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The Role of Bile Acids in Natural Resistance: Physico-Chemical Host Defence

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ABSTRACT

The toxic effects of endotoxin, the cell wall component-of Gram negative intestinal bacteria, under experimental conditions, can be induced only when they are administered.parenterally. However, in naturally occurring *entero-endotoxaemic diseases* (e.g., septic and various shocks, etc.), the endotoxin is absorbed from the intestinal tract. The cause and mode of translocation was unknown. The generally used experimental shock models differ from natural diseases only in the mode by which endotoxin enter the blood circulation. If the common bile duct of rats was chronically cannulated (bile deprived animals) orally administered endotoxin was absorbed from the intestinal tract into the blood circulation and provoked endotoxin shock. This *translocation of endotoxins* and the consequent shock can be prevented by sodium deoxycholate or natural biles. The bile acids split the endotoxin macromolecule into atoxic fragments. A similar detoxifying detergent action plays a significant role in host defence against infectious agents with a lipoprotein outer structure (e.g., so-called "big" viruses). This defence mechanism of macroorganisms based on the *detergent activity of bile acids* is called *physico-chemical defence system.* Since bile deficiency and the consequent endotoxaemia are important components in the pathogenesis of certain diseases (e.g., sepsis, intestinal syndrome of radiation disease, hepato-renal syndrome, parvovirus infection, herpes, psoriasis, atherosclerosis, etc.), bile acids may be used for the prevention and/or therapy of some clinical conditions such as the hepato-renal syndrome and psoriasis.

1. INTRODUCTION

In addition to immunological reactions, numerous other mechanisms contribute to host defence. One such mechanism is the *detergent action* exerted in the gut by bile acids produced in the liver. This was discovered during our studies on the absorption of endotoxins from the gastrointestinal tract. It is well known that endotoxins are capable of inducing a syndrome similar to septic shock when applied parenterally [1-4]. Experimentally induced endotoxin shock is based on an artificial situation, which differs from the natural disease with regard to the entry of endotoxin into the host organism. Under natural conditions,' during the so called entero-endotoxaemic . syndrome (e.g., various forms of shock) endotoxin will enter the circulation invariably from the

gastrointestinal tract. It is known that endotoxin impairs the movement of intestinal villi [5], but the mechanism of its absorption was not known. In spite of this lack of knowledge, intravenously or intraperitoneally injected endotoxin induced a shock syndrome in mammals, which is similar to the natural disease (entero-endotoxaemia) that occurs prior to death (diarrhoea, inactivity, circulatory disturbances). The pathological findings in animals that succumbed to endotoxin shock are similar to those seen in animals that died of natural disease (intestinal oedema, haemorrhages). There are major differences in the sensitivity of various species to parenterally given endotoxin, which shows a correlation with phylogenetic development [6-7]. However, when given orally endotoxin is not able to exert toxicity even in the most sensitive animal species [1]. The reason for this remained unknown for some time, because intestinal enzymes did not affect the endotoxin molecule.

In our initial experiments we observed that oral endotoxin was harmless when given to rats at doses, that were 500 to 3000 times higher than the parenteral.lethal dose. Moreover;- such treatment was harmless even when the gut mucosa had been damaged by histamine release induced by compound 48/80 and the animals had been sensitised ib lipopolysaccharide (LPS) toxicity by the intravenous application of lead acetate. It was also observed that endotoxin given orally to rats could be re-isolated from gut content by the method of phenol-water extraction. No endotoxin absorption could be demonstrated even when the animals were made extremely susceptible to endotoxin by the use of lead acetate [1]. Although the intestinal syndrome during radiation disease is due to *entero-endotoxaemia*, in irradiated animals endotoxin absorption can be demonstrated only on day 6 to 7 with sensitisation using lead acetate [8].

The above experiments did not shed light on the mechanism of endotoxin absorption from the gastrointestinal tract. The reason for the extreme tolerance of healthy animals to orally given* endotoxin could not be explained. Research was restricted to the study of animals/individuals with natural disease or, alternatively, of animals parenterally injected with endotoxin. There was little interest in the problem of *endotoxin absorption,* which is the prerequisite for the biological effect of endotoxin.

The lack of knowledge stimulated our interest in the problem of endotoxin absorption. We have been inspired by the *in vitro* experiments of an American research group, which observed that the treatment of endotoxin with a bile acid, sodium deoxycholate, resulted in the production of small atoxic units, which did not exert toxicity, but it was possible to revert to the toxic form if sodium deoxycholate was removed by dialysis. If protein was present in the system, the process became irreversible because the endotoxin fractions were adsorbed to the protein molecules. In this case it was necessary to extract the endotoxin (e.g., with phenol-water) and then it was possible to restore the molecule with some loss [91. On the basis of these findings we considered the possibility that bile acids may play an important role *in vivo* in the *detoxification of endotoxin.* If indeed this was the case, it was possible to recover orally given endotoxin from the gastrointestinal tract by extraction even though it was present there in the 'fragmented', atoxic form.

Our hypothesis was further supported by the pathological observation that faecal samples of calves and piglets suffering from Escherichia coli (*E. coli)* diarrhoea were deficient in bile acids and contained, significant quantities of neutral fat [10]. We predicted that bile deficiency was presentgThis hypothesis was also supported by the pathological observation that in newborn animalsjthat succumbed to *E. coli* diarrhoea, the *gallbladders* were fully loaded, indicating the lack of release into the intestinal-lumen. This resulted in acholic faeces [11]. We observed 9^ema auid a yellow colour in the small intestine in mice given endotoxin parenterally, which •suggested infiltration with bile acids. Perhaps parenterally injected endotoxin triggered the release of bile acids into the small intestine as a defence reaction but in this case no protection

Table I Deaths due to chronic bile fistula and orally administered ^{3}H-endotoxin in rats.

Group	Treatment	Death ratio (dead/total)
1	^{3}H-endotoxin (5 mg per os at 0 hr) + lead acetate (5 mg iv at 3 hr)	0/5
2	Bile fistula + lead acetate (5 mg iv)	1*/5
3	Bile fistula + ^{3}H-endotoxin (5 mg per os after 0 Hr) + lead acetate (5 mg iv after 3 hr)	5/5

* Died of intercurrent bile peritonitis.

could be achieved [12,13]. It is known that bacterial endotoxin chemically is a lipopolysaccharide molecule, in which the toxic moiety has been identified a$ the lipid A portion-rich in fatty acids [14,15]. On the basis of the above observations one may suggest that bile acids detoxify endotoxin within the gastrointestinal tract.

2. EXPERIMENTS ON BILE-DEPRIVED RATS

We designed experiments for the clarification of this question *in vivo.* Because rats do not have gallbladders, it was possible to produce bile deficiency by the *cannulation of the common bile duct.* With this technique we successfully produced a *chronic bile deficiency*, which was suitable for the study of endotoxin absorption from the gastrointestinal tract. We favoured the hypothesis that in healthy animals orally given endotoxin, or the endotoxin released from Gram-negative bacteria within the gastrointestinal tract, did not cause clinical symptoms because bile acids which are present in the gut on a permanent basis, will take apart the endotoxin molecule due to the detergent action. Non-toxic small fractions are produced which are adsorbed by protein molecules present in the gut. It was anticipated on this basis that bile deficiency was necessary for the development of entero-endotoxaemia. This hypothesis has been proven experimentally in rats [16,17]. Endotoxin labelled with tritium or radioactive chromium was not absorbed in normal animals after oral application [18]. No clinical symptoms were produced by such treatment. In contrast, if such endotoxin was given to rats having a bile fistula for 1-2 weeks, which led to bile acid deficiency, the animals succumbed to endotoxin shock (Table I). It was possible to detect radioactive endotoxin in the blood of these animals (by the measurement of ^{3}H and ^{51}Cr activity), and we could sensitise such animals further to endotoxin by treatment with lead acetate [19]. These experiments revealed that bile deficiency is required for the absorption of endotoxin from the gastrointestinal tract. In further experiments we treated the endotoxin with sodium deoxycholate prior to oral application to rats having bile fistulas. Complete protection was observed even when the rats had been sensitised to endotoxin by treatment with lead acetate (Table II). Identical results were obtained when bile was used from rats, pigs and cows for the restoration of missing bile acids. Such treatment prevented the development of endotoxin shock [6]. The ligation of the bile duct in rats increased the blood cholesterol, bile acid and bilirubin levels; as shown in Table III.

These experiments provided proof for the role of bile acids in host defence against endotoxin, and indicated that bile deficiency leads to the absorption of intestinal endotoxin, both of which have Clinical significance. Other investigators confirmed our results in animal experiments and also in clinical situations (endotoxaemia of patients suffering from bile duct occlusion and

Table II Sodium deoxycholate prevents deaths due to endotoxin in rats with bile fistula and lead acetate sensitisation.

Group	Treatment	Death ratio (dead/total)
1	Bile fistula + lead acetate (5 mg iv)	1*/6
2	Bile fistula + LPS (10 mg per os 0 hr) + lead acetate (5 mg iv + 3 hr)	5/6
3	Bile fistula + sodium deoxycholate (40 mg per os) + LPS (10 mg per os) + lead acetate (5 mg iv + 3 hr)	2/6

* Died of intercurrent bile peritonitis.

Table III The effect of ligation of the common bile duct in rats on serum bile acid, cholesterol, lipoprotein and bilirubin levels.

	Intact	Ligated
Glycocholic acid (μmol/l) (n = 15)	1.4 ± 0.84	45.5 ± 43.45
Glycodeoxicholic acid (μmol/l) (n = 15)	2.1 ± 0.79	7.36 ± 5.68
Cholesterol (mmol/l) (n = 15)	2.26 ± 0.34	3.5 ± 0.30
HDL (mmol/l) (n = 15)	0.55 ± 0.16	0.58 ± 0.18
LDL (mmol/l) (n = 15)	.36 ± 0.42	2.69 ± 1.04
Bilirubin (μmol/l) (n = 15)	7.0 ± 1.22	115.00 ± 89.67
Bilirubin D (μmol/l) (n = 14)	4.4 ± 1.30	45.00 ± 34.41

icterus), see for example *Bailey* [20], *Cahill* [21] and later *Gajfin* [22]. It was found that the treatment of patients suffering from icterus with bile acids prior to surgery prevented the impairment of renal function and the development of renal deficiency [21-24]. Our experimental observations made it possible to prevent a severe clinical disease. It seems obvious that this beneficial effect of bile acids is based on the physico-chemical, surface-active, detergent action. Our results support the hypothesis that the inactivation of endotoxin in the gastrointestinal tract by bile acids is due to the detergent action [25]. For this reason we studied other detergents in addition to sodium deoxycholate that are present in the bile of various animal species. Our goal was to find out whether or not they are capable of detoxifying endotoxin by fractionation of the molecule.

3. THE EFFECT OF VARIOUS DETERGENTS

Studies were done also with some commonly used *detergents* (e.g., sodium lauryl sulphate, cetylammonium bromide, polyoxyethylene stearate, Tween 20, benzalkonium chloride). Two rapid methods were developed for the determination of their activity. One approach was to inject a mixture of a lethal dose of endotoxin and a tolerated concentration of the detergent intraperitoneally into animals. If the detergent applied could detoxify endotoxin, the mortality of the animals was decreased. According to the second procedure the animals were sensitised to endotoxin by lead acetate. In this case microgram quantities of endotoxin could be used, mixed with detergent and given intravenously. Here it was also necessary to titrate detergent toxicity P?19[r] *[ts use]- Again, a decrease in mortality indicated the anti-endotoxic effect of the detergent investigated. These experiments indicated that *sodium deoxycholate* and *bile* obtained from vari-

ous animals (e.g., cow, pig, rat, and rabbit) showed 100% efficacy in protection, whereas sodium lauryl sulphate gave 80%, cetylammonium bromide 60%, and benzalkonium chloride 20% protection against the lethal dose of endotoxin, and Tween 20 was ineffective. We concluded that the best results were obtained with bile acids or with natural bile [26].

Our experimental results made it possible to explain the pathomechanism of entero-endotoxaemia in new-born babies, which occurred frequently some time ago, but decreased in number lately because of improved hygienic conditions in hospitals. However, this disease still has veterinary significance because 10-15% of newborn calves and piglets will fall victim to entero-endotoxaemia. It is likely that in a proportion of newborn animals bile production (probably because of disturbed liver function) or bile secretion into the small intestine (probably because of the lack of cholecystokinin) does not coincide with parturition, which leads to bile deficiency. There is a rapid penetration of Gram-negative bacteria into the gastrointestinal tract of newborn animals, which release endotoxins that would absorb in bile-deficient animals and cause disease and death. The situation is analogous to rats having a bilq fistula and receiving endotoxin by the oral route. It follows that such newborn animals would be protected if given after parturition detergent preparations, optimally bile acids, that would prevent disease. Such treatment would restore the bile content in the gut which would enter the enterohepatic circulatiop,.stimulate the production of bile acids and their secretion into thé gut, in,other words initiate the function of this fundamental host defence mechanism. ,

4. PROTECTIVE EFFECT OF BILE ACIDS PREPARATIONS AGAINST ENDOTOXIN SHOCK

On the basis of the above experiments therapeutic preparations of bile acids (DETERTOXON-S and B) have been produced which seem to be beneficial for the treatment of entero-endotoxaemia in newborns [27]. Comparative pathological studies conducted during these therapeutic trials demonstrated that these preparations were able to decrease mortality due to *E. coli* diarrhoea. For instance, in a cattle herd 195 calves received conventional treatment and 23 of them died, whereas after the treatment of 234 calves with the bile acid preparation only 6 were lost because of *E. coli diarrhoea.* Essentially similar results were obtained during the trial in other herds. The results were similar though inferior in a pig farm where 120 piglets were lost out of 2027 untreated animals, whereas 68 died of 1803 bile acid treated animals due to *E. coli* diarrhoea [28].

These results are significant because of the experience gained during several decades that the treatment of entero-endotoxaemic newborn animals with drugs or vaccines is of limited value. Vaccines provide protection only against specific strains that have been used for its production and become useless against other strains that frequently occur in a given population. This is a serious limitation, as approximately 150 different *E. coli* strains are known simply on the basis of O serotypes. For this reason the induction of specific immunity seems impossible. Perhaps the new, so-called pilus antigen vaccines or the use of DNA vaccines will improve the situation [29,30]. The problem is similar when antibacterial agents (antibiotics, sulphonamides) are used for treatment. Resistance will occur within a short period of time, which renders these drugs ineffective. One may hope, on the basis of our results, that this disease could be prevented by the restoration of bile acid deficiency. Current evidence indicates that in *E. coli* diarrhoea the entero- and protein toxins [30] could facilitate the translocation of endotoxin by causing damage to the gut mucosa. Such damage would inhibit or prevent the secretion of *cholecystokinin*, which

in turn would lead to bile retention and allow the absorption of endotoxin into the bloodstream, resulting in endotoxaemia and shock. This explanation is compatible with those views that emphasise the fundamental pathogenic role of endotoxin in these conditions.

The above examples of animal disease have comparative pathological significance and serve as models for human conditions. It is well known that a significant proportion of the human population suffers from problems of bile secretion and of gallbladder function *{Bertók Jr.* and *Bertók*, unpublished observations). It is possible that the use of bile acid preparations would be of advantage in such patients.

5. PHYSICO-CHEMICAL HOST DEFENCE

The pathophysiological significance of these findings may be summarised as follows: A unique host defence mechanism has been discovered the significance, of which is likely to lie way beyond protection against bacterial endotoxins. We call this mechanism the *physico-chemical defence* of the organism. The basis of this defence mechanism is the detergent effect of bile acids [6,31,32]. One may pose the question whether or not the protection based on detergent action provides defence against enterotoxaemia only or perhaps there are other situations in which endotoxin plays a role.

6. THE ROLE OF BILE ACIDS IN INTESTINAL ISCHAEMIA

We found in subsequent experiments that *intestinal ischaemia* is aggravated by bile deficiency [33,34]. Moreover, it was found that bile production is decreased under these conditions [35]. This was further supported by the observation that the administration of bile acids (500 mg pulverised and radiation-sterilised pig bile) to dogs with intestinal ischaemia prevented death in 60-70% of the animals [36]. The protective value of bile acids was also indicated in rats suffering from 'strangulated ileus', where endotoxin plays a fundamental role in the pathogenesis. Bile acids given into the intestinal lumen (20 mg pulverised and radiation-sterilised pig bile) prolonged the survival of the animals by 7 hours (50%) [37]. These results indicate that the use of proper bile acid preparations will provide new opportunities for the treatment of patients with acute abdominal conditions.

7. THE EFFECT OF BILE ACIDS ON VIRUSES

One may ask the question whether or not the protective effect of bile acid detergents is limited to bacterial endotoxins. *Theiler* observed that the virus of yellow fever and other 'arthropod-borne' viruses (belonging to the Toga group) are inactivated by bile or sodium deoxycholate. In contrast, thepoliovirus, the virus of mouse encephalitis and endocarditis and Coxsackie viruses resist such treatment [38]. This observation served as a basic principle for the classification of viruses according to which two groups, sodiunv deoxycholate sensitive ('big viruses') and resistant ('small viruses') could be distinguished. It is interesting that nobody, including *Theiler,* recognized the pathological significance of these observations despite the fact that *Theiler* was actually working with the virus of yellow fever when he discovered the antiviral effect of bile. If we compare the bile acid sensitive and resistant viruses, it becomes clear that the viruses having

lipoprotein capsule are sensitive and those that do not possess such a capsule are xesistant. It follows that bile acids would affect *in vivo* all the viruses that possess lipoprotein 'capsule' (peplon). Those without capsules are all resistant. Therefore, it is logical to 'assume that the detergent effect of bile acids would act *in vivo* on all those viruses that have lipoprotein capsules and that this fact could be used for protection against such viruses. In an experimental model using Aujeszky's disease virus, a herpes virus, we demonstrated the protective effects of bile acids. It was found in rats that bile deficient animals contracted disease after the oral administration of Aujeszky's disease virus whereas intact rats were resistant [39]. It is possible that temporary or partial bile acid deficiency plays an important role in infections with herpes viruses that may occur after alimentary overloads (e.g., weddings and other feasts).

8. BILE ACIDS AND PSORIASIS

In our view, *psoriasis,* a condition considered to be inherited in a polygenic manner [40], is also associated with bile or bile acid deficiency, as in most cases supplementation of the usual treatment with bile acids will diminish the severity of the clinical signs within^ short period. One of my co-workers (Klára Gyurcsovics) treated'a total of 551 psoriasis patients with bile acids (Suprachol®; Acidum dehydrocholicum).(given orally for 1-8 weeks. The clinical efficacy of the treatment was evaluated by means of the Psoriasis Area Severity Index (PASI score). During this treatment 434 patients (78.8%) became asymptomatic. Of 249 psoriatics receiving the conventional therapy, only 62 (24.9%) showed clinical recovery during the same period of time ($p<0.05$). The curative effect of bile acids was even more pronounced in the acute form of psoriasis (95.1% of the patients became asymptomatic). Two years later 319 out of the 551 acute and chronic psoriasis patients treated with bile acid (57.9%) were asymptomatic, compared to only 15 out of the 249 patients (6.0%) receiving the conventional treatment ($p<0.05$). At the end of the two-year follow-up, only 10 out of 139 acute psoriasis patients (7.2%) receiving the conventional therapy and 147 out of 184 such patients (79.9%) treated with bile acids were asymptomatic ($p<0.01$). On the basis of clinical observations (digestive disorders, ultrasonographically confirmed gallbladder changes, etc.) it may be assumed that the deficiency of bile acids can indeed play a role in the pathogenesis of psoriasis. In bile acid deficiency the absorption ('translocation') of endotoxins becomes possible [41], and this will trigger the release of inflammatory cytokines in the skin of individuals having a hereditary predisposition. If the absorption of endotoxins is prevented by bile acid supplementation, the release of inflammatory cytokines can be blocked [42]. Probably this is the mechanism that explains the success of our treatment approach. Thus, the effect that cytokines have been recognized to exert in the pathogenesis of psoriasis suggests the indirect role of bacterial endotoxins. However, this effect probably occurs only if bile production or excretion is deficient (cholecystokinin deficiency, disturbance of cholesterol metabolism and bile production). Thus, the treatment of psoriasis with bile acids should actually be regarded as supplementation of a physiological substance rather than a medicinal therapy. In our view, its effectiveness, safety, simplicity and low cost make this treatment modality suitable for a wider use in the *therapy of psoriasis* [42]. The successful treatment of psoriasis with bile acids is an example demonstrating that the recognition of physico-chemical defence has practical importance. Thus, recognition of the role of bile acids enables us to provide cause-based treatment to psoriatics who have mostly been given only symptomatic treatment so far.

9. BILE DEFICIENCY AND PARVOVIRUS INFECTION

The importance of bile acids is further emphasised by the observation that the symptoms of acute *patyovirus* infection, which causes severe intestinal haemorrhage in dogs, closely resemble experimental endotoxin shock. During autopsy the characteristic finding is a dilated, tightly filled gallbladder. Endotoxin could be detected in the serum of such dogs using lead acetate sensitised animals [43]. These observations suggest that parvovirus induced mucosal damage in the small intestine, which leads to haemorrhage, is likely to inhibit cholecystokinin (CCK) synthesis and CCK deficiency leads to bile retention. Partial bile acid deficiency occurs in the gut, which makes the absorption of endotoxin possible. It is likely that the sick dogs die of endotoxin shock [43].

10. BILE DEFICIENCY AND RADIATION-INDUCED INTESTINAL-SYNDROME

It is clear that the intestinal syndrome associated with radiation disease is caused by bacterial endotoxins. Because bile acids are capable of endotoxin detoxification and bile deficiency permits the absorption of endotoxins into the bloodstream ('translocation'), one may ask the question whether or not bile deficiency would play a role in radiation disease. For this reason we studied in rats the effect of experimental bile deficiency on the development of radiation-induced intestinal syndrome. It was observed that the rats having bile fistulas died of radiation disease within five days, whereas the control irradiated and sham-operated irradiated animals survived for 11 days. Apparently bile deficiency accelerated the development of endotoxaemia in this model [44].

11. BILE-ACID COMPOSITION OF VARIOUS SPECIES

In relation to the importance of bile acids in host defence against endotoxins one may ask the question whether or not bile composition plays a role in the endotoxin sensitivity/resistance of various species [45]. We have observed earlier that there are major differences in bile acid composition of bile obtained from sensitive (e.g., man, cow, guinea pig) and resistant species (e.g., birds, fish) [39]. It is interesting to note that there is a direct correlation between endotoxin sensitivity and radiation sensitivity of various species. Perhaps the ontogeny of the production of bile acids in chickens bears relevance to the fact that chicken embryos show endotoxin sensitivity up to 11 days of age, which is the time of the initiation of bile acid synthesis in the liver. Older chicks or mature animals can only be made susceptible to endotoxin by lead acetate treatment. It is likely that bile acids function not only in the gut but also in the liver in addition to acyloxyacyl hydrolase enzyme discovered in 1989 to be active in the detoxification of bacterial endotoxins [46].

12. ENDOTOXIN INACTIVATION BY LIVER/BILE ACIDS

We have observed, as have others, that liver homogenates from healthy rats are capable of inactivating small (microgram) quantities of endotoxins. In contrast, if the rats received lead acetate intravenously prior to the preparation of liver homogenates, no endotoxin inactivation was

Table IV Proven and presumed roles of bile acids and endotoxins in certain pathological processes.

Intestinal infections
Surgical / septic shock
Intestinal ischemic shock
Tourniquet shock
Gastro-intestinal syndrome of radiation disease
Hepato-renal syndrome
Enteroviral infections (e.g., parvovirus)
Herpes viral infections
Psoriasis
Atherosclerosis
Endocrine disorders

observed [39]. Similar results were obtained if we incubated natural bile fluid with lead acetate. Such bile solutions lost their capacity to detoxify endotoxin. It is likely that lead acetate damages the side chains that play an important role in the detergertt'effect of bile acids, and for this reason the modified bile acids are unable to detoxify endotoxin. This phenomenon may play a role in the lead acetate induced endotoxin sensitivity *in vivo* [39].

We demonstrated that after the dissociation of endotoxin by bile acids to its basic units, the detoxified material maintains the capacity to induce endotoxin tolerance [31-32]. It is possible that this observation will lead to the isolation oV non-toxic endotoxin subunits, which will be able to stimulate natural resistance without toxic side effects [39].

13. CONCLUSIONS

In conclusion, one may suggest that a physico-chemical defence mechanism, which is based on the detergent action of bile acids, provide the body with a general defence, which is not limited to bacterial endotoxins, but is also effective against other agents (e.g., viruses) that possess lipoprotein or lipid structures. This novel mechanism may be added to the known defence mechanisms, which are based on bile acids produced in the liver and present in the *enterohepatic circulation.* It is interesting to note in this respect that bile acids are the most important products of cholesterol metabolism, which are reusable end products that affect the production of steroid hormones (by the adrenal gland and gonads). It is possible that bile acids play a role also in the pathophysiology of endocrine and reproductive abnormalities, which remains to be elucidated [47] (Table IV).

It is possible that deficient bile production or secretion leads to the absorption of minute amounts of endotoxin, which may play a role in the induction of atherosclerosis. We showed in cholesterol-treated rabbits that endotoxin significantly increases the development of atherosclerosis [48]. Recently several authors recognized his fact [49-60]. Partial or temporary bile acid deficiency may occur for several reasons: CCK deficiency, disturbance of bile secretion and deficient bile production due to liver damage, leading to endotoxin absorption. In turn, the absorbed endotoxin will act on sessile phagocytes fixed to the wall of blood vessels according to known pathways (e.g., endotoxin-binding protein and CD14 endotoxin receptor), which leads to the production of cytokines and other biologically active materials. These mediators, in conjunction with elevated cholesterol levels, could precipitate and initiate plaque formation. On this basis one may suggest that the systematic investigation of bile production and secretion from a young age and correction of deficiencies would decrease the development of atherosclerosis and

the development of related diseases.

It is clear from the facts presented here that bile acids and endotoxins play a significant role in many more pathological conditions than were reasonably anticipated. It is likely that further investigation of these questions and the application of experimental results to patients will lead to new approaches in clinical medicine.

REFERENCES

1. Bérezi I, Bertók L, Baintner K, Veress B. Failure of oral E.coli endotoxin to induce either specific tolerance or toxic symptoms in rats. J Path Bact 1968;96:4S 1-486.
2. Bertók L. Bacterial endotoxins and their effects (Hungarian). Orv Hetil 1998; 139:1947-1953.
3. Gans H, Matsumoto K. Are enteric endotoxins able to escápe from intestine? Proc Soc Exp Biol Med 1975;147:736-741.
4. Gans H, Matsumoto K. On the escape of endotoxin from the intestine. In: Urbaschek B, Urbaschek R, Neter E, editors. Gram-negative bacterial infections. Wien: Springer Verlag, 1975;429-436.
5. Ihász M, Karika Gy, Ludány Gy. Effect of endotoxin on motion of intestinal villi (Hungarian). Orv Hetil 1968;109:1381-1384.
6. Bertók L. Role of bile in detoxification of lipopolysaccharide. In: Schlessinger D editor. Microbiology. Washington: American Society for Microbiology, 1980;91-3.
7. Bérezi I, Bertók L, Bereznay T. Comparative studies on the toxicity of E.coli lipopolysaccharide endotoxin in various animal species. Can J Microbiol 1966; 12:1070-1071.-
8. Bertók L, Kocsár L. Die -Rolle> des Endotoxins beim intestinalen Syndrom der Strahlenkrankheit. Wiss Z Karl Marx Univ Math Naturvviss 1974;23:65-66.
9. Rudbach JA, Anacker RL, Haskins WT, Johnson AG, Milner KC, Ribi E. Physical aspects of reversible inactivation of endotoxin. Ann N Y Acad Sci 1966;133(8):629-635.
10. Mouwen JM, Schotman AJ, Wensing T, Kijkuit CJ. Some biochemical aspects of white scours in piglets. Tijdschr Diergeneeskd 1972;97(2):65-72.
11. Bertók L. Role of endotoxins in E.coli induced pathological processes. (Hungarian) Magy Állatorv Lapja 1998;120:668-672.
12. Bertók L. Role of endotoxins and bile acids in the pathogenesis of septic circulatory shock. Acta Chir Hung 1997;36:33-36.
13. Bertók L, Tóth B. Studies on the pathogenesis of the edema disease of swine. I. the effect of the purified lipopolysaccharide endotoxin of Escherichia coli on mice. Acta Vet Acad Sci Hung 1963;13:421-428.
14. Westphal O, Ltideritz O, Bister F. Über die Extraction von Bacterien mit Phenolwasser. Z Naturforsh 1952;7b:148-155.
15. Rietschel ET, Brade H, Hoist O, MQller-Loennies S, Mamat U, Zahringer U, Beckmann F, Seydel U, Brandenburg K, Ulmer AJ, Mattern T, Heine H, Schletter J, Loppnow H, Schönbeck U, Fiad HD, Hauschildt S, Schade UF, Padova FD, Kusumoto S, Schumann RR. Bacterial Endotoxin: Chemical constitution, biological recognition, host response, and immunological detoxification. In: Rietschel ET, Wagner H editors. Pathology of septic shock. Current Topics in Microbiology and Immunology, Berlin: Springer-Verlag, 1996;216:40-81.

16. Kocsár LT, Bertók L, Várterész V. Effect of bile acids on the intestinal absorption of endotoxin in rats. J Bacteriol 1969;100(l):220-223.
17. Kocsár L, Bertók L, Várterész V. Function of bile acids in the intestinal-absorption of endotoxin in rats. Ann Immunol Hung 1974;17:49-52.
18. Kocsár L, Bertók L, Varga L. Double labeling of endotoxin with chromium and tritium. Ann Immunol Hung 1973;17:71-72.
19. Selye H, Tuchweber B, Bertók L. Effect of lead-acetate on the susceptibility of rats to bacterial endotoxins. J Bacteriol 1966;91:884-890.
20. Bailey ME. Endotoxin, bile salts and renal function in obstructive jaundice. Brit J Surg 1976;63:774-781.
21. Cahill CJ. Prevention of postoperative renal failure in patients with obstructive jaundice - role of bile salts. Brit J Surg 1983;70:590-595.
22. Gaffin SL. Control of septic shock - present day concept. S A J Hosp MedM982;40: 118-124.
23. Dimond T, Parks W. Perioperative management of obstuctive jaundice. Br J Surg 1997;84: 147-149.
24. Kisida E, Hegedűs Z, Bertók L, et al. Prevention of the postoperative hepatorenal syndrome by means of bile acid (preliminary reportj (Hungarian). Magy Seb 1996;49:1-7.
25. Bertók L. Effect of bile acids on endotoxin vitro and in vivo (physico-chemical defense). Bile deficiency and endotoxin translocation. Ann N Y Acad Sci 1998;851:408^10.
26. Bertók L, Kocsár L. Studies on some biological properties of surfactant treated endotoxin in rats. Ann Immunol Hung 1972;16:77-81.
27. Kocsár L, Bertók L, Várterész V et al. Produce for the production of ionizing radiation-activated bile acid content preparation for the treatment of entero toxemias of newborns (Hungarian). Budapest: Hungarian Patent 162.789/A-61-k-27/02,17/00.1973.
28. Bertók L. A new possibility for the increase of natural immunity (nonspecific resistance): the radiodetoxified endotoxin (Hungarian). Orv Hetil 1999;140:819-827.
29. Kohoe M, Sellwood R, Shipley P, et al. Genetic analysis of K88-mediated adhesion of enterotoxigenic Esherichia coli. Nature 1981;291:122-128.
30. Nagy B. Pathology and specific prevention of enteral infections of calves and piglets [Doct Thesis Hung Acad Sci]. (Hungarian). Budapest; 1992.
31. Bertók L. Physico-chemical defense of vertebrate organisms: the role of bile acids in defense against bacterial endotoxins. Perspect Biol Med 1977;21:70-76.
32. Bertók L. Physico-chemical defense system (Hungarian). Biológia 1977;25:127-134.
33. Karika G, Bertók L, Kisida E. Effect of bile deficiency on the survival of rats in the shock induced by occlusion of artéria mesenterica cranialis (Hungarian). Magy Seb 1978;31: 164-166.
34. Karika G. Role of bile acids in the endotoxemia abdominal catastrophies [PhD Thesis Hung Acad Sci]. (Hungarian). Budapest; 1981.
35. Karika G, Fehér T, Bertók L. Change of the bile production in the experimental mesenteric occlusion of rats (Hungarian). Kíséri Orvostud 1980;32:221-224.
36. Karika G, Bertók L, Kisida E, et al. Effect of bile acid treatment in the shock of dogs induced by the occlusion of artéria mesenterica superior (cranialis) (Hungarian). Kíséri Orvostud 1978;30:491-493.
37. Karika G, Bertók L, Kisida E. Effect of intraluminally given bile on the survival of rats in the strangulated ileus (Hungarian). Magy Seb 1978;31:355-357.
38. Theiler M. Action of sodium deoxycholate on arthropod borne viruses. Proc Soc Ex Biol

Med 1957;96:380-388.
39. Bertók L. Role of endotoxins and bile acids in the pathogenesis of septic circulatory shock. ActaChirurg Hung 1997;36:33-36.
40. Dobozy A, Horváth A, Hunyadi J, et al. Dermatology (Hungarian). Budapest: Eklektikon Kiadó;1998.
41. BelewPW, Rosenberg EW, Skinner RB jr', et al. Endotoxemia in psoriasis. Arch Dermatol 1982;118:142-143.
42. Gyurcsovics K, Bertók L. Role of bile acids in the pathogenesis and therapy of psoriasis (Hungarian). Orv Hetil 2000;141:915-917.
43. Szaniszló F, Bertók L. Demonstration of endotoxaemia in case of hyperacute parvoviral enteritis in dogs (Hungarian). Magy Állatorv L 1990;45:109-112.
44. Bertók L Jr, Sztanyik LB, Bertók L. Possible role of bile deficiency in the development of intestinal syndrome of acute radiation disease. Acta Physiol Hung 1992;79:29<32.
45. Haslewood GAD. The biological importance of bile salts.' Amsterdam: North-Holland Publishing Company; 1978.
46. Munford RS, Hall CL. Purification of acyloxyacyl hydrolase, a leukocyte enzyme that removes secondary acyl chains from bacterial lipopolysaccharides. J Biol Chem 1989;264(26): 15613—15619.
47. Bertók L. Endotoxins and endocrine system. Domest Anim Endocrinol 1998;15:305-8.
48. Horváth A, Bertók L, Horváth I, Lapis K, Hortoványi E, Hársing J, Kocsis I, Bozóky Z, Greskovits D. Additional data on antigen use for immunisation against atherosclerosis (in press).
49. Havel RJ. Role of the liver in atherosclerosis. Arteriosclerosis 1985;5:569-580.
50. Liao W. Endotoxin: possible roles in initiation and development of arterosclerosis. J Lab Klin Med. 1996; 128:452^60.
51. Liao W, Flórén CH. Hyperlipidemjc response to endotoxin. A part of the host-defense mechanism. Scand J Infect Dis 1993;25(6):675-682.
52. Chawla A, Saez E, Evans RM. Don't know much bile-ology. Cell 2000;103:1-4.
53. Cerwinka WH, Cheema MH, Granger DN. Hypercholesterolemia alters endotoxin-induced endothelial cell adhesion molecule expression. Shock 2001;16:44-50.
54. Charach G, Rabinovich PD, Konikoff FM, Grosskopf I, Weintraub MS, Gilat T. Decreased fecal bile acid output in patients with coronary atherosclerosis. J Med 1998;29:125-136.
55. Kalayoglu MV, Indrawati, Morrison RP, Morrison SG, Yuan Y, Byrne GI. Chlamydial virulence determinants in atherogenesis: the role of chlamydial lipopolysaccharide and heat shock protein 60 in macrophage-lipoprotein interactions. J Infect Dis 2000; 181 Suppl 3: S483-489.
56. Moseley RH. Bile-ology. Gastroenterology 2001;121:218-220.
57. Saikku P. Chlamydia pneumoniae in atherosclerosis. J Intern Med 2000;247:391-396.
58. Wiedemann CJ, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F, et al. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. J Am Coll Cardiol 1999;34:1975-1981.
59. Wiedermann CJ, Kiechl S, Schratzberger P, Dunzendorfer S, Weiss G, Willeit J. The role of immune activation in endotoxin-induced atherogenesis. J Endotoxin Res 2001;7: 322-326.
60. Wiedermann CJ, Kiechl S, Willeit J. Is endotoxemia a risk factor for atherosclerosis? Wien Klin Wochenschr 2001; 113:77-79.