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Experimental Study of Endogenous Endotoxin Absorption

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Three absorption routes of endotoxin derived from the intestine of shocked rabbits, namely via portal vein, via intestinal lymphatics and via transperitoneal routes have been studied. As the intestinal circulatory disorders, superior mesenteric artery occlusion (SMAO) and superior mesenteric vein occlusion (SMVO) were induced. Furthermore, in order to investigate the transperitoneal route, fecal peritonitis was induced. Detection and quantitation of endogenous endotoxin in plasma and lymph were carried out using synthetic chromogenic substrate, peptide-4-methylcoumarin amide (MCA). In the SMAO group, endotoxin levels in portal plasma exceeded levels in lymph from the thoracic duct throughout the experiment, and in the SMVO group, the relationship was reversed. In peripheral arterial blood, endotoxin levels were significantly lower in rabbits with thoracic duct lymph drainage than in those with intact lymphatic system. In rabbits with fecal peritonitis, endotoxin levels in lymph from the thoracic duct exceeded levels in portal plasma throughout the experiment.

Based on the results described above, intestinal lymphatics was expected to play more important role than the other routes in appearance of systemic endotoxemia in non-septic shock.

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Key words: Endogenous endotoxin, Superior mesenteric artery occlusion (SMAO), Superior mesenteric vein occlusion (SMVO), Thoracic duct lymph, Transperitoneal absorption

1. Introduction

It has been reported that systemic endotoxemia has been found in circulatory disturbances of intestine and liver failure, in spite of the absence of a focus of bacterial infection, so-called nonseptic endotoxemia. The endotoxemia has been clearly indicated by many investigators, particularly by Tamakuma and his associates[1], to originate in endotoxin escaping from the intestine. Tamakuma, *et al.* also reported that endotoxemia was eliminated by the injection of nonabsorbable antibiotics into the rabbit intestine, and that at the same time this prevented shock and death. However, with regard to the routes along which endotoxin escapes, many investigators, especially Gans and Matsumoto[2] and Cuevas and Fine[3,4], have recorded conflicting results. There have been three possibilities regarding the routes of absorption of endotoxin derived from the intestine in nonseptic shock. The first of these is via the portal vein, the second is via intestinal lymphatics, and the last involves a transperitoneal route, by which intestinal endotoxin enters the peritoneal cavity transmurally. Although many studies of the first route have already been done, there have been very few reports on the latter two routes. The purpose of the present study is to determine, under circulatory disorder conditions of the intestine, which routes of entrance into systemic circulation are actually favored in endogenous endotoxin absorption, and what quantities of endotoxin are absorbed.

2. Materials and Methods

2.1 Animals

Fifty-five adult albino rabbits of both sexes, weighing between 2 and 3.5 kg, were used throughout these experiments. Animals were overnight fasted prior to the experiment, water being allowed ad libitum, and then were anesthetized with intravenous sodium pentobarbital (nembutal, Abott Laboratories, Chicago), 30 mg/kg of bqdy weight, and supplemented as required. All animals breathed room air spontaneously. A polyethylene catheter was introduced into the abdominal aorta through the right femoral artery for recording of arterial blood pressure and for blood sampling.

2.2 Experimental procedures

Experimental animals were divided into six groups as shown in Table 1. In Group I, ten rabbits were subjected to superior mesenteric artery occlusion (SMAO) with thoracic duct lymph drainage (TDLD). In Group II, ten rabbits were subjected to SMAO without TDLD and with a cervical sham operation. In Group III, ten rabbits were subjected to superior mesenteric vein occlusion (SMVO) with TDLD. In Group IV, ten rabbits were subjected to SMVO without TDLD and with a cervical sham operation. In Group V, ten rabbits were subjected to fecal peritonitis with occasional TDLD. Finally, in Group VI, five rabbits were subjected to only laparotomy with TDLD (The control group). All surgical procedures were carried out under rigidly aseptic conditions.

Table 1 *Groups in the experiment.*

Group	Number	Treatment
I	10	SMAO with TDLD
II	10	SMAO without TDLD & cervical sham operation
III	10	SMVO with TDLD
IV	10	SMVO without TDLD & cervical sham operation
V	10	fecal peritonitis with occasional TDLD
VI	5	laparotomy with TDLD

SMAO: superior mesenteric artery occlusion
SMVO: superior mesenteric vein occlusion
TDLD: thoracic duct lymph drainage

2.2.1 A technique for collecting lymph from the thoracic duct

Following a left lower cervical incision, the external jugular vein and left subclavicular vein were exposed. Finding the orifice of the thoracic duct, two ligations approximately 3 cm apart were placed on the upper and lower parts of the jugular vein centering around the orifice. Between the two ligations thereafter, all draining veins into the jugular vein were transected. A soft silicon catheter with an internal diameter of 2.0 mm (Fuji system, Tokyo) was cannulated into the jugular vein and lymph from the thoracic duct drained spontaneously into a graduated test tube containing no anticoagulant. Throughout the period of drainage intake of intravenous normal saline solution, 20 ml/hr, was adjusted so that signifiacnt fluctuation in body weight was avoided (Fig. 1).

2.2.2 A technique for superior mesenteric artery occlusion

The anesthetized animals were placed on their right side during the following procedure. A left lateral abdominal incision was then made and the superior mesenteric artery was exposed by a retroperitoneal approach. Next, the artery was occluded just distal to its origin from the aorta with a Bulldog

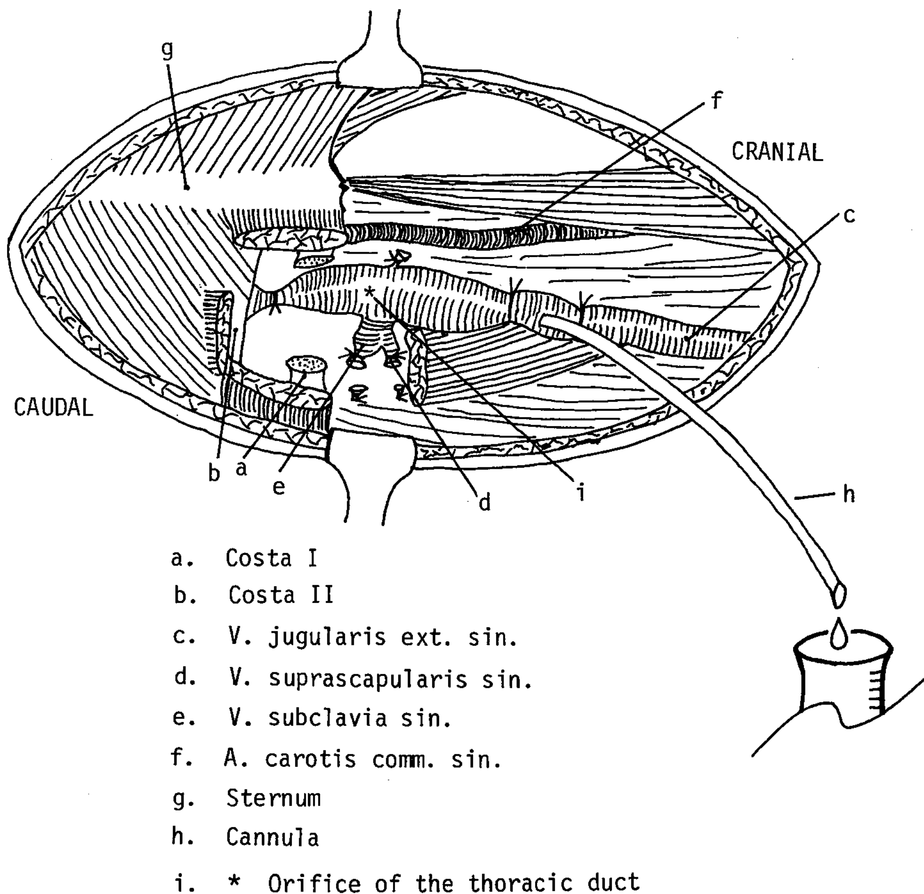


Fig. 1 Method for collecting lymph from the thoracic duct of rabbit.

clamp. This occlusion was continued for forty minutes and was released thereafter.

2. 2. 3 A technique for superior mesenteric vein occlusion

The superior mesenteric vein (SMV) was approached through a midline abdominal incision. The vein was identified as the lower part of the portal vein rather than the draining site of the splenic vein. A hard polyvinyl rod, 5 mm in diameter and 4 cm in length, was placed adjacent to the vein and within a loop of 3-0 Nylon thread so that the vein could be occluded by tying it tightly against the rod. Then the body wall was closed with interrupted silk suture. The occlusion was released after forty minutes by withdrawal of the vinyl rod through the sutured body wall.

2. 2. 4 Fecal peritonitis (Group V)

A laparotomy was performed through a midline upper abdominal incision, and a purse string suture was placed on the cecum wall. By tying the string, an artificial diverticle, approximately 1.5 cm in diameter, was made. After incising the top of the diverticle, about 0.5 cm in length, the abdominal wound was closed tightly with interrupted silk suture. No antibiotics of other drugs were administrated to the animals throughout the experiment.

2. 3 Collecting of samples

Lymph from the thoracic duct was drained spontaneously into a graduated tube which was exchanged every 15 minutes in Groups I, III, and VI. In Group V, thoracic duct lymph was collected before, 6 and 12

hours after operation. Because of the difficulty of maintaining long term drainage of lymph from the thoracic duct and its sterility, different rabbits were used at each time interval in Group V. Blood samples were drawn in Groups I, II, III and IV immediately prior to operation, at 20 and 40 minutes after occlusion, and at 30, 60 and 120 minutes after release from the right femoral artery and portal vein. Portal venous blood was collected by direct puncture of the portal vein through a tuberculin syringe at each time interval. Three milliliters of arterial blood and one milliliter of portal venous blood were collected in a pyrogen-free syringe with a heparinized internal wall. The volume of blood removed (4 ml) was replaced with an equal amount of normal saline solution. The heparinized blood samples were immediately centrifuged at 4℃ for 30 minutes at 3,000 rpm, after which plasma was separated and transferred to endotoxin-free tubes. These tubes were then coded and stored at −20℃ until analyzed. In Group V, 5 ml of peritoneal fluid was collected at each time interval for bacterial counts.

2.4 Measurement of thoracic duct lymph flow

In Groups I and III, lymph from the thoracic duct was drained into the graduated test tubes spontaneously. The lymph flow was calculated as volume difference/collection time (15 min).

2.5 Measurement of arterial blood pressure

Peripheral arterial blood pressure was continuously monitored and recorded with a polygraph (Nihon Koden PM6, Tokyo) attached to a pressure transducer (GOULD Statham P 50) which was connected with the polyethylene cannula inserted into the right femoral artery.

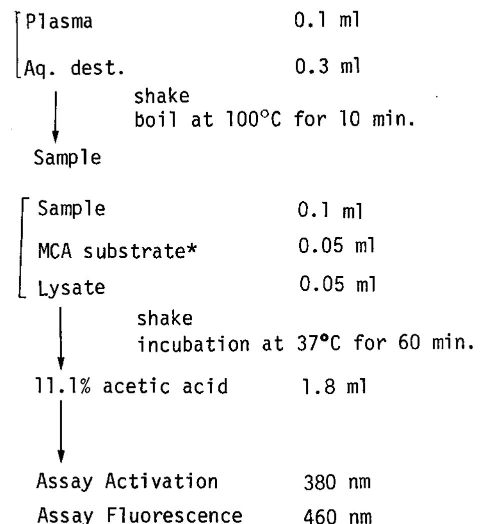
2.6 Measurement of samples

2.6.1 Glassware

All glassware used was washed with a neutral detergent, tap water and ion-exchanged water 3 times, wrapped in aluminum-foil, and then rendered endotoxin-free by drying at 250℃ for two hours.

2.6.2 Endotoxin assay in plasma and lymph from the thoracic duct

In the present study, the detection and quantitation of bacterial endotoxins in plasma and lymph from the thoracic duct were carried out according to the method of Harada *et al.*[5] using synthetic fluorogenic substrates (Boc-Leu-Gly-Arg-MCA, Peptide Institute, Osaka) found to be specific substrates for horseshoe crab clotting enzyme. Plasma and lymph samples were diluted 4-fold with pyrogen-free distilled water, and then were boiled at 100℃ for 10 minutes to eliminate inhibitors of the clotting reaction[6,7]. Pre-Gel (Teikoku-Zoki, Tokyo), as a horseshoe crab hemocyte lysate and MCA (4-methylcoumarin amide), as a fluorogenic substrate, were used. Assays of endotoxin were carried out in a water bath at 37℃. Incubation mixtures for the assay consisted of 0.05 ml of amoebocyte lysate, 0.05 ml of MCA substrate and 0.1 ml of the material tested. After 60 minutes, the reaction was stopped by the addition of 11.1% acetic acid solution (1.8 ml), after which the released AMC (7-amino-4-methylcoumarin) was measured by a fluorophotometer (Shimazu RF-510, Tokyo) at 460 nm of fluorescent emission and at



Endotoxin : E.coli 0127; B8 lipopolysaccharide B
*MCA : 4-methylcoumarin amide

Fig. 2 A method for assaying bacterial endotoxin in plasma and lymph.

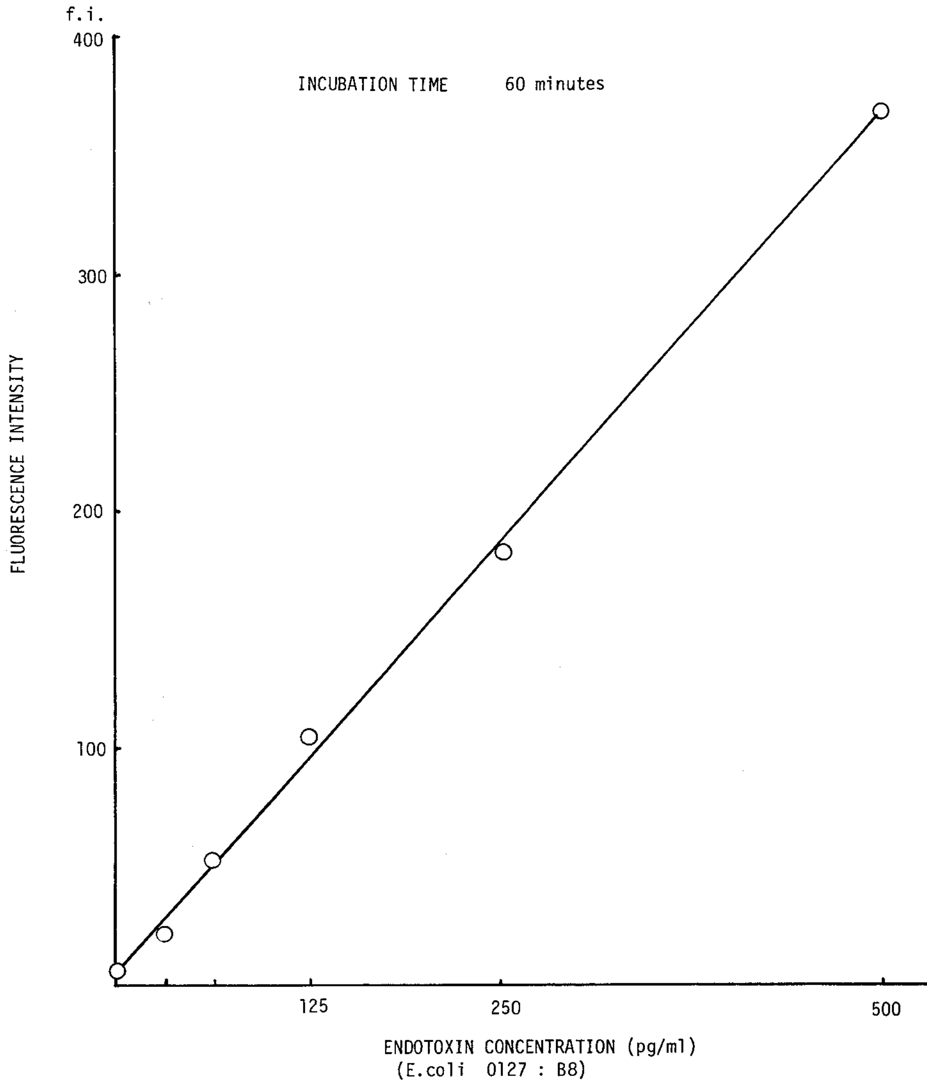


Fig. 3 Standard curve for assaying endotoxin levels using MCA-substrate.

380 nm of excitation light (Fig. 2). A standard curve for the quantitation of bacterial endotoxin was made by the above described method employing Escherichia coli 0127 : B_8 lipopolysaccharide B (Difco Laboratories, Detroit) as a standard (Fig. 3).

2. 6. 3 Hematology of rabbits with peritonitis

In Group V, white blood cell counts, hematocrit and platelet counts, before and 6 and 12 hours after operation, were determined by usual laboratory methods.

2. 6. 4 Bacterial counts in peritoneal fluid

In Group V, peritoneal fluids collected 6 and 12 hours after peritonitis were diluted tenfold serially with an autoclaved dilute solution (Na_2HPO_4 6.5 g, KH_2PO_4 4 g, tween 80 1 g, 1-cistein chloride 1 g, Agar 1 g in 1,000 ml distilled water). For aerobic and anaerobic cultivations conventional agar media (BBL Lab.) and TEP media (Eiken, Tokyo) were used, respectively. Anaerobic plates were incubated in a GASPAK jar (Baltimore Biological Laboratories Cockeysville, Maryland) at 37℃ for 24 hours.

2.7 Statistical analysis for data

Statistical analysis was done using the Student's paired and unpaired T-test. A P value of less than 0.05 was considered to be statistically significant.

3. Results

3.1 Hemodynamic changes

In Group I, peripheral arterial blood pressure was stationary at the preocclusive level after occlusion of the superior mesenteric artery. However, at the time of the release, blood pressure precipitously fell to approximately 50 mmHg and showed a representative declamping pattern. In Group III, blood pressure tended to fall immediately after occlusion of the superior mesenteric vein, and then gradually rose after the release. In Group V, blood pressure gradually declined until the animals died. In Group VI, all animals maintained an adequate blood pressure until 24 hours later (Fig. 4).

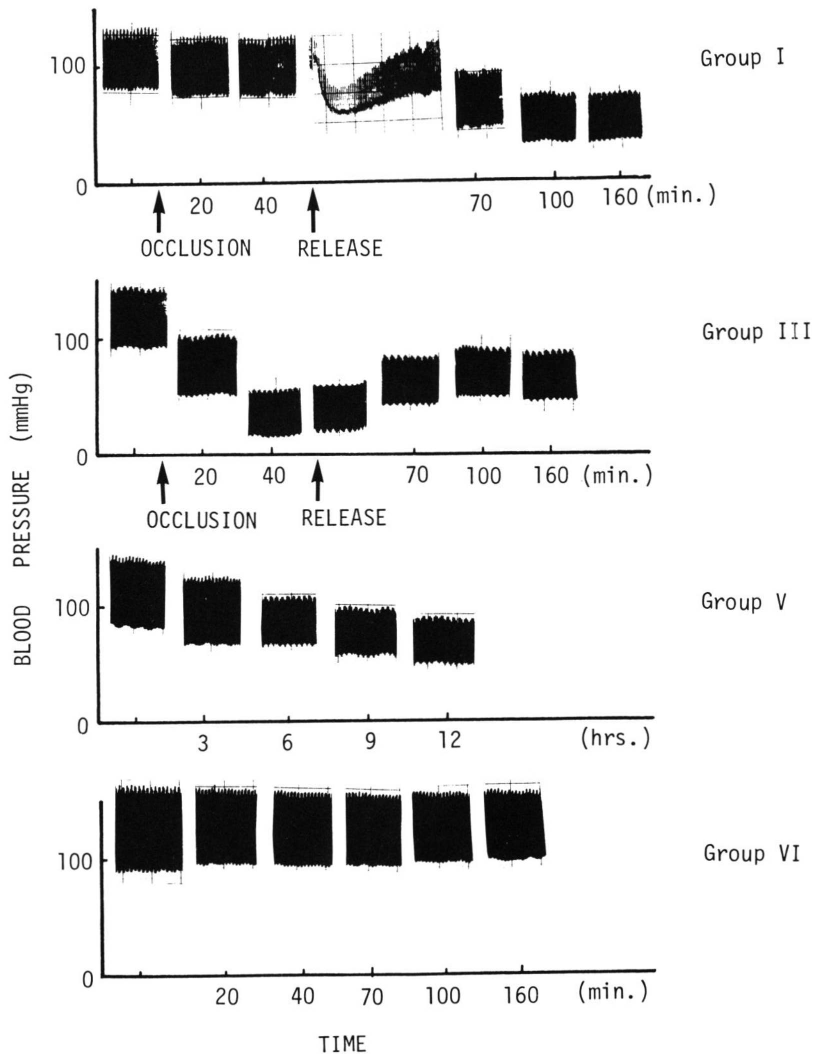


Fig. 4 Hemodynamic changes.

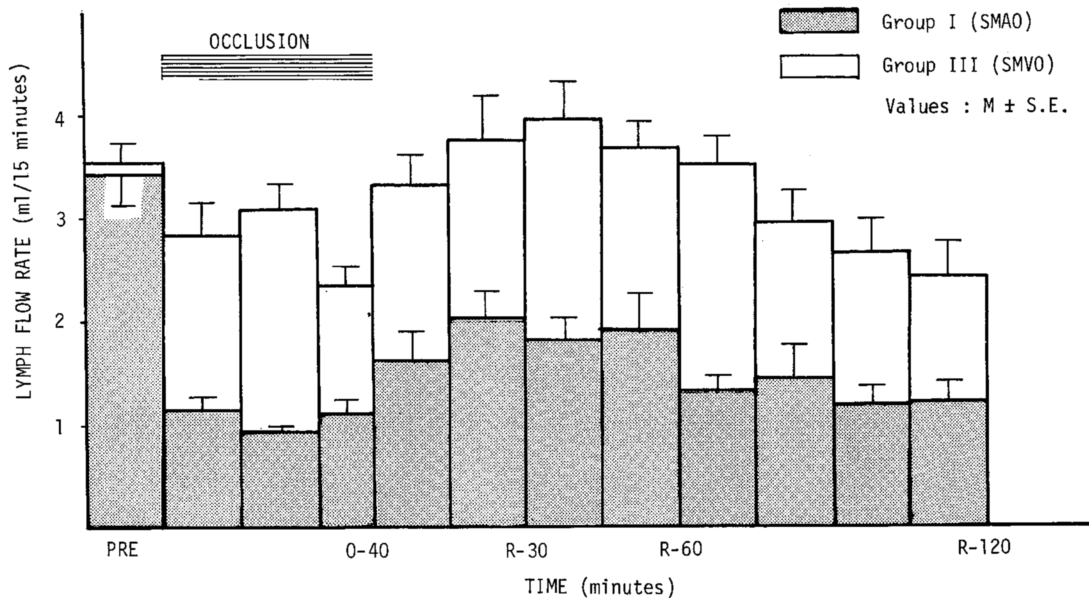


Fig. 5 Thoracic duct lymph flow rate in Groups I and III (N=10).

3.2 Thoracic duct lymph flow in Groups I and III

Results representing the means of all animals studied are expressed in Figure 5. In Group I, at the same time as occlusion of the SMA, the lymph flow rate decreased by 35 percent as compared to the preocclusive level ($P<0.05$). Then it slightly increased after the release. In Group III, the lymph flow rate decreased by 78 percent as compared to the preocclusive level ($P<0.005$) when the occlusion of the SMV was performed. It then began to rise abruptly after the release, with the maximum flow rate reached being 112 percent as compared to the preocclusive level. During the experiment in Group III it was observed that after the occlusion of the SMV, lymph from the thoracic duct became hemorrhagic and continued to be red in color throughout the experiment. The hemorrhagic component of the lymph measured as hematocrit varied from 2 to 24 percent and averaged approximately 4 to 5 percent.

3.3 Changes in endotoxin levels in lymph and portal plasma in Group I

The experimental data are summarized in Table 2. Endotoxin levels in lymph from the thoracic duct and portal venous blood at each time interval increased significantly as compared to the preSMAO levels ($P<0.005$). In particular, the increase from 40 minutes after the occlusion to 30 minutes after the release,

Table 2 *Changes in endotoxin levels in Group I (N=10).*

sample	before	after (minutes)				
		Occlusion		Release		
		20	40	30	60	120
Thoracic duct lymph (pg/ml)	36.6± 4.5	85±19.7*	184.9±58.8*	349.1±50*	355.3± 40.3*	252.2±33.1*
Portal venous blood (pg/ml)	114.5±11.8	284±37.3*	312.8±38.3*	502.7±85.2*	549.1±105.8*	515.4±88.7*

(1) values are mean ± standard error of mean.
(2) * significantly different from control levels
* $p<0.005$

from 184.9±58.8 to 349.1 ± 50 pg/m*l* in lymph and from 312.8 ± 38.3 to 502.7 ± 85.2 pg/m*l* in portal venous blood, was significantly marked ($P<0.005$). Endotoxin levels in portal venous blood at each time interval exceeded levels in lymph during the entire course of the experiment.

3.4 Changes in endotoxin levels in peripheral arterial blood in Groups I and II

Results are summarized in Table 3. In both groups, endotoxin levels in peripheral arterial blood at each time interval increased significantly as compared to the preSMAO levels ($P<0.005$). Endotoxin levels at each time interval in Group I were significantly lower than those of corresponding time intervals in Group II ($P<0.005$, $P<0.01$).

Table 3 *Changes in endotoxin levels in arterial blood in Groups I and II (N=10).*

Arterial blood	before	after (minutes)				
		Occlusion		Release		
		20	40	30	60	120
with TDLD (pg/m*l*)	17.7±4.7	31±6.4*	43.1±6.2*	77.6± 9.4*	67± 5.5*	53.6±6.8**
without TDLD(pg/m*l*)	35.7±7.2	64.7±8.1	73±7.7	135.6±13.4	118±10.1	78±5.3

(1) Values are mean ± standard error of mean.
(2) *,** significantly different from levels without TDLD
* p<0.005 ** p<0.01

3.5 Changes in endotoxin levels in lymph and portal plasma in Group III

Table 4 shows that endotoxin levels in lymph from the thoracic duct and portal venous blood at each collecting time increased significantly as compared to the preSMVO levels ($P<0.005$). At 20 minutes after SMVO, endotoxin levels in portal venous blood exceeded those in lymph. Thereafter, however, this relationship was reversed throughout the experiment.

Table 4 *Changes in endotoxin levels in Group III (N=10).*

sample	before	after (minutes)				
		Occlusion		Release		
		20	40	30	60	120
Thoracic duct lymph (pg/m*l*)	49.2± 6.6	171.4±17.2*	345±85*	790.4±93.2*	760.7±110.8*	775.8±131.7*
Portal venous blood (pg/m*l*)	92.2±12.2	208.3±27*	229.4±31.4*	321.7±32.6*	364.4± 29.2*	379.4± 25.4*

(1) Values are mean ± standard error of mean.
(2) * significantly different from control levels.
* p<0.005

3.6 Changes in endotoxin levels in peripheral arterial blood in Groups III and IV

Results are expressed in Table 5. In both groups, endotoxin levels in peripheral arterial blood collected at each time interval increased significantly as compared to the preSMVO levels ($P<0.005$). As in Groups I and II, endotoxin levels at each time interval in Group III were significantly lower than those of the corresponding time in Group IV ($P<0.005$, $P<0.01$).

3.7 Changes in endotoxin levels in Group V

As summarized in Table 6, endotoxin levels in lymph from the thoracic duct, portal venous blood and peripheral arterial blood increased significantly as compared to the preoperative levels ($P<0.005$).

Table 5 *Changes in endotoxin levels in arterial blood in Groups III and IV (N=10).*

Arterial blood	before	after (minutes)				
		Occlusion		Release		
		20	40	30	60	120
with TDLD (pg/ml)	23.5±7.7	55.1±17.2**	84.1±24.4*	112.2±22.9*	130.3±21.8*	133.9±24.8*
without TDLD(pg/ml)	35.8±7.4	143.4±27.8	300.2±56.7	380.8±60.3	307.7±33.4	259.3±18.1

(1) Values are mean ± standard error of mean.
(2) *,** significantly different from levels without TDLD
* p<0.005 ** p<0.01

Table 6 *Changes in endotoxin levels in Group V (N=10).*

sample	before	after (hours)	
		6	12
Thoracic duct lymph (pg/ml)	42.9± 5.6	329.2±50.1	340.3±48.2
Portal venous blood (pg/ml)	103.3±12	154.7±47.5*	316.8±30.6
Arterial blood (pg/ml)	28.2± 6.7	141.4±38.5	218.9±35.7

(1) Values are mean ± standard error of mean.
(2) * significantly different from levels 12 hours after peritonitis.
* p<0.01

Endotoxin levels 12 hours after peritonitis in three samples clearly exceeded levels 6 hours after peritonitis. However, with the exception of levels in portal venous blood (P<0.01) there were no significant differences between these endotoxin levels. Endotoxin levels 6 and 12 hours after peritonitis were higher in lymph from the thoracic duct than in portal plasma. A significant difference was shown in levels 6 hours after peritonitis (P<0.005).

3.8 Changes in hematologic data in Group V

Results are expressed in Table 7. As compared to the preoperative levels, white blood cell counts increased significantly 6 hours after peritonitis, but then decreased significantly 12 hours after peritonitis. Hematocrit levels at each time interval increased significantly as compared to the preoperative levels (P<0.01). Platelet counts also slightly increased as compared to the preoperative levels.

Table 7 *Hematological changes in Group V (N=10).*

	before	after (hours)	
		6	12
white blood cell counts $\times 10^3/mm^3$	7.25±0.2	10.3±0.9	3.3±0.7
hematocrit vol %	36.3 ±3.2	48.9±1.2	46 ±1.8
platelet counts $\times 10^4/mm^3$	33.5 ±4.7	37.4±3.5	36.6±8.1

Values are mean ± standard error of mean.

Table 8 *Bacterial counts in peritoneal fluid in Group* V *(N=10).*

	after 6 hours		after 12 hours	
	control gruop (N=3)	experimental group (N=10)	control group (N=3)	experimental group (N=10)
Aerobic culture method	N	5.28±0.15*	N	7.11±0.44
Anaerobic culture method	N	5.34±0.13*	N	7.26±0.41

N : negligible
(1) Values are mean ± standard error of mean.
(2) * significantly different from level 12 hours after peritonitis.
* p<0.005

Table 9 *Changes in endotoxin levels in Group VI (N=5).*

	before	after (minutes)				
		20	40	70	100	160
Thoracic duct lymph (pg/m*l*)	29.7± 8.2	26.7± 7.7	30.1±17.4	20.7±5.9	35.8±17.5	27.2±11.5
Portal venous blood (pg/m*l*)	87.1±21.5	97.8±13.5	79.3±12.1	93.3±8.9	103.5±15.7	90.2± 5.7
Arterial blood (pg/m*l*)	27.2± 7.7	26.7± 8.3	32 ± 6.5	30.6±8.5	29.6± 6.2	33.5±10.2

Values are mean ± standard error of mean.

3.9 Changes in bacterial counts in peritoneal fluid in Group V

Table 8 represents the logarithmic numbers of bacterial counts per milliliter of peritoneal fluid collected at each time interval. In aerobic cultivation, bacterial counts 6 hours after peritonitis were $10^{5.28\pm0.15}$/m*l*. These significantly increased to $10^{7.11\pm0.44}$/m*l* 12 hours after peritonits (P<0.005). In anaerobic cultivation, with an identical pattern to that in aerobic cultivation, a significant increase in bacterial counts was seen from $10^{5.34\pm0.13}$/m*l* at 6 hours after peritonits to $10^{7.26\pm0.41}$/m*l* 12 hours after peritonitis (P<0.005).

3.10 Changes in endotoxin levels in Group VI

Results are summarized in Table 9. Preoperative levels in lymph from the thoracic duct, portal venous blood and peripheral arterial blood remained substantially the same throughout the experiment.

4. Discussion

Since 1971, when the origin of the endotoxemia in nonseptic shock was elucidated to be lipopolysaccharide from the intestine, a large number of studies on endotoxin have been reported world wide. Direct evidence to support this elucidation has been dependent upon the development of techniques which permit the detection of endotoxin iteself. Recently, attention has been focussed by many researchers on the mechanism of endotoxin absorption from the intestine. Before invading systemic circulation, endotoxin, being as it is the lumen of the intestine, must first cross the intestinal mucosa. With regard to the mechanism of endogenous endotoxin crossing the mucosa, several possibilities have been suggested as the following being representative. 1) increase in the permeability of the intestinal mucosa to endotoxins[8]. 2) disorder in mucosal microcirculation due to intestinal circulatory disorder or increase in intraluminal pressure, which distends the intestinal wall[9]. 3) suppression of the preventive effect for mucosa by mucin[10]

and bile acid[11], which are so-called physiological surfactants of intestinal mucosa. On crossing the intestinal mucosa, endotoxins may be absorbed via submucosal capillaries and the intestinal lymphatic system, or may invade transmurally into the peritoneal cavity and be absorbed transperitoneally. In any case, endotoxin derived from the intestine may be transported into systemic circulation via the portal vein or via intestinal lymphatics, or via both. In the present study, ischemic (SMAO) and congestive (SMVO) conditions were performed on the intestine of the rabbit for modeling of intestinal circulatory disorders. In addition, fecal peritonitis was induced to investigate the routes along which endotoxin absorbed transperitoneally may enter systemic circulation. Although Sanford and Noyes[12] and Berczi *et al.*[13] studied endotoxin absorption derived from the intestine by means of intraluminal administration of ^{51}Cr labeled endotoxin, they were unable to demonstrate endotoxin absorption from normal intestines. Subsequently, Ravin *et al.*[14] and Koczar *et al.*[11] substantiated endotoxin absorption from the intestine under a low flow state condition. The detection of endotoxin using radioisotope, however, has been an inadequate method because of the discrepancies between the number of bacteria recovered from blood and tissues in shocked animals and the titers of detected endotoxin, as well as because of the presence of cross reaction[14]. In 1964, Levin and Bang[15] described a new method for assaying bacterial endotoxin which involved the gelation of hemocytes from the horseshoe crab by bacterial endotoxin. This gelation of the lysate, the so-called Limulus test, has been widely employed as a simple and very sensitive assay method for endotoxin. As a quantitative method modifying the Limulus gelation test, the MCA substrate (peptide-4-methylcoumarin amide) method, which was first described by Harada *et al.*[5], was employed in the present study. The MCA substrate has been recently introduced as a chromogenic substrate for blood clotting factors and their related enzymes[16]. A pro-clotting enzyme, which is contained in a hemocyte lysate from horseshoe crabs, is tranformed into an active clotting enzyme in the presence of Gram-negative bacterial endotoxin[17~23]. The clotting enzyme coagulates a clottable protein, so-called coagulogen, which is also contained in the hemocyte lysate. The enzyme, at the same time, cleaves the tri and tetrapeptide sequences of the MCA substrate (Boc-Leu-Gly-Arg-MCA), and liberates AMC (7-Amino-4-Methyl-Coumarin) as a fluorogenic substance. The AMC released is then measured spectrofluorophotometrically. Since a linear relationship is found to exist between the fluorescent intesity of the AMC released and the quantity of endotoxin ranging from 0.01 to 100 ng/m*l*, precise quantitation of bacterial endotoxin can be carried out. The recovery rate of this method has been reported to vary from 2 to 80 percent, corresponding to the amount of administrated endotoxin[24]. The normal flow rate of lymph from the thoracic duct is estimated as from 0.5 to 0.75 m*l*/15 min.[25~27] Lymph from the liver and the intestine normally comprises the majority of thoracic duct lymph flow, about 90 percent[28], and the intestine contributes approximately a half to three-fourths of the lymph in the thoracic duct[29]. As major factors capable of increasing lymph flow, a) arteriolar dilatation, b) postcapillary constriction, c) increased capillary blood volume or flow, d) increased tissue oncotic pressure and e) decreased plasma oncotic pressure have been reported[30]. In the present study, in both SMAO and SMVO the flow rate of lymph from the thoracic duct decreased abruptly at the same time as the occlusion of the vessels. Following release of the occlusion, the flow rate of lymph remained at a low level in SMAO rabbits, while in SMVO, on the other hand, the flow rate increased and exceeded the previous level. The finding of a decline in the lymph flow rate after SMAO can be accounted for by the fact, which has been demonstrated by Olinger *et al.*[31], that acute occlusion of the superior mesenteric artery caused a 45.2 percent decrease in portal venous flow, while the portal venous pressure decreased by 28.5 percent. Dumon and Mulholland[32] observed that end thoracic duct pressures in patients with liver cirrhosis increased to about three times the normal state. However, the present data on the lymph flow rate after SMVO were not supportive of their observation. The reason for this probably lies in the fact that superior mesenteric venous pressure was increased to the extent that it collapsed the intestinal lymphatic capillaries. With

respect to the absorption of endotoxin derived from intestinal bacterial flora, Cuevas and Fine[3] have recently emphasized that the main absorptive route for endogenous endotoxin was via the peritoneum in SMAO. To support their assertion, they reported that within 5 minutes after SMAO animals showed a systemic endotoxemia at a time when little or no endotoxin was in the portal venous blood. Furthermore they asserted lymphatic flow could not account for endotoxin in the blood within 5 minutes. On the other hand, Hau *et al.*[33] demonstrated that after contamination of the peritoneal cavity with E. coli, unaltered bacteria appeared in the thoracic duct lymph within 6 minutes of contamination. In the present data of SMAO, however, endotoxin levels in portal venous blood as well as in lymph from the thoracic duct during occlusion were high enough to be detectable. In addition, endotoxin levels in portal venous blood exceeded levels in lymph from the thoracic duct throughout the experiment. On the contrary in SMVO, endotoxin levels in lymph from the thoracic duct exceeded levels in portal venous blood at each time interval with the exception of levels 20 minutes after occlusion. Thus, it can be seen from the above results that in ischemic condition of the intestine (SMAO), endotoxins derived from the intestine predominantly enter systemic circulation via the portal vein, while in congestive condition (SMVO) they enter via intestinal lymphatics. There has been much discussion as to whether endotoxin is present in normal portal venous blood or not. Jacob *et al.*[34] and Prytz *et al.*[3], reported that endotoxin was detected by the Limulus gelation test in normal portal venous blood. Cuevas and Fine[3], on the other hand, disagreed with this observation. In view of the present data, it appears obvious that approximately 100 picograms per milliliter of endotoxin was quantitated, and this was clearly higher than the arterial control level. Many authors[28,30,36,37], particularly Glenn and Lefer[37], have reported that thoracic duct lymph collected from shocked animals contained high intestinal lysosomal enzyme activity (acid phosphatase, β-glucuronidase) and the myocardial depressant factor (MDF), which is produced in the pancreas of shocked animals. The activities of these enzymes and MDF in plasma were significantly lower in shocked animals with diverted lymph than in animals with an intact lymphatic system. In the present study, in both SMAO and SMVO rabbits, arterial endotoxin levels in animals with drainage of thoracic duct lymph were significantly lower than levels in animals without lymph drainage. In view of the above observations, it is reasonable to presume that endotoxin transported via intestinal lymphatics may play an important role in the appearance of systemic endotoxemia. As far as the fate of endotoxin absorbed transperitoneally is concerned, there are three possible routes, namely, via the portal vein or via the lymphatic system or via both. The mechanism of dissemination within the peritoneal cavity of foreign substances and the routes of dispersal from the peritoneal cavity have been extensively studied[38]. In the present study, fecal peritonitis was induced and was expected to result in exposure of a large absorptive surface to bacteria and their endotoxins. As a rule, it has been thought that of many kinds of intraperitoneal foreign bodies, crystalloids and microparticles are absorbed from the peritoneal surface via blood vessels, while on the other hand, colloid is absorbed via the lymphatic sytem, particularly via diaphragmatic lymph capillaries[38~47]. Although endotoxins are in a state of aggregation or dispersion under solvent and co-existing substance conditions, biologically active endotoxins are 10^6 to 10^7 in molecular weight and necessarily aggregate into particles of colloidal dimensions before antigenicity and toxic properties can be manifested[13,20]. Daniele *et al.*[48] demonstrated that when Escherichia coli endotoxin labeled by radioisotopes was placed in the peritoneal cavity, part of it remained while some escaped (about 21 percent), predominantly through the thoracic duct. Subsequently, Gans and Matsumoto[2] and Nolan *et al.*[49] demonstrated intestinal absorption of ^{51}Cr labeled endotoxin through a Thiry-Vella isolated gut sac in leadsensitized rats. From their studies, they speculated that the lymphatic system was more important than the portal vein in the passage of endotoxin absorbed transperitoneally into systemic circulation. In the present study, although endotoxin levels in lymph from the thoracic duct 6 hours after peritonitis apparently exceeded levels in portal venous blood, there were no significant differences between endotoxin levels in

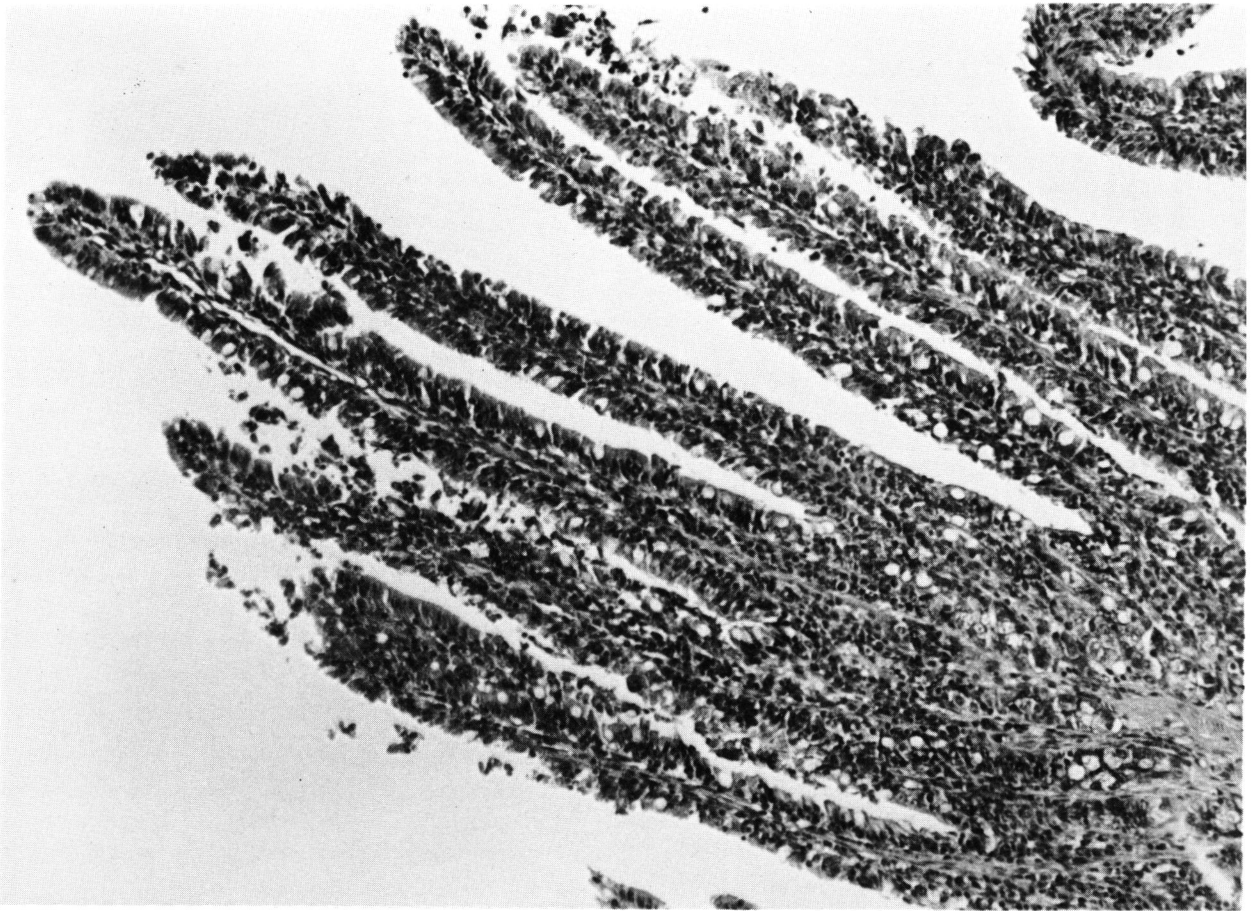


Fig. 6 Structure of intestinal mucosa in superior mesenteric artery occlusion (SMAO). Tissues stained with Haematoxylin and Eosin (× 400).

either sample 12 hours after peritonitis. As to lymph from the thoracic duct, endotoxin levels remained stationary and showed no fluctuations at each time interval. On the other hand, in portal venous blood, endotoxin levels, 12 hours after peritonitis were twice as high as levels 6 hours after peritonitis($P<0.01$). The above findings suggest, first, that endotoxins absorbed transperitoneally in fecal peritonitis enter systemic circulation predominantly via the lymphatic system, and second, that as time passes, the portal vein plays an equal role to that of the lymphatic system in the passage of endotoxin into the circulation. In gross laparotomy examinations at each time interval after peritonitis, intestinal serosa showed patchy deposits of fibrin and numerous filmy adhesions. In addition, there were petechial hemorrhages in the submucosa, and there was mild edema of the intestinal wall. Filler and Sleeman[50] have demonstrated an initial decreased absorption rate from the peritoneal cavity secondary to peritonitis caused by a mixture of Escherichia coli and hemoglobin. With regard to the bacterial counts of the present study, in both aerobic and anaerobic cultivations, the counts increased significantly during the period from 6 to 12 hours after peritonitis ($P<0.005$). This increase in bacterial counts in the peritoneal cavity can possibly be explained in two ways, first, by the proliferation of bacteria, and second, by a decrease in the absorption rate from the peritoneum such as that observed by Filler and Sleeman[50]. Furthermore, discrepancies in the decrease in the peritoneal absorption rate and the increase in endotoxin levels in portal plasma and lymph from the

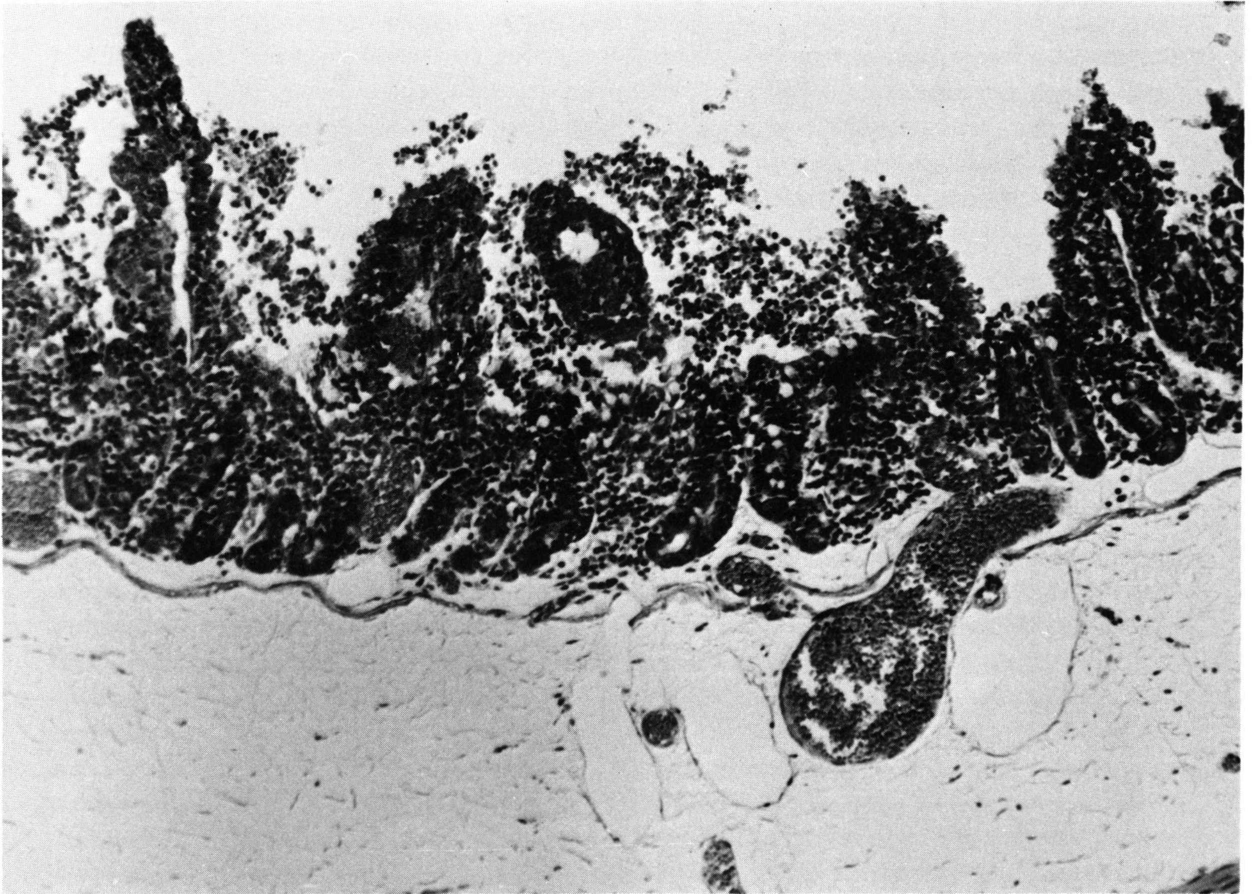


Fig. 7 Destruction of intestinal mucosa in superior mesenteric vein occlusion (SMVO). Tissues stained with Haematoxylin and Eosin (× 400).

thoracic duct can be accounted for by the fact that the decrease in bacterial clearance from the peritoneal cavity permits their continued growth and the production of large amounts of endotoxins. As regards the morphological changes in intestinal mucosa in the low flow state, destruction of mucosal microvilli, which is expressed in figures 6 and 7, was observed to be extremely higher in the congestive state (SMVO) than in the ischemic state (SMAO). In SMVO, the microvilli almost entirely sloughed off from the basement membrane, whereas partial destruction was observed near the top of the villi in SMAO. Those morphological observations appear to be enough to support the present data indicating that endotoxin levels in SMVO always exceed levels in SMAO.

5. Conclusions

The mechanism of endogenous endotoxin absorption has been studied under intestinal circulatory disorder conditions and fecal peritonitis. Several conclusions were obtained as follows.

1) Thoracic duct lymph flow decreased by 35 percent in SMAO and by 78 percent in SMVO, respectively, as compared to the preocclusive levels.

2) In SMAO, endotoxin levels in portal venous blood were higher than those in lymph throughout the experiment.

3) In SMVO, endotoxin levels in lymph from the thoracic duct were higher than those in portal plasma throughout the experiment.

4) In both SMAO and SMVO, peripheral arterial endotoxin levels with thoracic duct lymph drainage (TDLD) were significantly lower than those without TDLD.

5) In fecal peritonitis, endotoxin levels were higher in lymph from the thoracic duct than in portal plasma throughout the entire course of the experiment.

6) In fecal peritonitis, bacterial counts in the peritoneal fluid increased significantly as time passed.

7) There was much to suggest that main route of endogenous endotoxin absorption was via the portal vein under intestinal ischemic condition, via the intestinal lymphatics under the congestive condition, and via the lymphatic system in fecal peritonitis, respectively.

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内因性エンドトキシン吸収に関する実験的研究

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各種ショック状態時における腸管由来の内因性エンドトキシンの全身循環系への出現経路として，従来より三つの経路が考えられてきた．第一に門脈経路，第二に腸管リンパ経路，第三に腸管より一度腹腔内に出た後，腹膜より吸収され門脈及びリンパ系を介して吸収される経路である．今回家兎を使用し，上腸間膜動脈閉塞 (SMAO)，上腸間膜静脈閉塞 (SMVO) 及び糞便性腹膜炎モデルを作製し血行動態，胸管リンパ流量，門脈血，末梢血及び胸管リンパ液中のエンドトキシン量，さらに腹水中の細菌数の変動を測定した．SMAO 群においては，全経過を通じて門脈血中エンドトキシン値が胸管リンパ液中の値よりも高値を示し，SMVO 群では逆に胸管リンパ液中エンドトキシン値が高値を示した．末梢血中エンドトキシン値は，胸管リンパドレナージ群で非ドレナージ群よりも有意に低値を示した．腹膜炎群では，全経過を通じて胸管リンパ液中のエンドトキシン値が門脈血中の値よりも高値を示した．以上の実験結果より，内因性エンドトキシン吸収経路としての三つの経路は腸管の循環状態の変化によりそれぞれが主要なエンドトキシン輸送経路となり得るが，特に腸管リンパ経路が内因性エンドトキシン血症発現に関してより重要な役割を演ずる可能性が示唆された．