Clinical and Experimental Studies on Acute Intestinal Obstructions with Special Reference to the Cause of Death

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Clinical and experimental investigations were performed on the pathophysiology of death in acute intestinal obstruction, and the following results were obtained.

1) In clinical cases as well as in experimental animals, the number of intestinal coli-group bacilli was remarkably increased in intestinal contents both above and below the obstruction and in ascites. The increase was greatly inhibited by intraintestinal administration of fradiomycin, an unabsorbable antibiotic. Histological changes in other organs were suppressed, and the survival time of dogs was also remarkably prolonged by the treatment.

2) High molecular substances such as endotoxin, cellular components or DNA fraction liberated from Escherichia coli were increased in the blood and ascites from patients with acute intestinal obstruction. The increase was fairly well inhibited by the intraintestinal administration of fradiomycin. An intimate relation was consequently assumed between the increase of intestinal microorganisms, especially of coli-group bacilli and the development of clinical symptoms of ileus.

3) Blood acetylcholine level was extraordinarily elevated in ileus at the time of developing shock-symptoms accompanied by increase of catecholamine group. The elevation was greatly suppressed by fradiomycin. The change in blood acetylcholine level seemed to be parallel with that in blood concentration of the high molecular substance of E. coli, and a close relationship between them was suggested. The results substantiated the present authors' view that acetylcholine, one of the most important chemical mediators, was responsible for the development of shock-symptoms in ileus.

4) Comparative examination of inhibitory effects of pyribenzamine (anti-histaminics), cyproheptadine (antiserotonin), DV-79 (antiplasmin drug), atropine (anti-acetylcholine) and cortisone (adrenocortical hormone) on ileus symptoms revealed that only atropine, which has a strong anti-acetylcholine activity, could exert a strong preventive effect on the lethal action of the endotoxin and DNA fraction of the E. coli component.

The development of ileus brings about a remarkable increase of intestinal bacteria, particularly of the coli-group. This causes considerable damage to the intestinal wall at the obstruction and its surroundings, which are already afflicted by the circulatory disturbance. The change of intestinal flora brings about the

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appearance or increase in the blood of high molecular substances such as endotoxin and DNA fraction of Escherichia coli; the substances, in association with the catecholamine group, cause a hyper-elevation of acetylcholine, one of the most significant chemical mediators in the blood in ileus. This theory was previously advanced by us and now seems to be supported by experimental results. The hyper-elevation of acetylcholine causes severe disturbances in the living body which may lead to death.

We have made extensive clinical and experimental studies over a long period on the effect of ileus on the organism and especially on the causes of death of ileus patients. The cause and site of obstructions in ileus are variable, as well as its clinical type. The condition is also frequently accompanied by peritonitis or diseases of other visceral organs, and therefore an evaluation of its effect upon the organism is very complicated. As a consequence, the theories concerning the causes of death from ileus are as diverse as are those concerning its concept and development, and a series of different factors such as neural reflex stimulus, bacteria, body fluid deficiency, intoxication, etc., have been taken into consideration.

It has been reported since a long time ago that bacteria, especially intestinal bacteria, have a close relationship with the pathological features of ileus and the cause of death. More recent studies have confirmed that when animals with ileus are given antibiotics, or when ileus is experimentally produced in germ-free animals, their survival period is prolonged five to six times compared with that of animals not given antibiotics or of animals with normal bacterial flora. In view of such results, we have made both clinical and experimental studies on the causes of death in ileus with special reference to intestinal bacteria.

**MATERIALS AND METHODS**

In the clinical studies of ileus the contents of the closed loop of the intestine and peritoneal fluid were taken from 7 cases of the complicated ileus (3 cases of small intestinal volvulus and 4 cases of acute strangulated intestinal obstruction) at the operation. Blood was taken from the median vein before and after the operation, and the serum was separated.

In the animal experiments, normal dogs (in total 48 dogs), weighing 10-15 kg, were used. One group of dog was pretreated with Furadionycin, an unabsorbable antibiotics, and the other group was used as untreated control. In both groups of the dog complicated ileus was produced at the upper and under parts of the small intestine, and inlet pipes (Nelaton tube) were fixed at the upper and under parts of the intestine out of the closed loop, intestinal closed loop itself, and peritoneal cavity, respectively. The intestinal contents and peritoneal fluid were taken at time intervals as designed and the blood was taken from the femoral vein, and serum was separated.

The number of E. coli in the intestinal contents and peritoneal fluid from the ileus patients and dogs was counted by plate dilution method with deoxycholic acid agar media. Anti-E. coli endotoxin and anti-E. coli DNA rabbit sera were prepared by using lipopolysaccharide (Difco preparation), endotoxin of E. coli extracted and purified by Boivin et al.'s method, and DNA fraction of E. coli extracted and purified by Marmur's method, respectively, as antigens.

The change of the level of E. coli endotoxin or DNA in serum or peritoneal fluid from the ileus patients or dogs was examined by the sensitized red cell hemagglutination inhibi-
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Acetylcholine was extracted with acid-alcohol extraction by Stone's method and determined quantitatively and qualitatively with frog's rectus abdominis muscle by the method of Chang and Gaddum.

Ten times of LD_{50} of E. coli endotoxin or DNA were administered intraperitoneally to several groups of normal mouse weighing about 15 g. At every 3 hours after the administration, each one of the mouse groups were injected subcutaneously with either of 0.5 ml of 100 mcg/ml pyribenzamine, 0.5 ml of 100 mcg/ml cyproheptadine, 0.5 ml of 50 mg/ml DV-79 (transamine), 0.5 ml of 100 mcg/ml cortisone, or 0.5 ml of 200 mcg/ml atropin, respectively, in total 6 times; to mouse of the control group 0.5 ml of 0.85% NaCl was injected subcutaneously. The number of death in each group of animals was checked at 6, 12 and 24 hours after the administration and thus the protective effects of the above antiallergy drugs against the lethal effects of endotoxin and DNA fraction were tested.

RESULTS

1) Change of the number of intestinal microorganisms, especially coli-group bacilli in acute intestinal obstruction

At the time of operation in eight cases of ileus, three of small intestinal volvulus and five of small intestinal strangulation, coli-group bacilli were found remarkably increased in the contents of the obstructed intestines as compared with those in the normal intestine. Coli-group bacteria were found in ascites obtained at the time

![Fig. 1. Number of E. coli in loop contents and peritoneal fluid of patients with intestinal strangulated obstruction.](image-url)
of operation in three of the eight ileus cases, but they were not found in three other cases of this group (Fig. 1).

In addition to these investigations on patients, strangulation was experimentally produced in the upper part of the small intestine in dogs, and the intestinal contents and ascites were examined for coli-group bacilli. Subsequently, fradiomycin (neomycin) was administered intraintestinally, and the number of coli-group bacilli and histological changes in the intestines and visceral organs were investigated. The results were as follows:

a) In the untreated control dogs with ileus, a remarkable increase in the number of coli-group bacilli was found in the intestinal contents of the upper bowel, the lower bowel, and at the obstruction. Also, the number of these bacilli in ascites was found to gradually increase along with the length of the intestine, attaining the same level as that of the intestinal contents at the end of the ileus. This is in agreement with the observation of human cases (Fig. 2).

b) On the other hand, growth of the coli-group bacilli in the intestinal contents and ascites of dogs with ileus was greatly inhibited by oral administration of fradiomycin. Furthermore, in these cases histological changes in various organs were slight in comparison with those in untreated control animals. The survival time of treated dogs was also prolonged (Fig. 2).

Based on the results of these investigations, it was concluded that in ileus remarkable growth of intestinal bacteria, especially of coli-group bacilli, is closely related to pathological changes and the cause of death.

2) Studies on high molecular substances of the cellular components of E. coli liberated in blood after acute intestinal obstruction

Subsequently, we prepared immune rabbit serum against the endotoxin of Escherichia coli (pure preparation of Difco), a lipopolysaccharide extracted and purified from the bacteria cells by Boivin's method, and the DNA fraction was extracted and purified from the bacterial cells by Marmur's method. Using Noyes' method (Fig. 3), an inhibition test of sensitized red cell hemagglutination, we investigated whether the above-mentioned high molecular substances of the bacterial cells appeared in the blood of ileus patients.

Serum samples were taken daily from ileus patients both before and after operation, and inhibition tests using these samples were carried out on red cells sensitized with E. coli endotoxin and DNA fraction. The results obtained from the hemagglutination reaction with the anti-endotoxin and anti-DNA rabbit serum samples are shown in Fig. 4. Clinical history of a patient is as follows.

A male, aged 64, had strangulation of the small intestine, and 70 cm of the necrotic portion of the intestinal canal was removed. The inhibiting sensitized hemagglutination titer of the anti-endotoxin rabbit serum was 640 before the operation, which was lower than that for healthy subjects. On the first postoperative day it abruptly fell to 160, and the patient developed severe shock symptoms. On the second and third postoperative days
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Fig. 2. Change of the number of *E. coli* in loop contents and peritoneal fluid of dogs with strangulated obstruction.

Fig. 3. Inhibition test for sensitized red cell hemagglutination (Noyes et al.).

the titer was 320, and on the fourth day it rose to 640. After the fifth postoperative day it was restored to the normal level of 1,280, and in association with this the general condition of the patient improved greatly. Even before the operation, the inhibition titer for the sensitized red cell hemagglutination test of the anti-DNA rabbit serum was 320, which was lower than normal, and it fell to 160 on the first postoperative day. On the second day after the operation it rose to 320, and after the third day it was always 640. The restoration of serological titers to normal and the corresponding improvement of clinical symptoms were in parallel with the change in the contents of high molecular substances of *E. coli* (Fig. 4). The inhibition titer for the sensitized red cell hemagglutination test by the addition of ascites taken from this patient at the time of operation was 320 for the anti-endotoxin rabbit serum, and 160 in the anti-DNA rabbit serum.

In an experiment, strangulation was experimentally produced in the upper part of the small intestine of dogs and the following tests were performed.

1) In an inhibition test for sensitized red cell hemagglutination by addition of serum from untreated control ileus dogs, the titer with anti-endotoxin rabbit serum was 1,280 before the operation, but it decreased to 640 in four hours after the operation, and fell to 320 in eight hours (Fig. 5). The sensitized hemagglutination
Fig. 4. Detection of endotoxin and DNA fraction in a 64-year-old patient's serum with strangulated obstruction.

titer of the anti-DNA rabbit serum, which was 640 before the production of ileus, fell to 320 in four hours thereafter, and 160 in eight hours, becoming as low as 80 in later stages (Fig. 6). When ascites was applied, the titer of anti-endotoxin rabbit serum was 640 in four hours after the production of ileus, and 160 in eight hours (Fig. 7). The sensitized hemagglutination titer of anti-DNA rabbit serum was 320 in four hours after the production of ileus and 80 in eight hours (Fig. 8). In all of these observations a remarkable fall in the titers was noticed with the progress of ileus.

2) The tests on ileus dogs given fradiomycin orally revealed that titers very much higher than those in untreated control ileus dogs, indicating a strong inhibitory action of fradiomycin (Figs. 5–8).

Fig. 5. Detection of endotoxin in dog's serum with strangulated obstruction.
Fig. 6. Detection of DNA fraction in dog’s serum with strangulated obstruction.

Fig. 7. Detection of endotoxin in dog’s peritoneal fluid with strangulated obstruction.

Fig. 8. Detection of DNA fraction in dog’s peritoneal fluid with strangulated obstruction.
3) In contrast with this, the sensitized hemagglutination titer of anti-endotoxin rabbit serum, treated with serum or ascites samples taken from ileus dogs, fell abruptly and remarkably, when a large amount of the endotoxin had been infused into the dog’s intestine on both sides of the obstruction at the time of ileus production; the titer was as low as 160 even four hours after endotoxin injection (Figs. 5 and 7). The animals displayed shock-like symptoms and died early.

This is in agreement with the change in blood concentration of the high molecular substances of *Escherichia coli* in ileus patients and their clinical signs.

It was verified both clinically and experimentally that the endotoxin of *E. coli* and the DNA fraction of its cells increase greatly, not only in the blood but also in the ascites of ileus patients, and that this increase is inhibited and the clinical signs are improved by intraintestinal administration of fradiomycin, an unabsorbable antibiotic. It is therefore assumed that there is an intimate relation between the increase of *E. coli* components in the blood and the development of clinical symptoms of ileus.

3) **Change of the level of the chemical mediators in the blood, especially of acetylcholine in acute intestinal obstruction**

We⁶⁻¹⁰ had investigated in the past chemical mediators in ileus, and since 1951 we have been advocating that a hyperelevation of acetylcholine in the blood in ileus causes severe disturbances leading to death. As described above, since intraintestinal administration of fradiomycin remarkably prolonged the survival time of ileus animals, we examined clinically and experimentally the change in blood acetylcholine concentration in the development of ileus, and the effect of intraintestinal administration of fardiomycin. An example of clinical experience is presented as follows.

A male, aged 34, was admitted to the hospital with axial rotation of the small intestine. The jejunum was rotated 540 degrees counter-clockwise for a stretch of about 200 cm and had assumed a dark-red color with developing necrosis. An enterectomy was performed. The blood concentration of acetylcholine at the time of hospitalization was five times the normal level. On the first day of the operation the concentration became 10 times the normal, but thereafter it fell and gradually returned to an approximately normal level a week after operation. Blood pressure remained below 80 mmHg before, during and after the operation, and the patient developed severe shock symptoms. But on the second postoperative day the blood pressure gradually began to return to the normal level (Fig. 9). This is in agreement with the change in acetylcholine concentration.

The results of experimental studies were as follows.

1) Strangulation ileus of the upper small intestine was experimentally established in dogs, and it was found that the concentration of acetylcholine in blood increased abruptly from six hours after the establishment of ileus; in nine hours after the operation blood acetylcholine attained a value about 10 times that prior to ileus and the dogs developed shock symptoms. A parallelism between the blood acetylcholine level and clinical symptoms is thus indicated as in clinical cases.
2) Increase in the blood acetylcholine level in ileus dogs was well inhibited by preceding oral fradiomycin administration (Fig. 10).

We have already reported that blood catecholamines, especially the noradrenaline level, increase in the early stage of ileus and attain the maximum several hours after the establishment of ileus, but thereafter they decrease abruptly. During the development of shock the blood catecholamine level became lower than that before the establishment of ileus. This change in blood catecholamine level is closely associated with extraordinary increase of acetylcholine in the blood in ileus.

However, in ileus dogs to which fradiomycin had been administered, such changes of catecholamine level in blood and adrenal tissue, especially the noradrenaline and adrenaline granules in the latter, were fairly well inhibited.
These results strongly suggest that extraordinary increase in blood acetylcholine in ileus may be closely associated with the change in blood catecholamine level, and reconfirm our long-entertained view that the most significant blood chemical mediator related to ileus is acetylcholine. The substance was extraordinarily increased in this disease at the point of developing shock symptoms in comparison with the catecholamine group. It was further observed that this hypererelevation of blood acetylcholine level in ileus was inhibited most satisfactorily with fradiomycin, an unabsorbable antibiotic. On account of these findings, the change in blood acetylcholine level seems to be parallel with the change in blood concentration of the high molecular substances of *E. coli*, and to suggest a close relationship between them.

4) Studies on the relation between acetylcholine and the high molecular substances of *E. coli* components in acute intestinal obstruction

To ascertain further this relationship between the change in the blood acetylcholine level and high molecular substances of *E. coli* in the development of ileus, we examined the biological action of *E. coli* endotoxin and the DNA fraction of the bacterial cell component on animals. At the same time, the inhibitory effects of pyrbenzamine (antihistaminic) cyproheptadine (antiserotonin), DV-79 (antiplasmin), atropine (antiacetylcholine drug), and cortisone (adrenocortical hormone) on the fatal action of these high molecular substances were examined comparatively.

The toxicities (in terms of LD50) of the endotoxin and the DNA fraction were 189.8 and 769.4, respectively, for a mouse of 10 g (Figs. 11 and 12). Twice the minimum lethal doses of the high molecular substances were given to mice, and

![Fig. 11. Toxicity of endotoxin against mice.](image1)

![Fig. 12. Toxicity of DNA fraction against mice.](image2)
the preventive effects of the above-mentioned antiallergic agents on the lethal actions of the above-mentioned substances were examined.

1) The administration of endotoxin killed 10 to 20% of the animals of the control group and of the groups of pyribenzamine, cyproheptadine, DV-79 or cortisone treatment within 6 hours. The same dose of endotoxin killed 100% of the untreated and DV-79-treated animals, and 70 to 80% of the pyribenzamine-, cyproheptadine-, and cortisone-treated animals within 12 hours and 100% of all the groups within 24 hours. Contrary to this, none of the atropine-treated animals died within six hours, 60% of them survived 12 hours, and 40%, 24 hours (Fig. 13).

2) Within six hours after administration of the DNA fraction, 20 to 30% of the untreated control and the pyribenzamine-, cyproheptadine-, DV-79-, and cortisone-treated animals died; within 12 hours, 100% of the untreated animals and 40–80% of the pyribenzamine-, cyproheptadine-, DV-79-, and cortisone-treated animals died; and within 24 hours, 100% of all groups died. In contrast with this,
none of the atrophine-treated group died within six hours, 60% survived 12 hours, and 40% 24 hours (Fig. 14).

The above results confirmed for the first time that of various anti-allergic agents, only atropine, which has a strong anti-acetylcholine activity, could exert a strong preventive effect against the lethal action of the endotoxin and DNA fraction of the E. coli component.

This fact, together with the results of the aforementioned studies on the high molecular substances of E. coli components and acetylcholine level in the blood during the development of ileus, indicates that the appearance of the high molecular substances in the blood induces an abnormally high concentration of acetylcholine in the blood in association with the catecholamine release.

References