tially deproteinized filtrate of human serum diluted in an equal volume of M/15 buffer, with rice starch added, constitutes a simple medium for the propagation of *E. histolytica*. 3. Rice starch appears to act as a temporary inhibitor to bacterial multiplication in these cultures. Such suppression leads to more satisfactory growth of the amebae present. 4. Study of the biology of the bacteria in these cultures may give further information concerning the complex metabolic requirements of *E. histolytica*.

15855

Redistribution of Residual Blood Volume in Hemorrhagic Shock; Relation to Lethal Bleeding Volume.*

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When unrestricted hemorrhage is permitted from a major artery bleeding proceeds until death eventuates; the total amount of blood that has flowed out is called the *bleeding volume*. When death occurs hemorrhage has either ceased or is reduced to a negligible ooze even though a considerable amount of blood obviously still remains in the body; this remainder is called the *residual volume*. It is known that bleeding volume of animals is less than normal in shock, an observation that has often been used as evidence that the total blood volume is reduced in shock. The object of this study was to determine the magnitude of the residual volume and its distribution in the body after hemorrhagic shock as compared to that of normal animals.

Methods. Dogs weighing 7.9 to 23.3 kg were anesthetized with morphine sulfate and sodium barbital. Both femoral arteries and a femoral vein were cannulated for the purposes of bleeding, reinfusion, and the continuous recording of mean arterial blood pressure with a mercury manometer. A tracheal cannula was inserted. For determining the bleeding volume in the control animals blood was allowed to flow freely from a femoral artery cannula at the rate of 2 cc/kg/minute until bleeding ceased and/or death ensued. These events were virtually simultaneous in all cases. For producing hemorrhagic shock the animals were bled so that mean arterial pressure was maintained at 50 mm Hg for 90 minutes and then at 30 mm Hg for 45 minutes more, after which all withdrawn blood (heparinized, warmed, and filtered) was reinfused. The bleeding volume of the shock animals was determined in this way: One hour after reinfusion, hemorrhage was begun at the rate of 2 cc/kg/minute and was continued in the same manner until death as for the control animals.

After death the residual volume was determined without delay. The femoral cannulae were tied off. The chest was opened by midline thoracotomy. With suitable precautions to prevent blood loss the inferior vena cava was sectioned about 1 cm distal to the right atrium. Two cannulae were inserted; one distally into the inferior vena cava and the other proximally toward the atrium and the superior vena cava. The atrium was clamped across so as to occlude the right atrio-ventricular orifices. Through this arrangement as much venous blood as possible was collected by the upper cannula from the region of the body drained by the superior vena cava and the azygos vein, while blood from regions drained by the inferior vena cava was collected from the lower cannula. The blood still remaining in these 2 territories was next washed out. A cannula inserted through the wall of the left

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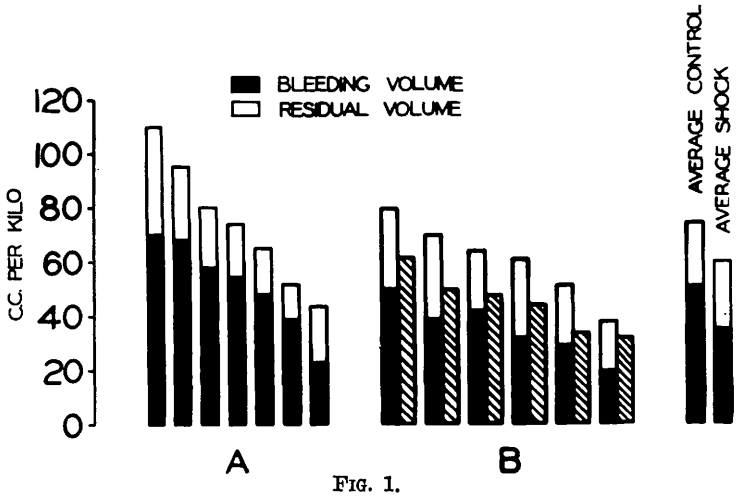

FIG. 1.

ventricle was passed through the aortic valve into the aorta; this was held in place by a ligature around the aorta. A solution of 0.9% NaCl and 1.0% sodium citrate was introduced into the aortic cannula under a constant pressure of 150 mm Hg. The mixture of blood and saline flowing from the 2 cannulae was collected separately. Perfusion was continued until the perfusate was clear and free of blood grossly. The oral and intestinal mucosa and other tissues examined after perfusion were invariably white; various muscles and the liver and spleen were relatively pale; no blood or bloody fluid could be expressed. In a few cases veins of the mesentery contained a light pink fluid indicating the admixture of a small quantity of blood; this was not regarded as significant. The blood still remaining in the right ventricle, pulmonary vessels and left atrium was drained as follows: A cannula was inserted into the left auricular appendage for drainage. The superior vena cava was clamped and the A-V clamp removed. By connecting the cannula in the central end of the inferior vena cava to a perfusion bottle blood was washed out.

The amount of blood contained in the perfusates was estimated through use of the conventional acid hematin method. A sample of arterial blood was taken from the animal just before death; it is assumed that the hemoglobin content of this sample was the same as that of the residual blood. The blood sample was diluted with the saline-citrate perfusion fluid so as to give a known dilution of the same magnitude as that estimated for the perfusate. Acid hematin was formed by the addition of hydrochloric acid in amount so as to give a final concentration of 0.1 normal. (It was determined that the saline-citrate solution did not affect the formation of acid hematin). Four or more different dilutions of this known solution were tested for light absorption in a Coleman spectrophotometer using a 450 γ filter. The curve plotted for these values became the reference standard against which the perfusate was compared. Appropriate amounts of the perfusate were prepared as above and absorption was determined. The concentration of hemoglobin in the perfusate was then calculated as compared to a concentration of 1.0 in the whole blood sample. This factor multiplied by the perfusate volume equals the volume of blood contained in the perfusate.

Results. Fig. 1-A shows the results obtained from 7 control animals, and Fig. 1-B those from 6 shock animals. (Eight experiments performed during development of technics are not reported here; their results, however, were consistent with those reported).

As shown in Fig. 1-A, bleeding volume in the control animals varied from 22.8 cc/kg to 70.7 cc/kg with an average of 51.4 cc/kg; residual volume varied from 12.9 cc/kg to 39.0 cc/kg with an average of 22.5 cc/kg;

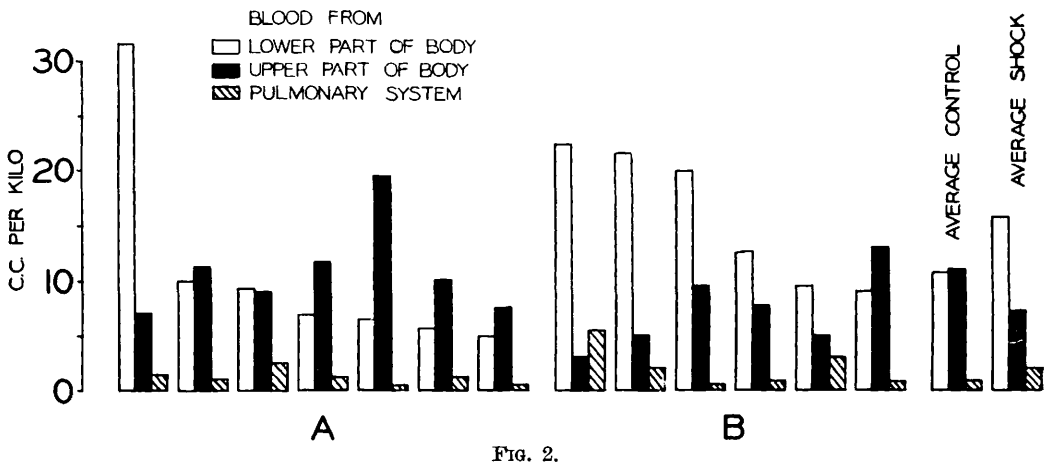

FIG. 2.

and the total blood volume varied from 43.5 cc/kg to 109.7 cc/kg with an average of 73.9 cc/kg. Fig. 1-B shows that the bleeding volume for the shock animals varied from 19.5 cc/kg to 50.2 cc/kg with an average of 35.2 cc/kg; residual volume varied from 17.9 cc/kg to 31.0 cc/kg with an average of 25.2 cc/kg; and the total blood volume varied from 37.4 cc/kg to 80.0 cc/kg with an average of 60.5 cc/kg. Results from the control animals are compared with those for the shock animals in tabular form below.

	Control	Shock
Avg total volume	73.9 cc/kg	60.5 cc/kg
Avg bleeding volume	51.4 "	35.2 "
% of total	69.2%	57.6%
Avg residual volume	22.5 cc/kg	25.2 cc/kg
% of total	30.7%	42.3%

The total blood volumes observed presumably represent the actual circulating volumes since perfusion should be unaffected by cardiodynamic alterations. While they are, on the average, less in shock dogs, this does not permit the conclusion that the total blood volume in any particular animal is reduced as a result of hemorrhagic shock. It is well established that the blood volumes per kg body weight of different dogs varies considerably. Moreover, Overbey *et al.*[1] recently reported that blood volumes estimated by dye methods decreased by only 0.7 ± 1.64 cc/kg during development of a similar type of hemorrhagic shock.

[1] Overbey, D. T., Ramirez, A., Wiggers, C. J., and Lawson, H. C., *Fed. Proc.*, in press.

The difference between the average bleeding volumes is considered significant. The bleeding volume is lower in the shock animal or, stated in another way, the heart pumps out a smaller percentage of available blood before the animal dies. There is, of course, a corresponding difference between the average residual volumes. Assuming that the total blood volumes are of the same order these data show the expected; namely, that blood which the heart of the shock animal cannot pump out of the body simply remains within it.

Fig. 1-B also shows the amount of blood withdrawn in order to lower the mean arterial blood pressure to 30 mm Hg during the initial hemorrhage (cross hatched bars). This volume varied from 31.6 cc/kg to 61.9 cc/kg with an average of 44.7 cc/kg. It is obvious that in every experiment this value exceeded the lethal bleeding volume after shock had developed.

The distribution of the residual volume is shown in Fig. 2-A for the control animals and in Fig. 2-B for the shock animals. In these graphs the volumes obtained respectively from the superior vena cava-azygos system, from the inferior vena cava system, and the cardiopulmonary system are compared at a glance.

It is obvious that as a rule the residual superior cava-azygos volumes exceed the inferior caval volumes in control animals. On the contrary, in every experiment except one

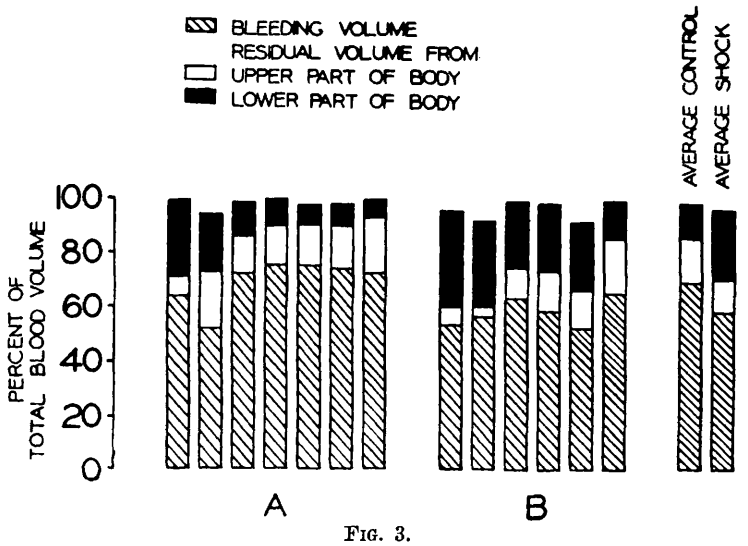

FIG. 3.

the reverse relation existed after development of shock. The pulmonary blood volumes were of such small magnitude that evaluation of differences becomes hazardous. While a little more blood seems to stagnate in the cardio-pulmonary circuit this is very insignificant compared to the great increase in the inferior caval territory. Results are compared in tabular form below.

	Control	Shock
Avg residual volume	22.5 cc/kg	25.2 cc/kg
Avg SVC-azygos volume	10.8 "	7.2 "
% of residual volume	48.0%	28.5%
Avg IVC volume	10.6 cc/kg	15.8 cc/kg
% of residual volume	47.1%	62.7%

(Note: Residual blood not accounted for above was recovered from the pulmonary system.)

Finally, all of the data obtained in this study are integrated in Fig. 3 as percentages of the total blood volume. They show that the residual volume remaining in the lower part of the body has increased proportionately, partly at the expense of the residual volume from the upper part of the body, but chiefly at the expense of the bleeding volume. It is obvious that bleeding volume is not a reliable criterion of total blood volume after development of shock.

Discussion. Reviews of recent literature[2,3] indicate that while reduction in blood volume is a concomitant of most common forms of shock, this is not the sole factor in development of irreversible circulatory failure. Furthermore, the development of shock without significant reduction in blood volume is not precluded. According to studies of Overbey *et al.*,[1] this is the case in a type of hemorrhagic shock which follows reinfusion of all blood withdrawn during preceding hemorrhage (standardized hemorrhagic shock procedure used in this laboratory). In this type of shock blood tends to pool in splanchnic areas as indicated by direct observations of intestinal capillaries,[4] development of passive congestion, edema, and hemorrhage in the intestinal mucosa,[5] persistence of high portal pressure,[6] and an apparent reduction in resistance in the mesenteric circuits.[7] With such premortal pooling in the splanchnic organs it may be expected that lethal bleeding volumes are reduced, not by virtue of a reduction in total blood volume but because it is not mobilized from regions of pooling before death from bleeding takes place. The partition of residual blood after lethal hemor-

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rhage in shock dogs reported in this investigation supports this interpretation.

Summary. 1. Seven control dogs anesthetized with morphine sulfate and sodium barbital were bled to death at the rate of 2 cc/kg/minute. Six dogs similarly anesthetized were first subjected to a standardized hemorrhagic shock procedure and were then reinfused with all blood previously withdrawn. They were bled to death 60 minutes after reinfusion at the same rate as the normal dogs. In each instance, the residual blood volume was determined after death by perfusion with a sodium chloride-sodium citrate solution under 150 mm Hg pressure. The residual volume was partitioned into fractions obtained from (1) the superior cava-azygos, (2) the inferior cava, and (3) the cardio-pulmonary systems.

2. The average total blood volume of the control animals slightly exceeded that of the shock animals, but the difference is not believed significant. The bleeding volume was significantly greater in the control animals, and correspondingly the residual volume was greater in the shock animal.

3. There was a significant shift in the distribution of the residual volume in the animal dying in shock. As compared to the controls, there was a marked increase in the residual volume of blood retained in the inferior vena cava territory and an accompanying decrease in the superior cava-azygos system.

4. The conclusion is reached that the reduced lethal bleeding volumes after transfusion of dogs in hemorrhagic shock are not significantly due to reduction in total blood volume but to pooling of greater volumes of residual blood in the splanchnic vessels. Changes in the cardio-pulmonary residual volumes are too small to affect lethal bleeding volumes.

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15856

Anesthetic Action of Beta-Dimethylaminoethyl Benzhydryl Ether Hydrochloride (Benadryl) in the Skin of Human Beings.*

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Beta-dimethylaminoethyl benzhydryl ether hydrochloride (benadryl) is one of the recent members of a series of synthetic antihistamine compounds which currently are receiving clinical trial and are showing promise of usefulness in the treatment of some types of allergy.[1-7] In regard to anesthetic action, only the first of this series of compounds, thymoxyethyldiethylamine (929 F), has been studied in detail. This compound was shown by Bovet and Staub[8,9] to possess antihista-

* Abridgment of thesis submitted by Dr. Leavitt to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Medicine.

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