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Tolerance to Blood Loss in Newborn Dogs. (24763)

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In the past it has been found that adult dogs seldom survived when 4-5% of body weight in blood was removed rapidly(1-6). Observations(7,8) that newborn animals were more resistant to respiratory hypoxia than adults suggested that tolerance to hemorrhage might also vary with age and prompted the experiments below.

Procedures. 213 mongrel dogs of various ages were anesthetized with ether, and blood removed rapidly from the heart with heparinized syringe and 19-gauge needle. Blood was weighed and hemorrhage defined in % of body weight. Hematocrit was determined by micro technic. After recovery from anesthesia, animals were given food and water *ad lib.* In surviving animals a second hematocrit was taken 24 hours later. In another group of 108 animals an intraperitoneal infusion of saline, equal to 5% or 10% of body weight, was given as pretreatment 1-4 hours before bleeding. Blood volume was determined in 14 dogs by Evans blue method of Gregerson (9).

Results. Fig. 1 shows % survival of dogs subjected to various amounts of hemorrhage. For simplification, results were presented in 2 categories: group A includes dogs 10 days and younger, group B older dogs. As shown in Fig. 1, when blood loss averaged 3.4% of body weight, survival was 52% in group A, and 57% in group B. However, when the hemorrhage averaged 5.2%, survival in group A was 22%, and in group B, 6%. Statistical tests of significance indicated the latter difference was not likely due to chance ($P = <0.001$).

In saline pretreated animals, absorption of fluid from peritoneal cavity was indicated by drop in hematocrit of about 10%. As shown in Fig. 1, saline failed to increase survival in either group A($P = >0.2$) or B($P = >0.4$) to a statistically significant degree. The results however, confirmed the finding of greater hemorrhage tolerance in younger animals.

Hematocrit changes with age have been described in detail elsewhere(10), and findings in our dogs were similar. In group A the initial hematocrit averaged 45% in those that recovered, compared to 37% in those not recovering. For group B these values were 32% and 30%, respectively. Dogs surviving 5.2% hemorrhage had a large decrease in hematocrit, the average value dropping about 50% at 24 hours.

Blood volume was not done on smallest animals because of technical difficulties, but results from 4 pups 10-16 days old showed average blood volume of 10.1% of body weight. In 10 dogs 6 weeks or older blood volume averaged 9.6% of the weight. These results are similar to those reported by others(11,12). Since blood volume was a fairly constant fraction of body weight at all ages, it was assumed that hemorrhage on weight basis imposed the same relative blood loss on all animals.

Discussion. The greater resistance of

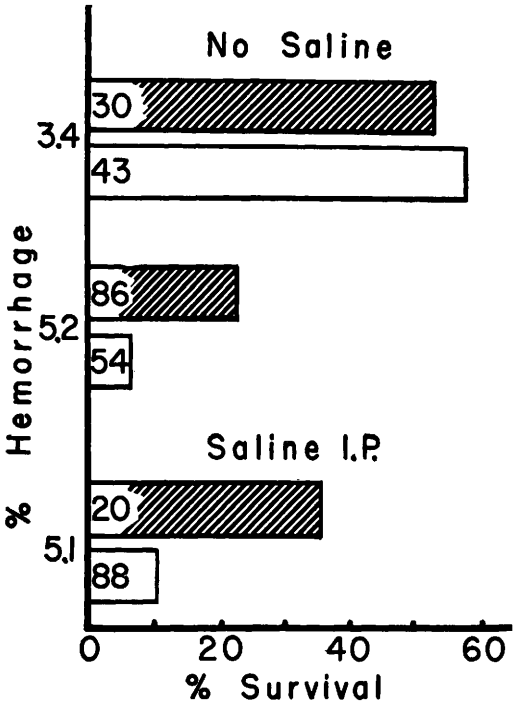

FIG. 1. Shaded bars, dogs 1-10 days old; open bars, dogs older than 10 days, including adults. Total animals in each group shown by numerals inside bar. Above, % survival of untreated dogs of various ages subjected to large rapid hemorrhage. Below, % survival of dogs pretreated with saline.

young animals to severe blood loss may be accounted for by one or more of the characteristics peculiar to the newborn. The central nervous system of neonatal dogs has been found highly resistant to respiratory hypoxia, and this may also imply high resistance to anemic hypoxia. The gasping movements seen after severe hemorrhage resemble those of hypoxia, and suggest cardiorespiratory failure as the cause of death rather than hypotensive shock, particularly since fatalities usually occurred within minutes after blood removal. Those surviving longer probably succumbed to hypotensive shock or cardiac trauma. Erythrogenic factors that may contribute to survival in the newborn are high red cell count, and larger amount and greater oxygen affinity of neonatal hemoglobin(8,13). That animals surviving a large hemorrhage averaged higher hematocrits than those succumbing to the same blood loss, suggests that high red cell volume favors recovery. In adult dogs pretreatment with adrenolytic drugs or surgical sympathectomy has been reported to increase tolerance to hypotension induced by hemorrhage(14). In the newborn dog, incomplete state of autonomic development may be a contributing factor in tolerance to blood loss. Numerous other differences between young and old animals undoubtedly contribute to hemorrhage resistance, since rats at 50 days of age, when anoxia tolerance has been lost, withstand greater blood loss than older animals(8,15).

In our experiments intraperitoneal saline failed to give protection against a large blood loss in either group A($P = > 0.2$) or B($P = > 0.40$), although a favorable effect was suggested. Others have reported some beneficial results when dogs were pretreated with i.v. saline before hemorrhage(16,17).

Summary. Dogs 10 days of age or younger had higher survival rates than older animals when subjected to rapid blood loss averaging 5.2% of body weight. No difference in survival was found when blood loss was 3.4%. Pretreatment with 5-10% of body weight in saline, given intraperitoneally 1-4 hours previously, failed to protect against subsequent hemorrhage.

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ECHO Virus Type 12, Isolation and Characteristics.*† (24764)

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Although ECHO viruses have received considerable attention within the past 4 years, adequate descriptions of many prototype strains have been lacking. This report concerns isolation and characteristics of prototype ECHO-12 virus. Included herein is a description of its properties in cell cultures, in laboratory animals and in serologic tests.

Materials and methods. Monolayer cell cultures of trypsinized rhesus monkey kidney (MKCC) were used in isolation, neutralizing antibody (NA) tests, preparation of virus pools, and plaque studies. Serologic methods have been described(1). Plaque studies were performed according to method of Hsiung and Melnick(2). Animals were immunized by repeated intramuscular injection of an emulsion containing equal volumes of Arlacel-Bayol F adjuvant and unconcentrated untreated virus-containing MKCC fluids.

Results. Establishment of ECHO-12 prototype virus. Prototype ECHO-12 virus, Travis 2-85-4,§ as first described by ECHO Virus Committee(3), was isolated in 1954(4) from rectal swab taken Aug. 18, 1953 from a healthy 6-year-old son of an American military family (Travis) stationed at Clark Air Force Base (CAFB) in the Philippines. A serum from this child taken 14 days earlier contained no detectable NA to this agent, whereas that collected 14 days after positive rectal swab had a titer of 1:128. Two and 3 months following virus isolation the serum titer was 1:32(1). Prior to acceptance as ECHO-12 prototype, 2-85-4 virus and its antiserum (titer 1:1,000 *vs.* 100 $TCID_{50}$) were tested reciprocally by our laboratory and those of Melnick and Sabin against 12 other original ECHO prototype viruses and their relatively low-titered antisera. These tests revealed no cross relationships. Subsequently, aliquots of ECHO-12, MKCC passage 5, were sent to Dr. Herbert A. Wenner and Microbiological Associates for preparation of hyperimmune monkey and rabbit antiserum respectively. The composite NA tests with monkey antisera (Wenner) obtained in 4 different laboratories have been reported(5). It was found that low dilutions of Wenner ECHO-12 antiserum neutralized significant amounts of ECHO viruses 1 and 8 and the original prototype ECHO-13 virus. In contrast, however, ECHO-12 virus was not neutralized by ECHO 1, 8 and 13 antisera (Wenner).

To investigate these findings the above 4 ECHO prototype strains were purified by plaque isolation or terminal dilution passage and antisera prepared to these purified viruses. The results obtained in our laboratory with terminal dilution purified ECHO-12 antisera (titer 1:2,048) against approximately 100 $TCID_{50}$ of purified ECHO viruses 1, 8, 12 and 13 are shown in Table I. ECHO viruses 1, 8 and 13 were not neutralized by this antiserum. No neutralization occurred when purified ECHO-12 virus was tested against antisera prepared to purified ECHO viruses 1, 8 and

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§ 2-85 is the number given this child and 4 is the number of specimen from which virus was isolated.