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Effect of Long-term Administration of Paromomycin Sulfate on the Level of Serum Albumin and γ-globulin in Human Cirrhosis

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The efficacy of administration of oral paromomycin sulfate on serum albumin and γ-globulin levels was studied in cirrhotic patients. After an observation period of 3 months, paromomycin sulfate at 2.0g per day or a placebo was administered for 6 months, and changes in serum albumin and in γ-globulin levels were examined every three months. Out of 16 cirrhotic patients treated with paromomycin, 11 (68.8%) showed significant increases in serum albumin compared with one out of 16 in the placebo group. Concerning γ-globulin, seven (43.8%) patients in the paromomycin group showed significant decreases compared with one in the placebo group. In addition, among the 11 cirrhotics whose endotoxemia decreased after paromomycin administration, eight (72.7%) showed significant increases in albumin level. It was suggested that paromomycin improves the serum albumin and γ-globulin levels in cirrhosis through the alleviation of endotoxemia caused by intestinal bacteria.

(Key Words:Paromomycin, Albumin, Cirrhosis)

INTRODUCTION

A number of studies have suggested that antibiotic treatment may protect against liver injury. Leach *et al.* (6) and Gyorgy (4) demonstrated that some absorbable and non-absorbable antibiotics were protective against hepatic necrosis caused by carbon tetrachloride and choline deficiency respectively. Subsequently, Broitman *et al.* (1) demonstrated that neomycin limited the development of cirrhosis in choline deficient rats. Since this preventive effect was abolished when purified endotoxin was added to the drinking water during the period of choline deficiency, they concluded that intraluminal endotoxin contributed to the cirrhotic process, and that eradication of endotoxin-producing bacteria was the mechanism involved in the neomycin effect.

It has been well established that endotoxemia develops frequently in patients with cirrhosis (2, 3, 8, 10), and we have demonstrated that the mortality in cirrhotics with endotoxemia is higher than in those without endotoxemia (10). These findings suggest the possibility that endotoxin might be a factor in the development of cirrhosis in man, and that nonabsorbable antibiotics might therefore be beneficial in the treatment of cirrhosis. Since serum albumin levels are closely related to the severity of cirrhosis, we conducted a controlled, double-blind trial on the efficacy of long-term administration of paromomycin sulfate, a non-absorbable antibiotic, on serum albumin levels in cirrhotic patients.

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MATERIALS AND METHODS

Thirty seven clinically stable cirrhotics were selected because it was mondatory that their general hepatic status did not change rapidly in the course of the study as determined by clinical examinations and liver function tests.

Each had been hospitalized in Tokai University Hospital between April 7, 1981 and November 3, 1981, but were outpatients when the study was carried out. The diagnosis of cirrhosis was made by laparoscopy and liver biopsy in all cases. They had not received any antibiotics previously for at least one month prior to the study.

The nature, purpose, and hazards of this study were explained to the patients and to their next of kin, and their written, informed consent was obtained. The protocol had been approved by the committee on human investigation, and the research was carried out according to Declaration of Helsinki.

After a three month control period without any antibiotics, the patients were randomly assigned to a schedule prepared from a table of random numbers and given either paromomycin sulfate (2.0 daily) or placebo (made of crystallized cellulose) for 6 months in a double-blind manner. Paromomycin sulfate or placebo was administered four times daily, 250mg after each meal and 250mg at bedtime. High calorie intake (40Cal/Kg) with a high protein diet (2g/Kg of protein) was indicated throughout the study in all cases.

Biochemical laboratory tests were performed every two weeks in all patients throughout the study period, and the mean and standard errors of serum albumin and γ-globulin levels every three months were calculated.

Biochemical laboratory tests included: total protein (biuret method, normal: 6.8 to 8.0g/dl), serum albumin (based on cresol green method, 4.2-5.2g/dl), serum alkaline phosphatase (method of the German Society for Clinical Chemistry, 79-219m U/ml), serum glutamic oxaloacetic transaminase (SGOT, mehtod of the Scandinavian Society for Clinical Chemistry, 9-27 I U/I), total bilirubin (modified Michaëlssson method, 0.5-1.0mg/dl) and serum electrophoresis.

Coagulation studies were also performed at the beginning of the study: plasma fibrinogen was estimated by the biuret method, and fibrinogen degradation products (FDP) by the staphylococcal clumping test. Platelets were counted by phase microscopy, and prothrombin time and activated partial thromboplastin time were also measured.

In some cases endotoxin-like activity was measured immediately after sampling by the Limulus assay as described by Levin *et al* (7). Blood-samples were obtained under aseptic conditions with pyrogen-free syringes. All glassware was rendered pyrogen-free by heating at 175°C for three hours (14). All procedures were carried out under sterile conditions. The amoebocyte lysate used in the Limulus assay was provided by Difco Laboratories (Detroit, Mich.). Heparinized plasma was shaken vigorously with chloroform (1;0.25) for four hours, the emulsion produced was centrifuged at 2,500 rpm for 10 minutes, and 0.1ml of the middle layer was taken and mixed with 0.1ml of pyrogen-free distilled water containing amoebocyte lysate. The mixture was incubated at 37°C for 24 hours and examined after 1, 4, and 24 hours.

The test was considered positive when definite gelation occurred. A negative control of pyrogen-free saline and a positive control of 10 ng of Escherichia coli endotoxin per ml were processed simultaneously to ensure accuracy of the assay. The sensitivities of all lots used were tested with the purified E. coli endotoxin standard from Difco Laboratories in the following manner: series 1: ten dilutions of standard endotoxin in saline were prepared and the lot preparations were tested with solutions containing 0.1 to 100 ng per ml of standard endotoxin. All the Limulus assays could detect as little as 0.5 ng of endotoxin per ml with good recovery. The results of each test were read by two independent observers who did not know the medication of the patients.

The statistical significance of differences was calculated by student's test.

RESULTS

Out of 37 cirrhotic patients studied, 19 were in the paromomycin group and 18 in the placebo group. Among the 19 patients in the paromomycin group, two patients were withdrawn because of possible side effects of paromomycin sulfate such as diarrhea, anorexia and abdominal pain, and another one was also withdrawn on account of development of a hepatoma. In the placebo group, one patient was withdrawn because of a rupture of the esophageal varices, and another patient was also withdrawn on account of a hepatoma. Finally, 32 patients completed the trial, 16 in the paromomycin group (group A) and also 16 in the placebo group (group B). Group A consisted of one case with alcoholic cirrhosis, 11 with posthepatitic cirrhosis and four with cryptogenic cirrhosis. Group B consisted of 10 cases of posthepatitic cirrhosis and six of cryptogenic cirrhosis. HBsAg was detectable in the sera of four and three patients respectively in each group by radioimmunoassay. There were no significant differences between the groups concerning age, sex, clinical symptoms, biochemical laboratory tests and Child's classification of liver damage (Table 1), nor were there any differences in blood coagulation studies between the two groups at the beginning of the study (Table 2). At the time of investigation, seven of the 16 in group A and five of the 16 in the group B were on diuretics.

Among the 16 patients in group A, 11 (68.8%) showed significant increases in serum albumin (Figure 1) compared with one out of 16 in group B (Figure 2). Of the 11 patients who showed significant increases in serum albumin level in group A, eight already showed an improvement in the first trimester. The amount of increment in serum albumin was more than 0.5g/dl in 11 cases (Table 3). Concerning the relationship between endotoxemia and serum albumin level, it was found that out of 11 cirrhotic patients whose endotoxemia disappeared with long-term paromomycine sulfate administration, eight showed significant increases in serum albumin level (Table 4).

Concerning the globulin level, seven (43.8%) of the 16 cirrhotics in group A showed significant decreases in serum γ-globulin level (Figure 3) compared with one out of 16 in group B (Figure 4).

Table 1 Cirrhotic patient characteristics

	Paromomycin (n = 16)	Control (n = 16)
Age (yr)	55.4 ± 3.1	57.2 ± 2.1
Male/Female	9/7	8/8
Symptoms		
Jaundice	(+)/(−)3/13	4/12
Ascites	(+)/(−)0/16	0/16
Encephalopathy	(+)/(−)2/14	1/15
Biochemical data		
T. P. (g/dl)	7.5 ± 0.2*	7.4 ± 0.3
albumin (g/dl)	3.7 ± 0.1	3.8 ± 0.2
γ-globulin (g/dl)	2.4 ± 0.1	2.2 ± 0.2
GOT (INU)	104 ± 16	146 ± 26
GPT (INU)	85 ± 19	105 ± 28
Al-P (INU)	214 ± 49	169 ± 24
T. T. T.	11.2 ± 1.5	9.7 ± 2.1
ChE (Knedel, U/ml)	2.1 ± 0.4	2.4 ± 0.7
Bilirubin (mg/dl)	1.6 ± 0.4	1.8 ± 0.7
Child's classification		
A	8	9
B	8	7
C	0	0

*Mean ± SEM

Table 2 Coagulation Study

Patients	Fibrinogen 200 ~ 400 mg/dℓ	Fibrinogen Degradation Products 0.49 ~ 2.38 μg/mℓ	Platelet Count > 100.000 / mm^3	Prothrombin Time 10.5 ~ 12.5 seconds	Partial Thromboplastin Time 40 ~ 100 seconds	Complement 30 ~ 40 CH50
Paromomycin group	5/16	7/16	9/16	13/16	1/6	12/16
control group	4/16	4/16	12/16	14/16	0/16	10/16

*The ratios indicate the number of abnormal tests to the total number of patients studied.

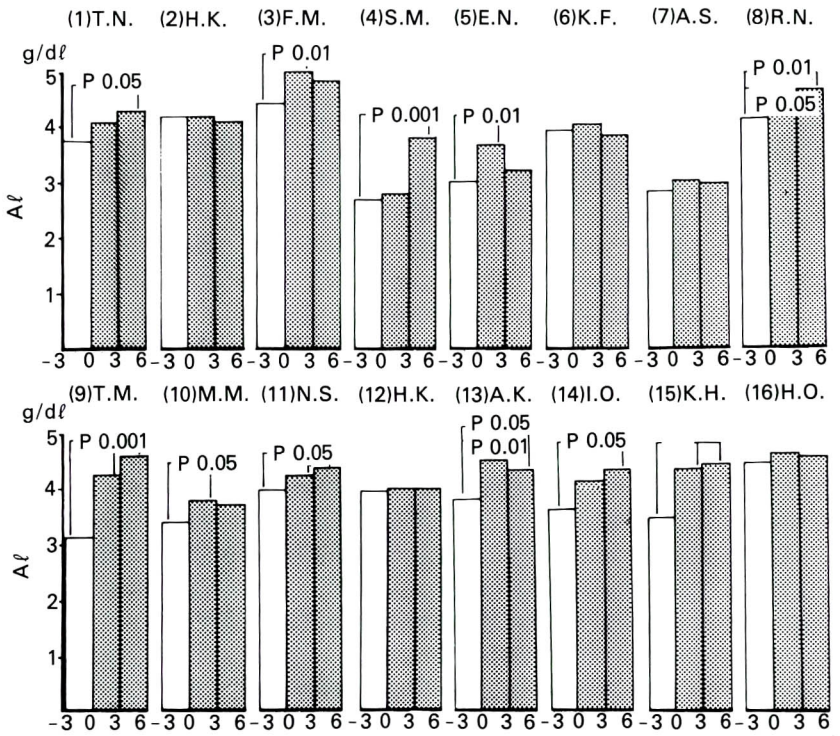

Fig. 1 Change in serum albumin level after paromomycin sulfate administration.
Each bar represents the mean of 3 months.
Figure under the horizontal axis show the month.

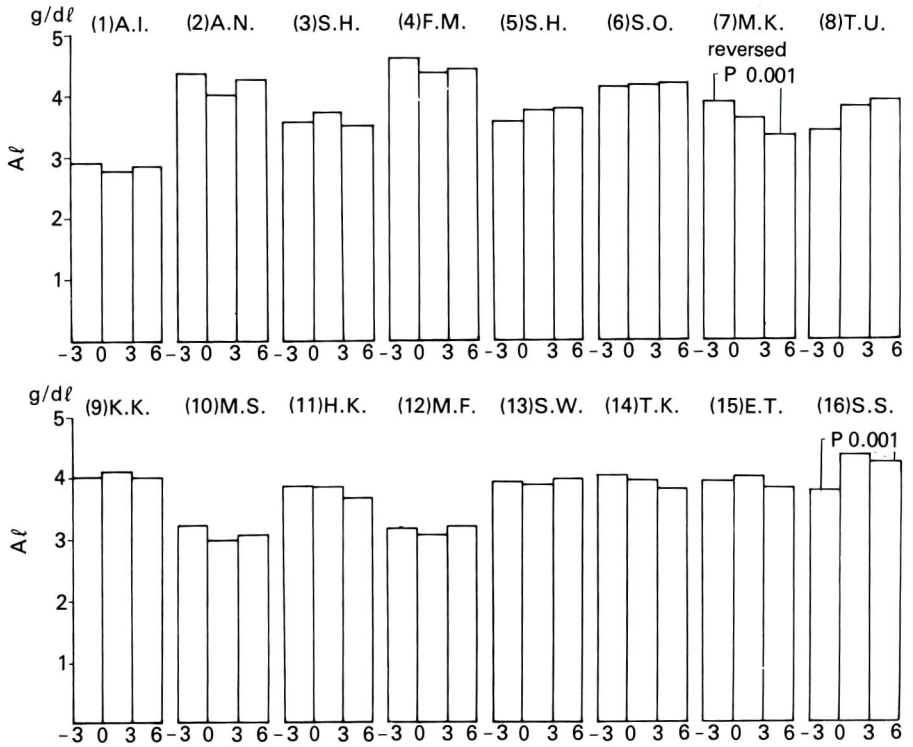

Fig. 2 Change in serum albumin level after placebo administration.
Each bar represents the mean of 3 months.
Figures under the horizontal axis show the month.

Table 3 Increment in serum albumin after paromomycin sulfate administration

Case No.	Increment of serum albumin
(1)	0.53 g/dl
(3)	0.56
(4)	1.08
(5)	0.64
(8)	0.47
(9)	1.41
(10)	0.38
(11)	0.36
(13)	0.67
(14)	0.70
(15)	0.91

Table 4 Correlation between endotoxemia and serum albumin in the paromomycin group

Case No.	endotoxemia (Limulus lysate test)				significant increase in serum albumin
	before administration	after administration			
		1 month	3 months	6 months	
(1)	(+ +)	(±)	(−)	(−)	(+)
(2)	(+ +)	(−)	(−)	(+)	(−)
(3)	(+)	(−)	(−)	(+ + +)	(+)
(4)	(+)	(−)	(−)	(−)	(+)
(7)	(+)	(±)	(−)	(−)	(−)
(8)	(+ +)	(−)	(−)	(−)	(+)
(9)	(−)	(−)	(−)	(+)	(+)
(11)	(+ +)	(−)	(−)	(−)	(+)
(12)	(+ +)	(−)	(−)	(+)	(−)
(13)	(+)	(−)	(−)	(−)	(+)
(14)	(+)	(−	(−)	(−)	(+)

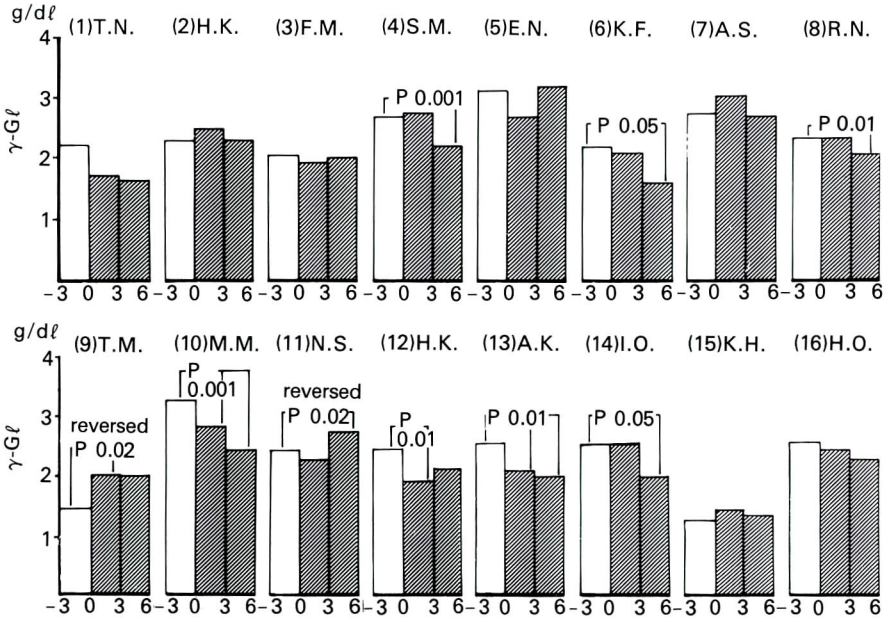

Fig. 3 Change in serum γ-globulin level after paromomycin sulfate administration. Each bar represents the mean of 3 months. Figures under the horizontal axis show the month.

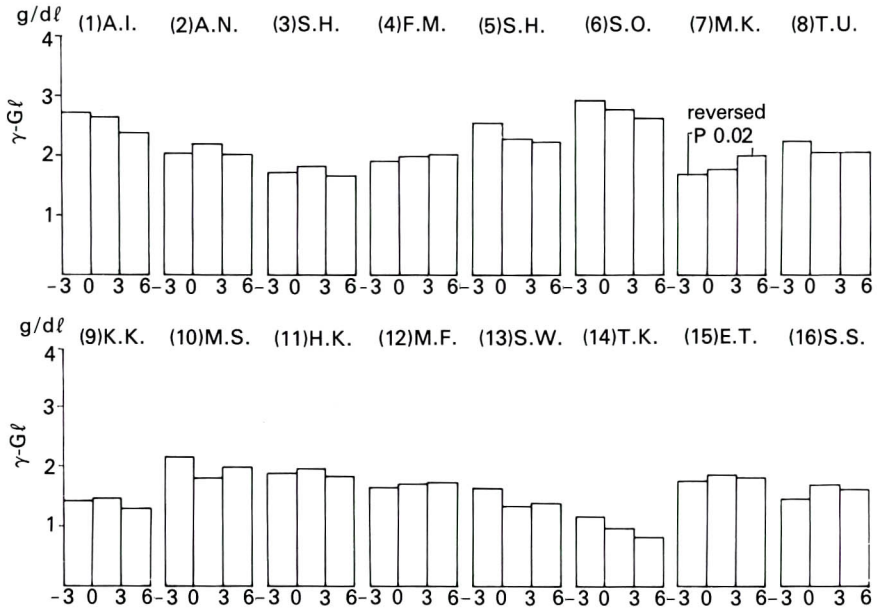

Fig. 4 Change in serum γ-globulin level after placebo administration. Each bar represents the mean of 3 months. Figures under the horizontal axis show the month.

DISCUSSION

Leach B. E. *et al*, first demonstrated that sulfonamide drugs protected against carbon tetrachloride-induced hepatic necrosis and death (6). Subsequently, Gyorgy P reported that aureomycin, streptomycin and neomycin effectively prevented hepatic necrosis in choline deficiency (4). Studying the development of cirrhosis in rats on choline-deficient diets, Rutenburg and his colleagues regularly found diffuse hepatic fibrosis within 300 days, but when absorbable antibiotics were added to the diet, cirrhosis was delayed by about 100 days. Moreover, when neomycin, a non-absorbable antibiotic, was used, significant fibrosis was prevented for as long as 750 days. They concluded that nonabsorbable antibiotics were more effective in preventing liver cirrhosis than the absorbable ones (9).

Broitman *et al.* confirmed this protective effect of neomycin administration against the development of dietary fibrosis and cirrhosis in rats fed choline-deficinet diets.

They also showed that this could be abolished by adding purified Salmonella typhosa endotoxin to drinking water during the period of the choline-deficient diet, and concluded that the absorption of intraluminal endotoxin contributed to the development of fibrosis and cirrhosis (1).

It is well known that endotoxemia develops frequently in patients with cirrhosis (2, 3, 8, 10), and significantly increases the mortality in such patients (10). On this basis, it is possible that endotoxin absorbed from the gut may be a factor in the development of cirrhosis in man, and that non-absorbable antibiotics might delay or reverse cirrhosis in man by decreasing endotoxemia. Long-term administration of non-absorbable antibiotics, such as neomycin or paromomycin, is known to reduce the numbers of bacteria in the intestine, especially gram-negative becteria (11, 13), resulting in a decrease in the amount of endotoxin produced in and absorbed from the intestine.

The present study suggested strongly that the severity of cirrhosis, as assessed by the serum albumin level, was significantly improved compared with the placebo group by the administration of paromomycin sulfate orally for 6 months to previously stable humans with cirrhosis. In fact, about 70% of the cirrhotics thus treated showed a significant increase in serum albumin during paromomycin therapy compared with 6% in the placebo group. This was generally correlated with the disappearance of endotoxemia since among 11 cirrhotics whose endotoxemia disappeared on paromomycin therapy, eight showed a significant increase in serum albumin level. These findings warrant study in a larger group of patients, utilizing liver biopsies and more quantitative tests of liver functions (e.g. the aminopyrine breath test) for a more reliable assessment of the status of the cirrhosis.

In our study, we also encountered a significant decrease in serum γ-globulin levels during long-term administration of paromomycin sulfate. It seems most likely that this decrease resulted from reduction of the number of bacteria in the gut, decreasing the production of bacterial antigens as well as endotoxin. Triger *et al.* reported a highly significant increase in titers of antibodies to E. coli, Bacteroides and rat colon antigen in the serum of pa-

tients with liver disease. They proposed that the diseased liver fails to sequester a significant portion of antigens absorbed from the intestine, which then become available to stimulate antibody formation accounting for the hyperglobulinemia observed in liver disease (12). Koga *et al.* (5) likewise found high titers of antibody to E. coli in the sera of cirrhotic patients, especially those with high γ-globulin titers.

In summary, the effects we observed on serum proteins due to the long-term administration of paromomycin sulfate to cirrhotic patients probably resulted in part from the reduction in the bacterial population of the intestine. This decreases the production of both endotoxins and bacterial antigens in the gut, with less available for absorption into the circulation. The decrease in endotoxin improves the levels of serum albumin, and the decrease in antigens in the circulation decreases the producation of antibody globulins. Further trials seem warranted using this approach to the therapy of cirrhosis.

REFERENCES

1) Broitman SA,Gottlieb LS and Zamcheck N: Influence of neomycin and ingested endotoxin in the pathogenesis of choline deficiency cirrhosis in the adult rat. J Exp Med 119: 633—641, 1964.
2) Caridis DT, Reinhold RB, Woodruff PWH and Fine J: Endotoxemia in man. Lancet I: 1381—1386. 1972.
3) Clemente C, Bosch J, Rodes J, Arroyo V, Mas A and Maragall S: Functional renal failure and haemorrhagic gastritis associated with endotoxemia in cirrhosis. Gut 18: 556—560, 1977.
4) Gyorgy P: Antibiotics and liver injury. Ann N Y Acad Sci 57: 925—932, 1954.
5) Koga A: The etiology of hypergamma-globulinemia in patients with liver cirrhosis has not yet been clearly elucidated. Acta Hepatol Jap. 21: 288—294, 1980.
6) Leach BE and Forbes JC: Sulfonamide drugs as protective agents against carbon tetrachloride poisoning. Proc Soc Exp Biol Med 48: 361—363, 1941.
7) Levin J, Tomasulo PA and Oser RS: Detection of endotoxin in human blood and demonstration of an inhibitor. J Lab Clin Med 75: 903—911, 1970.
8) Liehr H, Grün M, Brunswig D and Sautter Th: Endotoxemia in liver cirrhosis: Treatment with polymyxin B. Lancet I: 810—811, 1975.
9) Rutenburg AM, Sonnenblic KE, Koven I, Aprahamian HA and Reiner L: The role of intestinal bacteria in the development of dietary cirrhosis in rats. J Exp Med 106: 1—3, 1957.
10) Tarao K, So K and Moroi T: Detection of endotoxin in plasma and ascitic fluid of patients with cirrhosis: Its clinical significance. Gastroenterology. 73: 539—542, 1977.
11) Tarao K, Yamazaki R, Ito A, Odagiri S and Fukushima K: Fecal bacterial flora in the cirrhotic patients and effects of administration of the antibiotics or lactulose on it. Jap J Gastroenterol. 77: 1566—1574, 1980.
12) Triger DR, Alp MH and Wright R: Bacterial and dietary antibodies in liver disease. Lancet II: 60—63, 1972.
13) Weinstein L, Samet C and Meade R: Effect of paromomycin on the bacterial flora of the human intestine. JAMA 178: 891—897, 1961.
14) Yin ET, Galanos C and Kinsky S: Picogram-sensitive assay for endotoxin: gelation of Limulus polyphenus blood cell lysate induced by purified lipopolysaccharides and lipid A from Gram-negative bacteria. Biochimica et Biophysica Acta. 261: 284—289, 1972.