

17215. Infection as a Factor Causing Death in the Eviscerate Rat.

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The data of these experiments indicate that the extent of bacterial contamination during the procedure of evisceration in the rat is an important factor in determining the survival time. Survival was not affected by the administration of gelatin or of Tyrode's solution.

Methods. Male rats of the Sprague-Dawley strain were maintained on a diet of Archer Dog Pellets. The technic of evisceration, which is done in 2 stages, has been described by Ingle.¹ When the animals reached 250 ± 2 g, they were anesthetized (intraperitoneal injection of 18 mg of cyclopentenyl-allyl-barbituric acid sodium) and subjected to the second stage of evisceration. Intravenous infusions of solutions containing glucose (C.P. Dextrose, Merck) and insulin (Regular Insulin, Lilly) were made by a continuous injection machine which delivered fluid into the saphenous vein of the right hind leg at the rate of 20 cc per 24 hours. The glucose load was 44 mg per 100 g of rat per hour and the insulin dose was 4 units per 24 hours per rat. Temperature was constant at $26.5 \pm 0.5^\circ\text{C}$.

The time of survival was determined by use of a heart-beat amplifier (Model A, Upjohn) which was designed to amplify the D.C. potential generated by the heart beat to actuate a 6-point recording mechanism (Leeds and Northrop Micromax S). This apparatus gives a permanent visual record of an all-or-none response of the amplifier to the beating heart of 6 animals simultaneously.

Experiments and results. It is well known that the presence of inorganic ions other than sodium and chloride favor the survival of perfused isolated organs and tissues. In Experiment 1, a comparison was made of the survival times of eviscerate rats given Tyrode's solution with the survival times of similar rats given 0.9% sodium chloride solution. The amount of added glucose and insulin was the same in each group (see methods). Twenty-one rats given Tyrode's

solution had an average survival of 2822 minutes with a range of 2457 to 3580 minutes. An equal number given saline had an average survival of 2818 minutes with a range of 2350 to 3262 minutes. It was concluded that either the use of Tyrode's solution failed to prolong survival to a greater extent than saline or that the effect was small.

As the eviscerate rat approaches death, there is a significant decrease in the concentration of proteins in the blood plasma and there is a concomitant increase in the free fluid of the body cavities (Ingle, Prestrud, and Nezamis, unpublished data). It was considered possible that the loss of fluid could be decreased and survival prolonged by the intravenous administration of a solution of colloids which might remain in the blood and sustain its osmotic activity. A 5% solution of degraded and purified gelatin containing 0.9% sodium chloride (Plazmoid, Upjohn) was used. Glucose and insulin were added to the solution. Fourteen eviscerate rats given gelatin had an average survival of 2509 minutes with a range of 1985 to 3046 minutes. Sixteen similar rats given saline with glucose and insulin had an average survival of 2748 minutes with a range of 2364 to 3076 minutes. The administration of gelatin did not reduce the rate at which fluid accumulated in the body cavities. It was concluded that this preparation of gelatin did not prolong the survival of the eviscerate rats.

Although the normal rat has a high natural resistance to the infection of wounds, the resistance of the eviscerate rat to infection is very low. A few hours following evisceration the peritoneum and even the blood becomes heavily charged with bacteria. We have never observed septicemia and peritonitis following other operations in the rat. The following organisms have been recovered from the peritoneal fluid of rats 24 hours following evisceration: *Pseudomonas*, hemolytic streptococci, *E. coli*, staphylococci, and *Proteus vulgaris*. Our attempts to apply sterile

¹ Ingle, D. J., *Exp. Med. and Surg.*, 1949, **7**, 34.

technic, although adequate for all of the many other operative procedures which we have carried out on the rat, have been only partially successful in the eviscerate rat. The sterilization of the skin of the rat with a germicidal agent (Mercresin, Upjohn), the use of sterile instruments, the use of sterile solutions for the infusions, etc., have greatly reduced the extent of bacterial growth per unit time, and the peritoneal fluid of an occasional animal has remained sterile, but uniform success has not been attained. That infection is an important factor in limiting the survival of these animals is indicated by the following results.

In Experiment 3, 26 rats which were eviscerated without asepsis had an average survival of 2743 ± 109 minutes, whereas 26 rats which were eviscerated under "aseptic" conditions had an average survival of 3113 ± 67 minutes. The standard deviation of the difference (370) was 129. The ratio between the difference and its standard deviation was 2.9, thereby meeting the usual requirements of statistical significance.

Discussion. Although a solution containing potassium, calcium, magnesium, phosphate and bicarbonate ions in addition to sodium and chloride is superior to saline alone for the perfusion of isolated tissues, the administration of Tyrode's solution to the eviscerate rat failed to have a significantly more favorable effect on survival than physiological saline. The blood and tissues of the eviscerate rat contain an optimal concentration and distribution of these ions at the time of operation and it is probable that no lack of these substances develops during its survival.

Hepatic insufficiency is characterized by the development of ascites. The extent to which the loss of fluid from the blood into the body cavities may be due to a decrease in plasma proteins is not known to us. The administration of gelatin did not inhibit the rapid accumulation of fluid in the body cavi-

ties, nor did it prolong survival. Other plasma substitutes should be tested in the eviscerate rat.

The readiness with which the eviscerate rat develops septicemia and its low resistance to infection of the peritoneal cavity in contrast to the high resistance of the non-eviscerate rat offer interesting problems. The future success of our efforts to prolong the survival of the eviscerate rat will depend in part upon the outcome of our attempts to completely prevent infection. Even the partial success of our present efforts has significantly prolonged the survival of these animals. The average survival of the "aseptic" group of Experiment 3 is longer than has been reported in the literature for any series of liverless mammals. The longest survival of any single animal of our series to date is 63 hours and 47 minutes.

Summary. Eviscerate male rats were given continuous intravenous infusions of solutions containing glucose and insulin. The times of survival were determined by a heart-beat recorder. The substitution of Tyrode's solution and of 5% gelatin for 0.9% sodium chloride in the infusion fluid failed to prolong survivals. The rats which were eviscerated without sterile technic developed septicemia and peritonitis. The attempts to maintain asepsis during the procedure of evisceration were not uniformly successful, but the average survival of 26 rats operated under "aseptic" conditions was 3113 ± 67 minutes as compared to 2743 ± 109 minutes for 26 rats operated without asepsis. The maximum survival time recorded for any animal was 63 hours and 47 minutes.

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