 5 doses (5—20 μg) in the last five days of sensitization it reduced the anaphylactic death in guinea pigs. Pirogenal administered in a similar manner depressed the inflammatory reactions in rabbits which would have been expected to show both active and passive Arthus phenomena. Large doses enhanced the necrotic area in spite of the reduction of inflammatory reactions. This effect may be explained by the diminution of capillary permeability caused by endotoxin. (Pirogenal inhibits the increase in capillary permeability induced by histamine.)

Susceptibility and early hypersensitivity to endotoxin may play a part in resistance or in various disorders and non-specific damages. The role of delayed endotoxin hypersensitivity in these alterations is not evident.

10. The non-specific endotoxin therapy

Endotoxin can induce a specific protection, for example typhoid endotoxin protects against typhoid fever, etc. Some experiments on non-specific therapy of diseases caused by unrelated microorganisms and agents will be discussed below. The resistance-promoting activity of endotoxin and stimulation of non-specific antibody may be responsible for the beneficial result of the therapeutical trials described below.

FRICKE, PROBST and SCHUMACHER (1958) studied the effect of purified *Salm. abortus equi* endotoxin denoted Pyrexal. This preparation influences favourably both acute and chronic salpingitis in doses of 0,1—2,25 μg given intravenously. The above authors explain this effect as due to stimulation by endotoxin of the reticulo-endothelial apparatus, the properdin system, the leucocyte and pituitary-adrenal axis.

WESTPHAL and SIEVERS (1960), ARGENTON, BECKER, FISCHER, OTTO, THIEL and WESTPHAL (1961) carried out therapeutic trials with Lipid A prepared from *E. coli* endotoxin. They found that Lipid A given intravenously in doses of 100 μg (3—7 times) reverses the progress of optic nerve of neuritis previously unresponsive to prolonged conventional treatment. Apart from mild fever, encountered occasionally, this preparation did not show any toxic effect. According to the above authors this favourable effect of Lipid A is due to an intensive leucocytosis and an increase in phagocytotic activity.

The non-specific endotoxin therapy employed by Russian authors cited by PLANELLES and BUDNITZKAYA (1961) will be discussed below. They used Pirogenal (an endotoxin preparation with low toxicity). Pirogenal was administered intravenously in 1—50 μg amounts (on the average twice weekly) depending on individual susceptibility and progress of healing. KOGAN experienced good results with this preparation in traumatic lesion of the spine. 10—12 injections of Pirogenal had minimal effects in cases of multiple sclerosis as observed by KASATKINA. 27 patients with psoriasis have been treated with Pirogenal by STEIN and his collaborators. 7 of these showed definite healing, 13 moderate and 7 mild improvement. PATZKIH obtained good results in 20 cases of traumatic iridocyclitis on administering intravenously a single dose of 3,0 μg/kg Pirogenal. In a further 6 cases the treatment had to be repeated. According to NYESMEYANOVA, Pirogenal improved restoration of nervous function and diminished scar formation after transection of the spinal cord. POLEZAYEV and his collaborators state that Pirogenal promotes regeneration of damaged heart-muscle and improves the inflammatory reaction of the connective tissue.

It is probable that several factors discussed previously are involved in the beneficial non-specific effect of endotoxin treatment in some diseases.

11. The chemical detoxification of endotoxin

Because of the widespread use of endotoxin in medical practice and for theoretical reasons some authors attempted to detoxify endotoxins by chemical methods.

NOLL and BRAUDE (1961) treated coli endotoxin with $LiAlH_4$. They obtained a product by this treatment which lost its toxicity and pyrogenicity, but retained the whole antigenicity of the parent endotoxin. They claimed that such detoxification was connected with the reductive cleavage of fatty acids from esterbonds.

HASKINS, LANDY, MILNER and RIBI (1961) stated that the treatment of *Salm. enteritidis* endotoxin with $LiAlH_4$ diminished the toxicity. However, this diminution could not be correlated with the change in fatty acid content. FUKUSHI, ANACKER, HASKINS, LANDY, MILNER and RIBI (1964) found that 90 per cent of the coli endotoxin treated with $LiAlH_4$ remained biologically intact. 10 per cent of the endotoxin was converted to non-phosphorylated polysaccharide losing its toxicity and biological properties.

NOVOTNY (1963 and 1964) described 3 chemical methods to detoxify endotoxin: 1. transesterification with BF_3, 2. deacylation with potassium methylate and 3. treatment with a mixture of pyridine and formic acid. He obtained markedly detoxified (serratia) endotoxins by these treatments. Nowotny states: „... that not all biological activities of endotoxin are related to each other. Serological reactivity and antigenicity are not related to toxicity; similarly, the enhancement of nonspecific resistance and the adjuvant effect are related neither to the toxicity nor to the antigenicity of the preparations."

The detoxification procedures of Noll and Braude and that of Novotny were repeated with some modification in our laboratory. We used sodium methylate instead of potassium methylate and applied BF_3-acetic acid complex instead of pure BF_3. (TAKÁTS, VÉGH and KOVÁTS, unpublished experiments.) We studied *E. coli* 0:55 and *Serr. marcescens* endotoxins prepared by the Boivin method and precipitated with ethanol. We controlled their toxicity by their lethal effect on mice and Shwartzman reactivity. (The mouse lethality of both parent endotoxins was 5 μg/g, the Shwartzman reactive unit was 2,5 μg.) The phosphorous content and ester- and amide-bound fatty acid was estimated by the methods used by Nowotny. The changes in antigenicity of parent and modified endotoxins were checked with hyperimmune rabbit antisera by: 1. precipitation, 2. passive haemagglutination and 3. agar diffusion. (The number, site and intensity of the different antigen components of endotoxin preparations were assessed by agar gel diffusion. The parent endotoxins formed 3 precipitin lines, numbered: 1, 2 and 3, from the central serum well outwards).

Our results are summarized as follows:

In contrast to Noll and Braude and Novotny we found the efficacy of the above detoxification-treatments to be limited. The „detoxified endotoxins" retained much of their toxicity in our hands. Conversely, the antigenicity of the preparation was markedly affected by the chemical treatments.

The $LiAlH_4$ treatment caused a marked decrease in P-content and in a less pronounced decrease in fatty acid content. There was no significant change in the precipitation, haemagglutination and agar gel diffusion tests, nor any significant reduction of toxicity.

The BF_3-acetic acid complex-treatment, abolished the Shwartzman reactivity of the parent endotoxin, but did not diminish the mouse lethality. The endotoxin must have been profoundly altered by this treatment since the P-values were strikingly decreased, but fatty acid content enhanced. The original antigenicity disappeared. The precipitin and passive haemagglutination-titres and the intensity of precipitation lines were much diminished. (We suggest that the factor lethal for mice remaining after the BF_3-acetic acid complex-treatment is a lipid-like breakdown product of endotoxin.)

The pyridine-formic acid mixture also modified the parent endotoxin. The Shwartzman reactivity was much reduced, but the lethality for mice was only slightly decreased. A marked drop in P content and a slight diminution in the fatty acid content resulted from this treatment. There was a marked diminution of titer in the serological tests. The precipitin line nearest to the antigen well was absent, but the other 2 lines were unchanged.

The sodium methylate procedure (we used sodium methylate instead of potassium methylate) resulted in the greatest reduction in mouse lethality (though only to 50 per cent!). The Shwartzman reactivity of this preparation was moderately diminished. The P content increased and the fatty acid content was somewhat decreased. There were no changes in the serological tests, but the intensity of the 2 precipitin lines closest to the serum well was significantly less. This indicates a change in the antigen structure.

There were some chemical differences between the coli and serratia endotoxin preparations, but the effects of the different treatments were similar for each. The differences between the efficacy of the detoxification procedures in our own and in other laboratories may have arisen because (as mentioned by NOVOTNY, 1964) some endotoxins are more difficult than others to detoxify.

In spite of our failure to obtain any considerable degree of detoxification we observed some interesting changes in chemical composition, antigen components and biological action of endotoxin. We failed to find, however, any correlation between the phosphorous and fatty acid content (cleavage of ester linkages) of endotoxin and its antigenic character, or its biological action.

However, we consider that there was a correlation between the antigenic structure and biological activity of endotoxin. We observed decreased intensity of the 2 components forming the precipitin lines closest to the serum well in agar gel, which coincided with the reduction in mouse lethality of the sample. The antigen component of endotoxin forming the third precipitin line (close to the antigen well) might be responsible for the Shwartzman reactivity of endotoxin, since it was absent in the samples which failed to induce a Shwartzman reaction. It is probable that this component is also responsible for the serological reactivity, since if it is absent or much decreased the pre-

cipitating and passive haemagglutinating capacity of the samples is also markedly decreased.

The BF_3-acetic acid complex which we used instead of pure BF_3 yielded an interesting result, i. e. in this case the fatty acid content increased. However, the increase seems to be only virtual, because the result of this treatment may be the concentration of fatty acids at the expense of other constituents which might be eliminated during the strong chemical treatment.

Two other detoxification treatments should be mentioned. FREEDMAN and SULTZER (1962) described the treatment of endotoxin with acetic anhydride in the presence of anhydrous sodium acetate. This procedure reduced the pyrogenicity and lethality. The modified endotoxin was unable to induce tolerance, but increased resistance to infections. This alteration was reversible because mild saponification restored the original toxicity and pyrogenicity. KIM and WATSON 1964) inactivated endotoxin with reduced crystalline papain. Because of the probable presence of 3 ester linkages in endotoxin the responsible alteration could not be elucidated.

The above detoxification experiments do not allow a firm conclusion whether or not endotoxin has a „toxophore group" which is separable from the antigen portion. However, the recent work of RADVANY, HEALE and NOVOTNY (1966) strongly suggests that they are not separable.

According to these results it may be assumed that endotoxin has (at least) two toxophore groups: 1. group responsible for Shwartzman reactivity (it may be rather a pure antigenic group which has also toxic properties) and 2. another group responsible for mouse lethality.

Concluding remarks

Some authors are inclined to attribute the development of endotoxin resistance only to the mechanism which they examined and the possible role of other mechanisms is sometimes ignored.

In fact, several different mechanisms may participate in the induction of endotoxin resistance. A synoptic view of the known mechanisms suggests that they act independently. All the mechanisms can be fitted into the concept of endotoxin resistance.

The primary toxicity of endotoxin (susceptibility) is a non-specific property and resistance to endotoxin can be induced by non-specific substances. Several factors in endotoxin resistance are, naturally, non-specific. Such factors as the RES, leucocytes, the properdin system, lysozyme and adrenal cortical hormones may all play important parts in the defense of the organism against different non-specific damages and stresses. Such non-specific defense mechanism may also operate in the case of injuries caused by endotoxin?

But the role of certain immune factors may also be presumed in the induction of resistance. Bacteriocidins or perhaps „specific" opsonins (natural antibodies) appear after endotoxin treatment. It is difficult to deny their role in the resistance to endotoxin. Probably the non-

specific defense mechanisms and the antibodies can block the capacity of endotoxin to cause early hypersensitivity and also primary toxicity.

It may be supposed (see e. g. WATSON and KIM, 1963) that primary toxicity is connected with a special chemical part of the endotoxin molecule (this part may induce the non-specific defense) and its antigenic (or hapten) character is associated with another part. However, RADVANY, NEALE and NOWOTNY (1966) proved experimentally that toxic and antigenic properties may be present on the same macromolecule.

KUNIN, BEARD and HALMAGYI (1962) have shown that sera of rabbits immunized by *E. coli* 0:14 antigen contain antibodies that cross-react in the passive haemagglatination test with the lypopolysaccharides (or crude O antigens) of a wide variety of *enterobacteriacae* (among them different *Salm. typhosa* lipopolysaccharides). This cross haemagglutination is probably due to a common hapten which may be associated — but not necessarily identical — with the endotoxin. (The common hapten could not be demonstrated by direct bacterium agglutination tests.) Lipopolysaccharides of *Serratia marcescens, Br. abortus, Ps. aeruginosa* and Gram-positive strains did not cross-react in the above manner.

SUZUKI, WHANG, GORZYNSKY and NETER (1964) and WHANG, LÜDERITZ, WESTPHAL and NETER (1965) proved that the common antigen of Kunin is distinct from endotoxin. Endotoxin or Lipid A injected simultaneously with common antigen suppressed the antibody formation to common antigen.

In the above haemagglutination tests the *E. coli* 0,14 antisera cross-reacted with different typhoid endotoxins. We could prepare the skin of *E. coli* 0,55 resistant rabbits with S. typhosa 0:901 endotoxin for the elicitation of the Shwartzman reaction. This may mean that immunospecificity may be better demonstrated by this method (KOVÁTS and VÉGH, 1966).

Although the experimental evidence is only preliminary it is probable that the immunospecificity — in the preparation of the Shwartzman reaction in specifically resistant rabbits — depends on the different antigen components contained in different strains.

It is interesting that with the increasing number of endotoxin injections (i. e. with the increase of resistance) this immunospecificity disappears. Three explanations for this disappearance may be put forward. 1. The increased cross-reactive common antibodies suppress the immunospecificity. 2. The increased non-specific resistance overshadows the immunospecificity. 3. Both mechanisms are involved.

Apart from the Shwartzman phenomenon there are other phenomena, which imply immunospecificity in the endotoxin hypersensitivity, especially in its relation to resistance. Resistance is a convenient way to detect immunospecificity in endotoxin hypersensitivity because probably all species have similar hypersensitivity to unrelated endotoxins. With the elimination of sensitivity against some endotoxins sensitivity to other endotoxins can be demonstrated. Resistance can be induced by endotoxins deriving from unrelated strains only after longer exposure to endotoxin.

MERGENHAGEN and JENSEN (1962) and WATSON and KIM (1963) observed specificity in the development of endotoxin resistance.

Concerning the relationship between endotoxin resistance and resistance to infection it seems worth while to put forward a hypothesis on the basis of the known experimental data.

GORDON and BRUCKNER-KARDOSS (1959), GUSTAFSSON and LAUREL (1958), WOSTMANN and GORDON (1958) demonstrated that the reticuloendothelial function is incomplete and the gamma globulin level is very low in germfree animals;; neither of them show a tendency to increase if the animals remain in a germfree environment. If these animals are brought into conventional surroundings they are subject to bacterial contamination. Their reticuloendothelial function and gamma globulin level increase to normal.

Germfree animals display a marked resistance to endotoxin, but minimal resistance to infections. Conversely, conventional animals have a diminished endotoxin resistance, but more marked resistance to infection. If conventional animals are made resistant to endotoxin the result is an even more marked resistance to infection.

It may be concluded that the infection resistance is enhanced during the course of life together with the increase of the endotoxin susceptibility and hypersensitivity (hyperreactivity).

Infections may lead to endotoxin resistance, but in the cases of severe and long-lasting infections the result may be inverted: hyperreactivity to endotoxin may develop instead of resistance.

The development of endotoxin susceptibility and hypersensitivity may also be due to infections, but rather in the form of a permanent symbiosis with endotoxin-producing microorganisms. Endotoxin hypersensitivity may — or may not — be present at birth, but it increases in the course of life. Further research work on germfree animals could solve this problem.

How does symbiosis with microorganism induce and increase endotoxin susceptibility and early and delayed hypersensitivity?

It was already mentioned that RAVIN, ROWLEY, JENKINS and FINE (1960) have shown the slow but continuous absorption of endotoxin from the intestines, which is taken up by granulocytes and macrophages. These may be activated by absorbed endotoxin to promote the non-specific defense of the organism. *Thus, endotoxin would be a physiological activator of the non-specific defense mechanism.*

However, the opposite possibility should also be considered. It cannot be taken for granted that the symbiotic flora and the endotoxin released from them are harmless. These symbiotic strains sometimes cause severe infections (diseases) and now and then these bacteria and their endotoxins induce toxic and hypersensitivity alternations in tissues.

ADDENDUM

Relating to our hypotheses and the biological importance of endotoxin some additional data should be quoted.

DES PREZ and BRYANT (1966) suggest that platelet-damage by endotoxin (aggregation and release of platelet-agents) is the consequence of platelet phagocytosis of endotoxin which requires a definite small quantity of divalent cations. This platelet-endotoxin interaction may be a special instance of platelet damage exerted by antigen-antibody complex.

GREISMAN, YOUNG and WOODWARD (1966) state that continous *i. v.* infusion of *E. coli* endotoxin (total is under 1 μg) resulted in a refractory state of a single *i. v.* test injection of endotoxin in rabbits, but they remained fully responsive to other substances (influenza virus, old tuberculin, enterotoxin) known to release endogenous pyrogen. An immunological mechanism is supposed to participate in this effect. — Using smaller doses for the rapid induction of the refractory state a primarily specific mechanism running at a cellular level is assumed. However, with larger doses of endotoxin a non-specific mechanism is superimposed. If the interval between endotoxin injections is lengthened the cellular desensitization diminishes and tolerance becomes increasingly dependent upon antibodies directed against the common toxophore group.

Growing interest and the rising number of experiments emphasize the necessity to evaluate the general role of endotoxin in biology.

BENNETT (1964) said at the beginning of the endotoxin symposion held in New Brunswick, N. Y. in 1963: „Endotoxins possess an intrinsic fascination that is nothing less than fabulous. They seem to have been endowed by Nature with virtues and vices in the exact and glamorous proportions needed to render them irresistable to any investigator who comes to know them". „Along with these convenient properties endotoxins possess a range of biological activity that seems specifically designed to cut across all the categories into which research and researchers are presently classified and indexed. They can affect structure and function of numerous organs and cells, change tissue and blood levels of many (perhaps too many) enzymes, modify carbohydrate, fat and protein metabolism, raise or lower body temperature, increase or decrease resistance to bacterial and viral infections and other noxious stimuli (including themselves), cause haemorrhage and increase coagulation of blood, modify haemodynamics in every accessible anatomical site, cause or prevent shock, modify gastric secretion, destroy tumours, and affect the function of several endocrine glands. This spectrum of activity makes possible at least one prediction: an investigator in almost any biological field is likely to obtain a „positive" result if he tries endotoxin in the experimental system he is using."

Readers of the above work and the present monograph may think that the authors ascribe undue importance to endotoxin. Among biologically important agents there is no *primus inter pares*. Endotoxin is the main subject of our discussion, but not of all biology. However, it has a unique property of being both a physiological and pathological foreign substance of the body. In the concluding remarks of the sympo-

sion mentioned above BRAUN said that one of the common tendencies was to attach some unifying concepts to the actions of endotoxin. „I think that in many respects our hopes have been justified." ZWEIFACH stated at the same meeting: „For several days we have been discussing the mode of action and the site of action of endotoxin. I for one am somewhat confused and a little surprised that we still have not come to grips with this question."

The reader may easily see that there is much unsolved about the role of endotoxin in biological processes. The present monograph tried to offer some additional data and hypotheses. Further work will show to what extent our attempt succeeded.

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AUTHOR INDEX

SUBJECT INDEX

Felelős kiadó a Szegedi Orvostudományi Egyetem rektora
Technikai szerkesztő Pálfy Gyula tudományos munkatárs
Megjelent 525 példányban 9 (B/5) ív terjedelemben
Készült: Linó szedéssel, íves magasnyomással az MSZ 5601-59
és az MSZ 5603-55 szabványok szerint
67-5513 — Szegedi Nyomda