# RESULTS OF EXPERIMENTAL INTESTINAL STRANGULATION OBSTRUCTION IN GERMFREE RATS*

By EGIL AMUNDSEN, M.D., AND BENGT E. GUSTAFSSON, M.D.

(*From the Department of Germfree Research, Karolinska Institutet, Stockholm, Sweden*)

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Numerous factors influence the pathological physiology of intestinal strangulation obstruction. Those originating from the strangulation fluid, *i.e.* the fluid produced by the strangulated loop of intestine, have been a focus of interest for experimental studies during the past few years. This fluid is very toxic on intraperitoneal injection in healthy animals (1–3). Antibiotics diminish the toxicity (4–6) and there are findings which indicate that the content of *Escherichia coli* and of red blood cells in the strangulation fluid influence the toxicity of the fluid (7). The decisive influence attributed to the intestinal bacteria on the toxicity of the strangulation fluid (8, 9) prompted the following experiments in which clinical course, survival time, and toxicity of strangulation fluid were noted on germfree rats and conventional rats with experimentally produced low ileal strangulation obstruction.

## Material and Methods

Adult germfree albino rats of the Swedish strain of both sexes were used. The control group was comprised of exgermfree animals of the same strain which had been kept with the conventional animal colony for 2 to 3 months to aquire the conventional bacterial flora. The germfree animals were reared according to Gustafsson (10, 11), and fed diet D7 (11) and water *ad lib*. All animals were kept on raised screens and weighed 250 to 350 gm.

In both germfree and conventional animals experimental intestinal strangulation obstruction was produced and in some animals a rubber bag was also introduced to collect the strangulation fluid. Preliminary tests showed that the technique for the production of the strangulation obstruction was of importance, and after some trials the following technique was developed.

Using standard surgical procedures and nembutal anesthesia laparotomies were performed on the germfree animals in the standard isolator (11) and on the conventional animals in the operating room using aseptic technique. After a midline incision 3 to 5 cm long through the abdominal wall intestinal strangulation obstruction was produced by tieing off with silk each end of a 5 cm loop of ileum with the distal ligature located 5 cm orally to the ileocecal

junction. The two ends of the loop were then brought together and the loose ends of the two ligatures were tied around the mesentery and the intestine and care was taken not to obstruct the bloodflow in the mesenterial artery. This type of experimental condition will be referred to as closed loop strangulation obstruction without bag.

In additional groups of rats the same procedure as outlined above was followed except that the strangulated loop was put into a rubber bag. The ends of the ligatures were brought through the wall of the bag with a needle close to the opening of the bag. The two ends of the intestinal loop were brought together and the ligatures were tied around the bag to close it and prevent it from slipping. The intestine with the rubber bag was replaced in the abdomen which was closed in layers. This type of experimental condition will be referred to as closed loop strangulation obstruction with bag.

Closed loop strangulation obstruction without bag was produced in 10 germfree rats and in 10 conventional rats. Closed loop strangulation obstruction with bag was produced in 10 germfree rats and in 25 conventional rats.

After operation the animals were kept in separate cages. By observing daily the pan underneath the raised screens the feces production was registered to control the effect of the obstruction on the intestinal passage. The postoperative course was observed, survival time was noted, and postmortems were performed. Strangulation fluid collected from the bags was incubated in thioglycolate broth and glucose broth aerobically and anaerobically at 37°C. Toxicity tests of the strangulation fluid involved the injection of varying amounts and fractions of strangulation fluid intraperitoneally into conventional rats or adult male mice, the latter weighing 24 to 26 gm.

## RESULTS

*Postoperative Observations.*—The germfree rats seemed only slightly affected by the strangulation obstruction the first days after the operation. They moved around in their cages, and took food and water. Gradually, however, they appeared ill and succumbed after a period of extreme prostration and pallor of the skin. There was, however, a definite difference in appearance between the animals with and without rubber bags, the latter showing a better general condition than those with bags.

The condition of the conventional animals deteriorated more rapidly than that of the germfree rats. The conventional rats had also an initial but much shorter uneventful period when they moved around and took food and water, but they were not so lively as the germfree rats. Again there was a difference between the animals without and with rubber bags, as the conventional rats without bags were in a worse condition than those with the bags. This was just the reverse of the situation in the germfree rats.

*Survival Time.*—There were great differences in the mean survival time between the groups studied (Tables I and II, Text-fig. 1). The germfree rats without bags had by far the longest mean survival time of 240 hours, whereas the corresponding conventional controls with the same type of operation had a mean survival time of 44 hours (Table I). This difference was highly significant (Table III) which is also evident from the fact that the minimum survival time in the germfree group (88 hours) was longer than the maximum survival time in the conventional group (58 hours). The dispersion in the germfree group

was great and some animals lived for a quite long time. Thus 1 animal lived 19 days and 3 lived 15, 16, and 16 days respectively with complete intestinal strangulation obstruction. These values contrasted to the maximum survival time of 2½ days in the conventional group.

In the groups in which the rubber bag was used to collect strangulation fluid the difference in survival time between the germfree and conventional groups
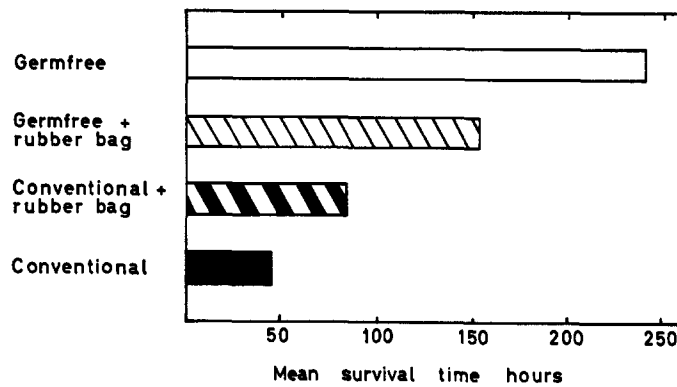


TEXT-FIG. 1. Mean survival times of rats with experimental intestinal strangulation obstruction.

TABLE I

*Survival Times of Germfree and Conventional Rats with Closed Loop Strangulation Obstruction, without Rubber Bag*

| Group | No. of animals | Survival time | | |
|---|---|---|---|---|
| | | Range | Mean | SD |
| | | *hrs.* | *hrs.* | *hrs.* |
| Germfree | 10 | 88–456 | 240 | 144 |
| Conventional | 10 | 30–58 | 44 | 11 |

was also statistically significant (Tables II and III). When the two conventional groups were compared the introduction of the bag was followed by a prolongation of the survival time and the difference was statistically significant. On the other hand there was a reversed tendency in the germfree animals as the germfree group with the rubber bag had a shorter mean survival time. This difference was, however, not statistically significant.

*Postmortem Examination.*—Although the animals were taking some food and water, all the germfree animals were emaciated at the time of death. The 3 germfree animals with the longest survival time had thus an average body

weight loss of 88 gm (35 per cent) of the initial mean body weight of 254 gm. The figures for the corresponding 3 conventional animals were an average loss of 27 gm (8 per cent) of the initial mean body weight of 320 gm.

The strangulated loop in the germfree animals was distended and contained a considerable amount of collagenous fluid, which had a very slight, sweet odor. In the conventional animals the loop contained less fluid, which was foul smelling, dark, and more liquid. The wall of the loop in both groups of animals was gangrenous, but no macroscopic perforations were seen. The part of the

TABLE II

*Survival Times of Germfree and Conventional Rats with Closed Loop Strangulation Obstruction with Rubber Bags*

| Group | No. of animals | Survival time | | | Strangulation fluid | |
|---|---|---|---|---|---|---|
| | | Range | Mean | SD | Range | Mean |
| | | *hrs.* | *hrs.* | *hrs.* | *ml* | *ml* |
| Germfree | 10 | 84 – 266 | 152 | 64 | 5 – 33 | 20 |
| Conventional | 25 | 40 – 130 | 82 | 23 | 9 – 32 | 20 |

TABLE III

*Statistical Analysis: P, Values in Group Comparisons*

| | | | |
|---|---|---|---|
| Germfree without bags | *versus* | Conventional without bags | p < 0.001 |
| Germfree with bags | *versus* | Conventional with bags | 0.01 > p > 0.005 |
| Germfree without bags | *versus* | Germfree with bags | p > 0.1 |
| Conventional without bags | *versus* | Conventional with bags | p < 0.001 |

intestinal tract proximal to the obstruction, including the stomach, was markedly distended in the germfree rats owing partly to odorless gas (Fig. 1). The degree of distension seemed to increase with the length of the survival time. In the conventional rats the distension of the intestine orally to the strangulation was less pronounced and the contents had a foul odor.

The intestinal tract distal to the obstruction was collapsed in both the germfree and the conventional animals and contained only mucous matter in the animals with the longest survival times. The cecum is normally enlarged in germfree rats with a relative weight of the content of about 5 per cent of the body weight in the strain studied (12). In the germfree animals with obstruction this ratio was down to 0.1 to 0.2 per cent which is lower than that (0.8 to 1 per cent) observed in intact conventional rats (12).

*Strangulation Fluid.*—Large amounts of fluid collected in the bags in some animals (see Table II and Fig. 2). The average amounts were the same in the germfree and conventional rats but there were great variations. In the rats

without rubber bags the amounts of fluid in the peritoneal cavities were quite small.

The strangulation fluid was dark red to dark brown in both groups of animals and was partially clotting after removal from the bags.

All the cultures of the strangulation fluid from germfree rats proved to be sterile. The tests from all the conventional rats showed growth of various organisms, the nature of which lies within the scope of forthcoming studies.

TABLE IV

*Toxicity Tests of Strangulation Fluid from Individual Rats*
0.5 ml injected intraperitoneally into male mice weighing 25 gm.

| Germfree | | Conventional | |
|---|---|---|---|
| Rat No. | No. of mice surviving 24 hrs. | Rat No. | No. of mice surviving 24 hrs. |
| 1 | 2/2 | 1 | 0/2 |
| 2 | 2/2 | 2 | 0/2 |
| 3 | 2/2 | 3 | 0/2 |
| 4 | 2/2 | 4 | 0/2 |
| 5 | 2/2 | 5 | 2/2 |
| 6 | 2/2 | 6 | 0/2 |
| 7 | 2/2 | 7 | 2/2 |
| 8 | 1/2 | 8 | 0/2 |
| 9 | 2/2 | 9 | 2/2 |
| 10 | 2/2 | 10 | 2/2 |
| | | 11 | 1/2 |
| | | 12 | 2/2 |
| | | 13 | 0/2 |
| | | 14 | 2/2 |
| | | 15 | 2/2 |
| Pooled fluid | 2/2 | Pooled fluid | 0/2 |

*Toxicity Tests.*—The testing for toxicity of the strangulation fluid was initially carried out by injecting it intraperitoneally into rats. This required comparatively large amounts of fluid. Conventional mice were chosen as test objects after it was found that the fluids which proved toxic on intraperitoneal injections in rats, also were toxic on intraperitoneal injections in mice. Those fluids which were not found toxic on intraperitoneal injections in rats, were not toxic on intraperitoneal injections in mice. The use of these latter enabled testing to be carried out with smaller amounts of fluid.

On testing the fluid collected from individual conventional rats, it was found that only 7 out of the 15 fluids tested were lethal in the dosage given (0.5 ml/ 25 gm body weight, Table IV). The pooled fluid was toxic, however.

The strangulation fluids collected from individual germfree rats were not found toxic and neither was the pooled fluid from these animals toxic when tested in the same way as the fluid from conventional rats (Table IV).

The pooled fluid was freeze-dried and after it had been redissolved was administered in increasing doses based on the dry-weight. It was then found that the freeze-drying had not noticeably affected the toxicity and that the toxicity decreased on dilution and increased as did the dry-weight content, (Table V). The fluid from the germfree animals, treated in the same way, was not found toxic in any instance even in a concentration with a dry-weight content 4 times that of the original pooled fluid (Table V).

The pooled strangulation fluid from conventional animals which had been

TABLE V

*Toxicity of Freeze-Dried Pooled Strangulation Fluid Injected Intraperitoneally into Conventional Mice*

| Dose multiple of dry-weight of original fluid | No. of mice surviving 24 hrs. | |
| --- | --- | --- |
| | Fluid from germfree rats | Fluid from conventional rats |
| 0.25 | 4/4 | 4/4 |
| 0.5 | 4/4 | 4/4 |
| 1.0 | 4/4 | 2/4 |
| 2.0 | 4/4 | 0/4 |
| 4.0 | 4/4 | 0/4 |

proved to be very toxic was filtered through a Millipore filter with a pore size 0.22 $\mu$. The filtrate was injected into 4 mice in doses of 0.5 ml/25 gm body weight. None of the mice died.

### DISCUSSION

It is evident both from the postoperative course and the considerable prolongation of the survival time that the absence of the flora in germfree animals profoundly changes the course of intestinal strangulation obstruction. The findings stress the importance of the bacteriological factors; *i.e.*, inflammation and toxins in the pathogenesis of intestinal strangulation obstruction. In the absence of the flora animals could withstand the changes due to the blocked intestinal passage such as loss of fluid, tissue dehydration, loss of electrolytes, and impaired acid-base balance, for the astonishingly long time of almost 3 weeks. The toxic principles seem to be carried by the strangulation fluid and to be connected to the microorganisms or their toxins and furthermore to be carried by some particles which do not pass a microfilter. This was substantiated by the fact that our toxicity tests of the strangulation fluid collected in germ-

free animals all were negative and showed no bacterial growth whereas all strangulation fluids from the conventional animals showed massive bacterial growth.

The importance of the strangulation fluid in the pathophysiology of intestinal strangulation obstruction is also indicated by the fact that the introduction of the rubber bag in conventional rats prolonged the survival time, although the presence of the bag in the peritoneal cavity must be a deleterious factor. This is indicated by the somewhat decreased survival time in the germfree animals with rubber bags when compared with germfree animals without bags. The prolongation of the survival time in the conventional rats when the rubber bag was used is well in agreement with the findings of other investigators (1–3, 8) in dogs and rabbits.

It might be asked whether other factors than the absence of the flora might favor the germfree animals under the circumstances studied. A constant finding in germfree animals is a distended cecum with a content making up 5 per cent of the body weight and this cecal content could act as a supply for water and electrolytes. It is very unlikely, however, that this reservoir might be of any importance in the great movements of fluids and minerals that must take place during the 3 weeks that some germfree animals live and lose up to 33 per cent of their body weight. The cecum of germfree animals in the weight classes studied here contain about 10 ml of water. This must be compared with the daily need of about 15 to 25 ml of water for these rats.

The great difference in survival time between the germfree and the conventional rats could conceivably depend on a higher general resistance to shock in the germfree animals. Experimental work with hypovolemic shock (16), burn shock (17), endotoxin shock (18), and tourniquet shock (19) does not, however, indicate any such higher resistance of the germfree animals.

After total body x-radiation in lethal doses germfree rats (20), chicken (21), and mice (22) showed, in contrast, a better survival time than did the conventional counterparts.

The importance of the bacterial factors in strangulation obstruction has been stressed by several workers (1–9, 13–15) but there is some discussion whether additional factors are needed (7, 13–15). Barnett and Clippinger (23) showed in 1959 that the survival time of dogs with closed loop strangulation obstruction increased by a factor of 2 (from 29 to 56 hours) by the administration of penicillin. In our study the total absence of the flora in animals with strangulation obstruction was associated with a prolongation of the survival time by a factor of 6 (from 44 to 240 hours).

## SUMMARY

Experimental low ileal strangulation obstruction has been produced in germfree and conventional rats. The mean survival time was 240 hours in the

germfree rats and 44 hours in the conventional controls. 4 of the 10 germfree rats survived 15 or more days, whereas the 10 conventional animals were all dead within 2½ days.

The strangulation obstruction fluid from the germfree animals was sterile and non-toxic when injected into mice even after a fourfold concentration. The same fluid from the conventional animals contained a great number of microorganisms and caused death within 24 hours when injected intraperitoneally into mice.

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EXPLANATION OF PLATE 55

FIG. 1. Digestive tract of germfree rat with experimental strangulation obstruction for 19 days. *A*, stomach; *B*, strangulated loop of ileum; *C*, cecum.

FIG. 2. Rubber bag with strangulation obstruction fluid in a germfree animal 5 days after operation. To the left of the ligature is a bubble of air trapped at the operation. The strangulation loop can be partly seen in this area. The blur to the extreme left is due to another air bubble caught in a fold of the bag.
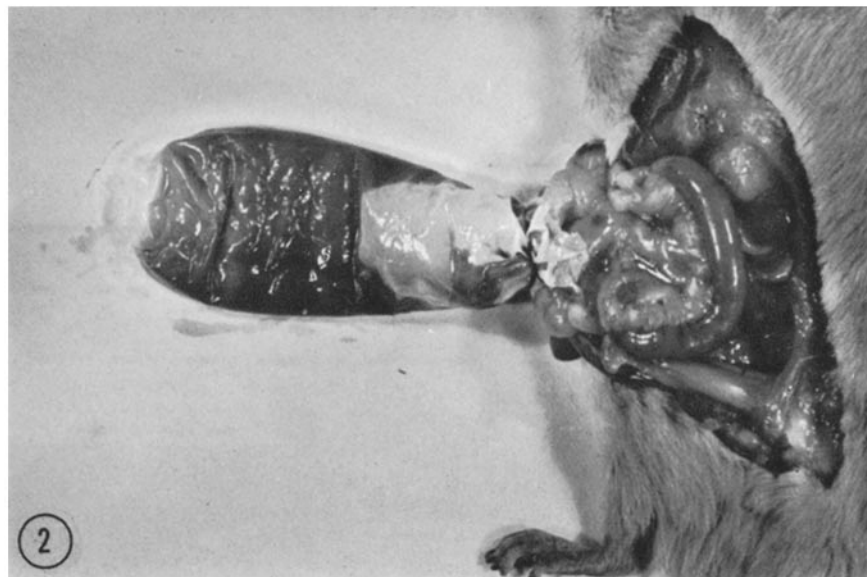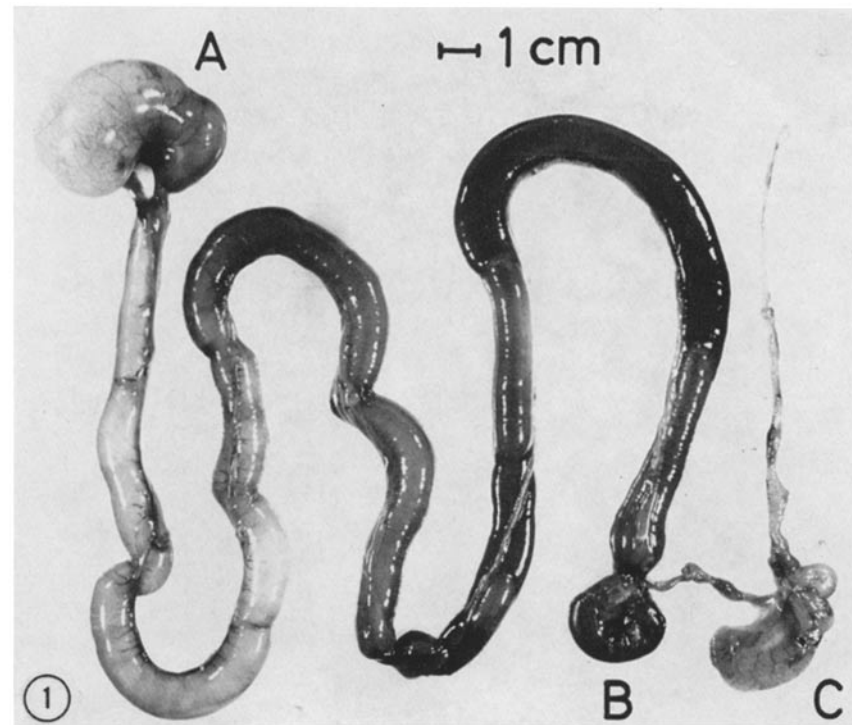
(Amundsen and Gustafsson: Intestinal strangulation obstruction)