THE EFFECT OF BLOOD IN EXPERIMENTAL PERITONITIS*

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Duff Allen,[1] in 1927, showed that the presence of blood in the pleural cavity was an important factor in the production of experimental empyæma. The addition of one to two cubic centimetres of autogenous blood to broth cultures of pneumococci and streptococci injected into the pleural cavity of dogs gave a much higher incidence of empyæma in these animals than in those receiving similar cultures without blood. From his experiments, Allen concluded that blood increased the incidence of empyæma and believed that under such conditions an increase in virulence of bacteria might be the result.

The peritoneum of animals is well known to be resistant to infection, especially by artificial means. In attempting to evaluate various means of treatment in the experimental animal, the problem of developing a satisfactory peritonitis became one of primary importance. The introduction of organisms into the peritoneal cavity of rabbits and guinea-pigs has failed to produce peritonitis consistently.

The belief that blood may have a deleterious effect by reason of its being a good culture media existed many years before Allen's publication. Thus Schumann,[12] in 1921, in his monograph on ectopic pregnancy, discusses this feature of blood in the peritoneal cavity. The usual result of blood in the peritoneal cavity in ruptured ectopic pregnancy when the hæmorrhage is not so rapid as to cause death or immediate surgical attention, seems to be the formation of an hematocele.

This Schumann explains on the basis that blood acts as a foreign body, setting up peritoneal irritation and aseptic peritonitis, with exudate and the formation of adhesions. He further states that the real gravity of hematocele lies in its susceptibility to infection. "Given a mass of blood mixed with fibrinous exudate and in intimate contact with the intestinal walls, infection by the ubiquitous colon bacillus is a natural sequence and the conversion of this clot to a pulvic abscess is naturally a common occurrence." He states, moreover, that in most cases the infection is of such low grade that the tissues do not break down, but perimetritis occurs.

The possible analogy of peritoneum to pleura as regards its reaction to blood was further explored by Sparks and David,[13] in 1929. Basing their experiments upon those of Allen, they injected various amounts of autogenous blood and various amounts of broth culture of staphylococci, streptococci, and colon bacilli into the peritoneal cavity of dogs, rabbits and guinea-pigs. Peritonitis was produced only by the streptococci in rabbits and in the control animals the incidence of peritonitis was the same as in those receiving blood. Dogs and guinea-pigs were resistant in all cases. In the experiments of Sparks and David the dogs received twenty to 100 cubic centimetres of blood and ten cubic centimetres of broth culture, the rabbits three cubic centimetres of blood and three cubic centimetres of culture, and the guinea-pigs one cubic centimetre of blood and one centimetre of culture. The authors concluded that autogenous blood together with varying types of pathogenic organisms injected into the peritoneal cavity of dogs, rabbits and guinea-pigs does not predispose to the production of peritonitis.

Hermann[6] also attempted to use blood to aid in the production of peritonitis in rabbits. He found that attempts at modifying the virulence of the fecal flora by incu-

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bation with rabbit's blood or by the simultaneous injection of blood with organisms were unsatisfactory. He stated that the rabbits died more consistently when blood was added, but necropsy did not show peritonitis. No protocols were published of his experiments on the effect of the addition of blood.

From the data in the literature it is difficult to evaluate the effects of blood in the peritoneal cavity on the production of experimental peritonitis. It has never been shown that the presence of blood in the peritoneal cavity has any effect upon the production of peritonitis.

The effect of blood in the peritoneal cavity without infection has been studied by Sabin and absorption rates have been studied by Florey and Witts.[4] Sabin has shown that the presence of blood in the peritoneum sets up a mild peritoneal reaction into which macrophages soon enter to take up and phagocytize the red blood-cells. The contri-
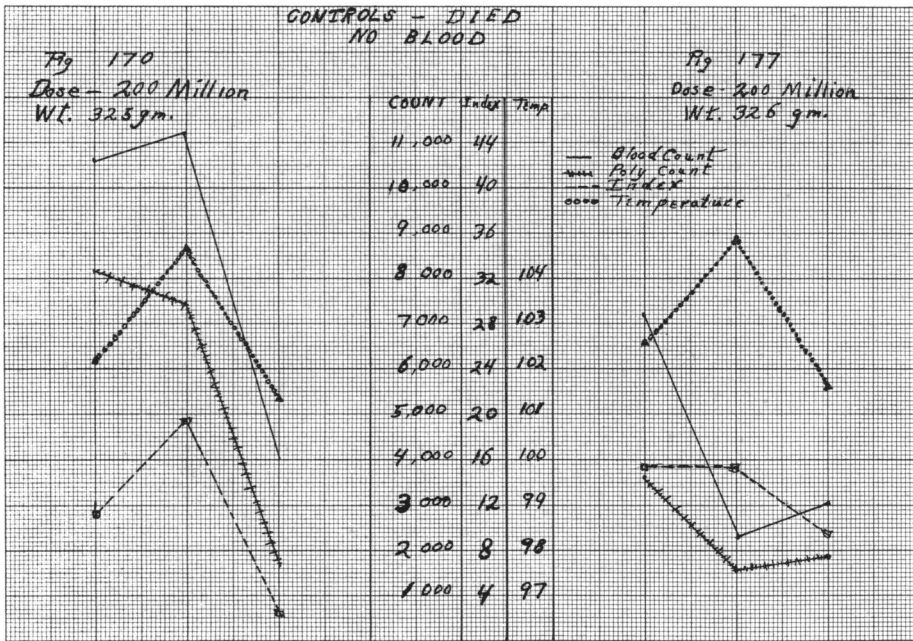

FIG. 1.

bution of Florey and Witts is of value in that they demonstrate the slowness of complete absorption of blood from the peritoneal cavity. Thus even relatively small amounts of blood are not completely absorbed for at least forty-eight hours. They, too, cite the danger of infection, mentioning the death of several animals from septicæmia after septic punctures of the abdominal wall. They demonstrated blood to be absorbed via the thoracic duct at a steady slow rate. They believed that the rate of absorption of red cells was influenced by the intra-abdominal pressure and by the depth of respiration. Sweet and Smythe,[16] in 1921, demonstrated similar facts concerning the absorption of blood from the peritoneal cavity.

Many foreign substances have been studied in relation to the production or treatment of peritonitis. Steinberg and Goldblatt[15] have demonstrated that any mechanism which decreases absorption from the peritoneal cavity in the presence of infection within that cavity favors the development of a peritonits. Any mechanism which permits of normal or increased absorption is associated with recovery of the animal and failure to produce a typical peritonitis. Absorption was determined in terms of the number of

organisms recovered from the peripheral blood and the thoracic duct lymph. These authors showed that recovery was associated with bacteræmia, but in the development of peritonitis no organisms could be recovered from the blood and only few from the lymph. Gum tragacanth was used to decrease absorption and was uniformly associated with the development of fatal peritonitis.

Bruce Morton[9] has, moreover, shown that a plastic exudate of any kind, excited by organisms, or by irritating chemicals, such as turpentine, markedly decreases absorption.

Various other methods of interfering with absorption so as to favor the production of peritonitis have been applied. Gum tragacanth has been used most frequently in this connection in the production of experimental peritonitis. Hermann obtained a higher incidence of peritonitis in experimental animals as a result of the injection of living bacteria in those animals which had received a previous injection of killed organisms.
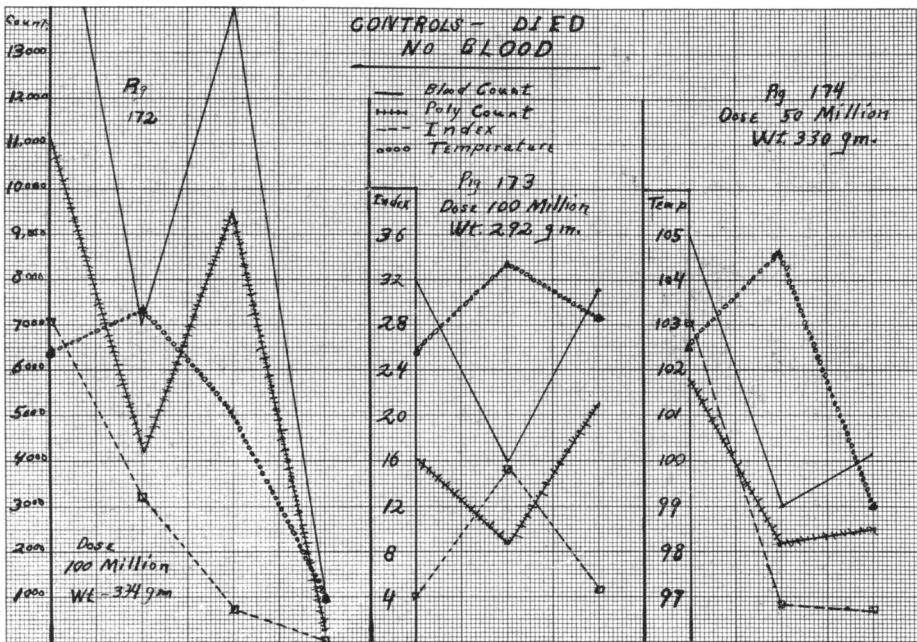

FIG. 2.

He believes that this is due to an increased local reaction rather than to a decrease in absorption. Hypertonic glucose and saline solutions have been studied by Reschke,[11] in 1921, as a possible means of decreasing absorption from the peritoneal cavity. The introduction of eight to nine grams of dry glucose placed in the peritoneum of rabbits through a laparotomy wound resulted in a decreased absorption of bacteria and toxins. Since high concentrations of sugar act as a bacteriostatic agent; it is difficult to draw conclusions from his results.

Starling and Tubby,[14] in 1894, as well as Orlow,[10] in 1895, showed that absorption is slowed in proportion to the concentration of organic material of fluids placed in the peritoneal cavity. These authors pointed out the fact that serum is very slowly absorbed from the peritoneal cavity.

From the foregoing results one would expect that blood should decrease absorption from the peritoneal cavity, since it sets up a local reaction and simultaneously increases the protein or organic content of the injected fluid.

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From the previously cited experiments one would expect that these factors would favor the production of peritonitis when organisms are injected with or subsequent to the injection of blood.

Material and Methods.—Since Meleney[7] has found that the colon bacillus is the organism most commonly found in peritonitis, we have used this organism throughout these experiments. Guinea-pigs were chosen for these experiments because they were found to react more uniformly than other laboratory animals.

In our early experiments two strains of colon bacilli isolated from the peritoneum of a patient operated upon for a ruptured appendix were used.
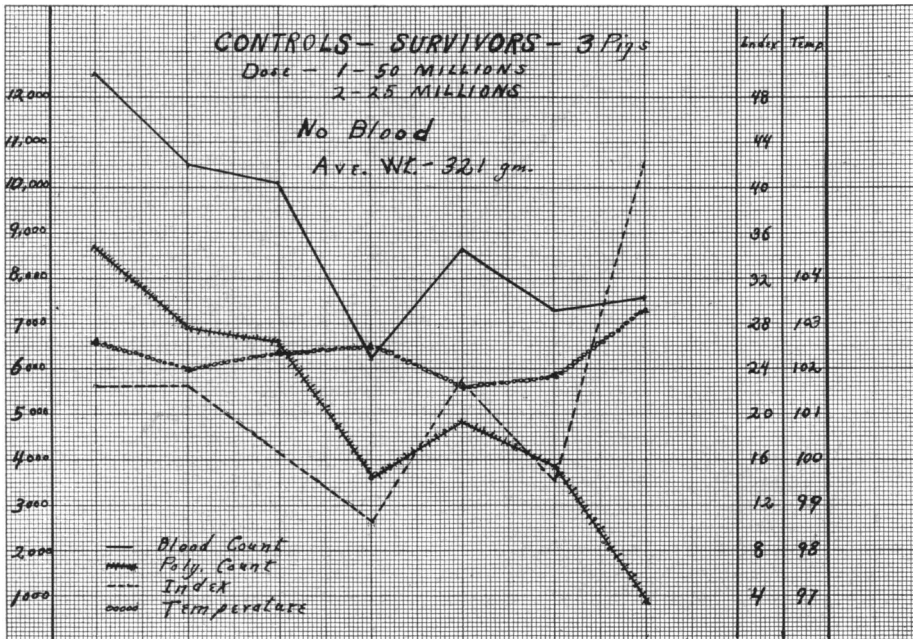

FIG. 3.

The results from the experiments using these strains were quite variable. Similar results were found using a strain (C-20) grown through several generations on laboratory media. Experiments with this strain, using larger or smaller amounts of broth culture, seemed to show that peritonitis and death would occur in a certain number of cases, but even the largest doses occasionally failed to produce peritonitis. Smaller doses might have the desired effect on one day and fail to produce peritonitis on the next. This confusion led to the employment of a wide range of dosage of organisms with inconclusive results.

In the earlier experiments two important factors were not controlled. Known amounts of broth cultures were used without regard to the number of bacteria present. There may be a wide variation in the bacterial count of broth cultures, ranging from 50 to 400 million bacteria per cubic centimetre in

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the case of the colon bacillus. In order to control the number of bacteria used, a Gates turbidimeter was employed and all doses determined in terms of millions of bacteria.

The other factor not controlled in the earlier experiments was the variability of the toxicity of the strain used. It was desirable to have on hand a strain in which a fairly constant minimal lethal dose would always produce peritonitis. Various strains in the Pepper laboratory of the University Hospital were tested and two fairly satisfactory strains were found. Dr. Frank Meleney,[8] of New York, kindly contributed two other strains, one of which was found very satisfactory. However, we noted that there was much vari-
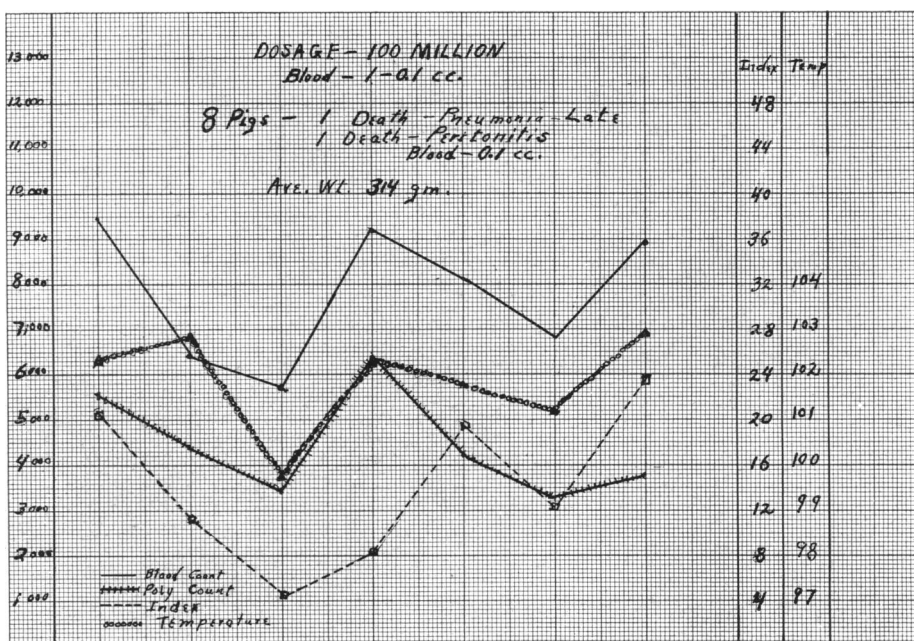

FIG. 4.

ability in the toxicity of the strains tested from Pepper laboratory, although they satisfactorily produced peritonitis. We had noted the development of substrains in most of the strains we had used, but we attached little significance to this phenomenon. Doctor Meleney called our attention to the significance and importance of the development of substrains in relation to variability in toxicity.

Substrain development, as we interpret it, is not the well-known dissociation of bacteria into smooth, rough and intermediate colonies, as described by Theobald Smith and others. There seems instead a definite separation into two substrains, and this seems to occur at any time as the organisms are growing or transferred on artificial media. One substrain is glistening white on its surface and opaque to transmitted light. This substrain of colon bacilli is always toxic when produced in a toxic strain. The other is dark on its sur-

face, but readily transmits light. It may be almost entirely non-toxic. Whenever this separation into substrains occurs and the non-toxic out-number the toxic colonies, the minimal lethal dose of the injected organisms will be much increased. It is then necessary to subculture from the toxic substrain to restore the toxicity. This explains the necessity of determining the minimal lethal dose of any strain on the day before its use to be reasonably sure that the expected effect will occur.

With these refinements in technic an experiment was devised accurately to determine the value of blood in experimental peritonitis. The minimal lethal dose of the strain used was determined as being slightly below 100
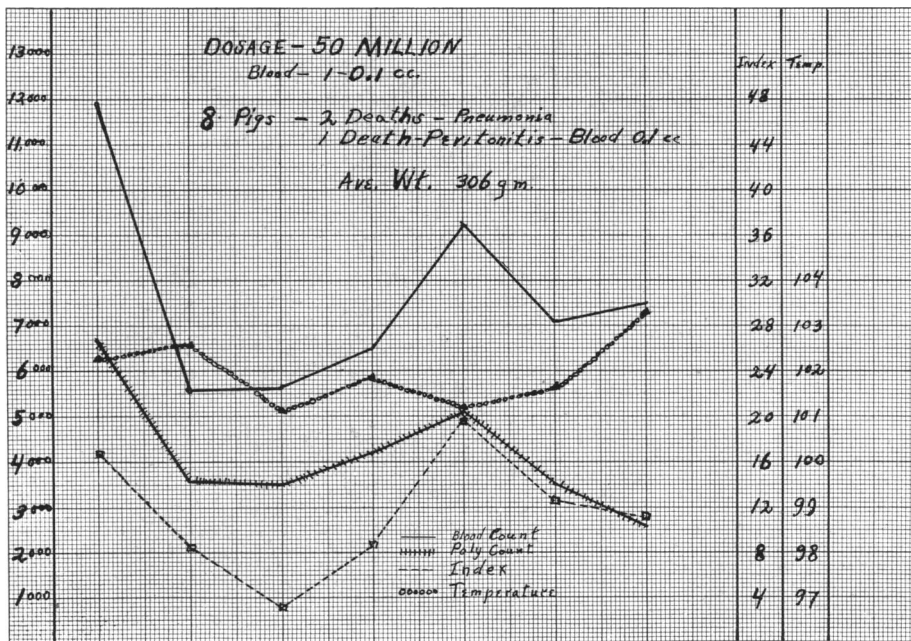

FIG. 5.

million bacteria for a guinea-pig of about 300 grams in weight. Six guinea-pigs were injected with doses of 400, 350, 300, 250, 200, and 100 million bacteria. All the animals died of peritonitis. The last, receiving 100 million bacteria, died in seventy-two hours with a severe plastic peritonitis. The remaining animals died in from two to four hours.

Thirty-four guinea-pigs were used next day, of which ten were used as controls. Blood counts, differential counts, including Schilling indices, were taken from ear veins. Rectal temperatures were taken and smears were made from the peritoneal cavity by needle puncture; all at similar intervals so that all data was collected as nearly simultaneously as possible. Injections were made with a tuberculin syringe through a 22-gauge needle. The animals had been on a uniform diet for some time before the experiment and their weight was nearly uniform. Housing, diet, and all other conditions

were kept uniform during the experiment. When an animal died it was autopsied as quickly as possible, cultures were taken from the peritoneum and microscopical sections were made of its viscera. Blood and peritoneal smears were stained with Ramonowski's stain. The total white blood-cell count and the total polymorphonuclear count were chosen for illustration with the Schilling index as giving the most significant findings. High percentages of monocytes were frequently observed in the differential count, but they were too variable to illustrate.

Autogenous blood was not used in these experiments. Defibrinated sheep's blood collected under sterile conditions was used instead. Broth cul-
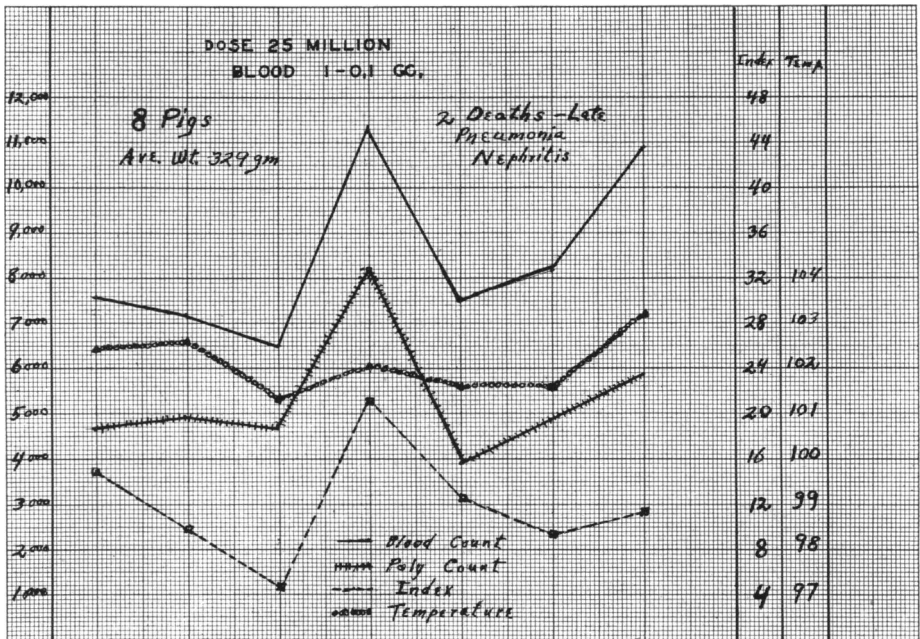

Fig. 6.

tures were thoroughly mixed and shaken, the count determined by the turbidimeter, and the calculated dosage injected simultaneously with blood. Sufficient amount of fluid could not be withdrawn from the peritoneal cavity for cell count without definitely changing dosage relationships, so only very minute amounts were used to make smears.

Results.—In the ten control animals the minimal lethal dose was between fifty and 100 million bacteria. Twenty-five million bacteria did not cause peritonitis or death in either of two animals in which no blood was injected in the peritoneum. When fifty million bacteria were injected, one of the two animals developed peritonitis and died. The six animals receiving 100 million bacteria or more all died of peritonitis.

Twenty-four animals received blood as well as bacteria. These were divided into three groups receiving 100, fifty and twenty-five million bacteria respectively. Each of these groups of eight animals was further subdivided into four groups of two animals receiving 0.1 cubic centimetre, 0.2 cubic centimetre, 0.5 cubic centimetre and one

cubic centimetre of defibrinated sheep's blood injected with the bacteria. Of those receiving 0.2 centimetre or more blood in the peritoneal cavity with the organisms, none died of peritonitis. Four animals of this group died of pneumonia and did not have peritonitis at death as evidenced by a study of microscopical sections. These animals lived for a period of two, six, nine, and eleven days following the injection of the organisms and blood.

Of the animals receiving only 0.1 cubic centimetre of blood with the organisms, one of the two animals receiving 100 million, and one of the two receiving fifty million bacteria developed peritonitis and died on the second and fifth days following the injection. Of the two animals receiving 0.1 cubic centimetre of blood and twenty-five million bacteria one died of pneumonia seven days after receiving the intraperitoneal injection. The mortality and occurrence of peritonitis are illustrated in Table I. When sufficient blood was injected with the organisms (0.2 cubic centimetre or more) the animals did not develop peritonitis even when a dose which killed all controls was injected.

TABLE I

Gross Mortality

Controls

Dosage	200 M	100 M	50 M	25 M
Animals...................	2	4	2	2
Peritonitis................	2	4	1	0
Deaths....................	2	4	1	0

Experimental

a. 100 M Organisms

Blood	1 cc.	0.5 cc.	0.2 cc.	0.1 cc.
Animals...................	2	2	2	2
Peritonitis................	0	0	0	1
Deaths....................	0	1	0	1

b. 50 M Organisms

Blood	1 cc.	0.5 cc.	0.2 cc.	0.1 cc.
Animals...................	2	2	2	2
Peritonitis................	0	0	0	1
Deaths....................	1	0	1	1

c. 25 M Organisms

Blood	1 cc.	0.5 cc.	0.2 cc.	0.1 cc.
Animals...................	2	2	2	2
Peritonitis................	0	0	0	0
Deaths....................	0	0	1	1

In the controls which died (Figs. 1 and 2) there was a general tendency to a temporary rise of two to three degrees Fahrenheit, in temperature, followed by a drop to below the normal level as the animal approached death. The Schilling index dropped in all fatal cases. Occasionally there was a temporary rise before the sudden drop occurred. The percentage of polymorphonuclear cells was not markedly disturbed in the fatal cases, although there was a drop in total count in nearly every instance.

In the three surviving controls (Fig. 3) the temperature was not so greatly altered. There was a temporary drop in the nuclear index, which later rose to above the normal level. There was a rise in the polymorphonuclear count at the end of twelve hours, but this dropped to a point well below normal by the end of eighty-four hours. The total

count rose rapidly after the injection and later dropped to the normal level. The discrepancy between the total count and the polymorphonuclear count represents a rise in the lymphocytes which continued to remain high.

The animals receiving 0.2 cubic centimetre or more of blood and 100 million bacteria showed a primary drop in temperature, Schilling index and total white blood-cell count, but there was a rapid return to normal with no disturbance of the polymorphonuclear percentage. (Fig. 4.). One of the animals receiving 0.1 cubic centimetre of blood and 100 million bacteria died of peritonitis within forty-eight hours. The findings in this animal were in general similar to the controls who died of peritonitis.

The curves obtained from the animals receiving fifty and twenty-five million bacteria with 0.2 cubic centimetre or more of blood are quite similar to that obtained from the corresponding group receiving 100 million bacteria. (Figs. 5 and 6.)

The high incidence of pneumonia (five of thirty-four) can be explained either on the bases of intercurrent respiratory infection, or upon the fact that the organisms, quickly passing from the peritoneum, set up foci in the lungs.

Peritoneal smears showed that in the fatal cases of peritonitis bacteria increased rapidly. In the control animals surviving, bacteria first increased, then decreased until at the end of eighty-four hours they were usually absent. Animals with blood in the peritoneal cavity showed a very rapid disappearance of bacteria, so that at the end of thirty-six hours no bacteria were found in the smear. The smears of these animals did not contain the large number of bacteria seen in those of the control animals. Table II shows typical findings in the smears of each group.

TABLE II

Peritoneal Smears

Guinea-pig No. 172. Injected intraperitoneally with 100 million bacteria. Result.—Death within twenty-four hours from peritonitis.

	2 hrs.	4 hrs.	12 hrs.
Polymorphonuclear cells	28	63	many
Lymphocytes	3	0	0 per H.P.F.
Red blood-cells	0	40	many
Macrophages	0	0	0
Bacteria	7	many	innumerable

Guinea-pig No. 171. Injected intraperitoneally with twenty-five million bacteria. Result. —Survived.

	2 hrs.	4 hrs.	12 hrs.	36 hrs.	60 hrs.	84 hrs.
Polymorphonuclear cells	3	115	47	84	53	3
Red blood-cells	4	30	0	0	0	0
Macrophages	0	3	4	14	31	31
Bacteria	1	many	30	5	50	0

Guinea-pig No. 179. Injected intraperitoneally with 100 million bacteria and one cubic centimeter of blood. Result.—Survived.

	2 hrs.	4 hrs.	12 hrs.	36 hrs.	60 hrs.	84 hrs.
Polymorphonuclear cells	44	222	150	32	67	3
Red blood-cells	137	832	61	103	22	3
Macrophages	0	0	4	2	6	0
Bacteria	1	9	2	0	0	0

Only one culture of the peritoneal cavity of autopsied animals was sterile and this was one of the animals receiving blood which died of pneumonia. The animals receiving

blood seemed to have less growth in the culture, but too many extraneous factors enter here to draw any conclusions from this fact.

The pathological sections of these animals were interesting. All showed mild nephrosis, cloudy swelling of the liver and acute splenitis. In the animals dying with peritonitis there was marked swelling and œdema of the peritoneum with occasional subserous infiltrations. Pneumonia was diagnosed only when the alveolar spaces were filled in the stage of either red or gray hepatization, since all lungs showed some engorgement and filled capillaries. All adrenals showed poorly stained cells with vacuolization. Some of the adrenals showed derangement of architecture as well and this was especially true when peritonitis was the cause of death.

In order to determine whether dilution of the injected organisms by blood was responsible for the variation in the results seen in the two groups of animals, a third group of six animals was used. These animals received 100 million bacteria diluted with 0.1 cubic centimetre to one cubic centimetre of sterile broth. All these animals died of peritonitis within forty-eight hours.

Discussion and Conclusions.—It would seem from these results that blood injected with organisms not only gives no predisposition to peritonitis but offers a moderate degree of protection against it, at least in the case of the colon bacillus. The control animals receiving a minimal lethal dose all died, yet only two of sixteen receiving a minimal lethal dose with varying amounts of blood died from peritonitis, and these animals received a small amount of blood. Certainly doses below the minimal lethal dose were not raised to minimal lethal by the employment of blood. That this effect was not the result of mechanical dilution was later proven by the addition of broth in varying quantities to minimal lethal doses of bacteria without effect. Peritoneal smears also indicate that blood seems to hasten the disappearance of bacteria from the peritoneum. This may be by reason of greater rapidity of absorption, or by increased rapidity of destruction of the bacteria. This power of protection is not sufficiently great, nor can analogy be drawn with sufficient clarity to human peritonitis to justify its clinical application in any way at present.

The results from these experiments would lead one to believe that the pleura and peritoneum as serous membranes do not respond in a similar manner to the presence of blood introduced with organisms, since Allen has shown that the addition of a small amount of blood increases the incidence of empyæma of the pleural cavity when certain organisms are injected.

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