Coagulation Changes of Hemorrhagic Shock in Baboons

CLIFFORD M. HERMAN, M.D., ROSS B. MOQUIN, M.D.,
DAVID L. HORWITZ, M.D., PH.D.

From Division of Experimental Surgery, Clinical Medical Sciences Department, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

THERE is considerable controversy over the nature and extent of coagulation changes which occur in hemorrhagic shock. It has generally been stated that hemorrhage leads to *hypercoagulability* followed by a hypocoagulable state,[1, 10, 11] as found in studies conducted in dogs and using a reservoir technic in which blood is drawn into a reservoir until blood pressure falls to a pre-determined level. The reservoir blood, anticoagulated with ACD or heparin, is reinfused as needed to maintain blood pressure at the desired level. Hardaway has recently criticized the reservoir technic, stating that the blood stored outside the body is somehow altered to promote disseminated intravascular coagulation upon reinfusion.[6] Furthermore, dogs have been shown to differ significantly from primates in their response to hemorrhagic shock, thus minimizing the clinical applicability of dog studies.[23]

We, therefore, desired to study hemorrhagic shock in a primate using a nonreservoir technic. Because the baboon is being used increasingly more in studies of hemorrhagic shock,[2, 16, 19] that animal has been used in these studies. Hawkey and Symons have shown the coagulation systems of man and baboon are similar with the exception of higher activities of certain individual factors, especially VII and VIII.[8] The similarity of coagulation mechanisms lends additional value to the use of the baboon as a model for study of clotting changes in shock.

Methods

Five adult male baboons (*Papio doguera*) weighing from 19 to 38 Kg., were used after quarantine for at least 30 days, during which they were skin-tested for tuberculosis and given routine courses of benzathine penicillin (300,000 units), intramuscular iron (Imferon, 3 cc.), and an antihelminthic (thiabendazole). They were maintained on a diet of Tek-Lad primate food and fresh fruit until the day before the experiment. On the morning of study, they were sedated with Sernylan (phencyclidine hydrochloride), 1 mg./Kg., and polyethylene catheters were placed in the femoral artery and through the femoral vein to the right atrium. The arterial and

Submitted for publication March 1, 1971.

From the Bureau of Medicine and Surgery, Navy Department, Research Task No. M4305.05. 3007.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care."

venous catheters were connected to pressure transducers and a recording device. They were temporarily disconnected to permit withdrawal of blood samples. The animals were then placed in a prone position with padded axillary supports, and small incremental doses of intravenous pentobarbital were given as the animals became sufficiently conscious to struggle against the supports. No more than 50 mg. per hour of pentobarbital was given.

After obtaining baseline measurements and blood samples, blood was withdrawn over a 15-minute period until the mean arterial pressure was 60 mm. Hg. It was maintained at this level for 1 hour, withdrawing more blood or infusing Ringer's lactate as needed to keep the desired stable pressure level. After the hour at a mean arterial pressure (MAP) of 60 mm. Hg, blood samples were drawn and then further blood was withdrawn over a 5-minute period until the MAP fell to 40 mm. Hg. It was maintained at this level for one additional hour by further blood withdrawal or infusion with Ringer's solution. All of the shed blood was withdrawn into 500 ml. capacity plastic bags containing 2,250 units of heparin in 30 ml. of normal saline.

Following the second hour of shock, Ringer's lactate was infused. The total volume given (including that administered during the shock period) equalled 2½ times the amount of blood shed, and the infusion was given over a 15-minute period. One hour after the onset of the resuscitation period blood samples were withdrawn, followed by reinfusion of the shed blood. One hour after reinfusion, the final blood samples were drawn.

A second group of six animals was used as a control group. They were positioned and sedated as the experimental group, but they were not bled and were not infused with Ringer's lactate solution.

Arterial blood gases and pH were measured with an Instrumentation Laboratories gas analyzer. Cardiac outputs were determined by the indocyanine-green dye-dilution method, using a Beckman Cardiodensitometer. Hematocrits were determined by the microcapillary method, white blood counts by Coulter counter, and platelet counts by direct count using phase contrast microscopy.

Coagulation Studies

Fibrinogen was measured by the method of Parfentjev,[18] Euglobulin lysis time (ELT) by the method of Celander and Guest,[22] and serial thrombin time as described by Reid.[22] Quick one-stage prothrombin time (PT) was determined using Simplastin (Warner-Chilcott), and activated partial thromboplastin time (PTT) using Platelin Plus (Warner-Chilcott). PT and PTT were also performed on plasma diluted 1:1 with saline, in an effort to detect increased levels of clotting factors. Factor VIII (antihemophilic globulin) was quantitated using a commercial factor VIII deficient plasma (Dade) in the activated PTT determination. Fibrin split products (FSP) were tested for using rabbit antiserum to human fibrinogen (Hyland) according to the method of Marder and Shulman.[14]

Statistical Methods

Two-way analysis of variance was used to test for homogeneity of each parameter measured. Sample time (corresponding to stage of shock or resuscitation) was compared with animal-to-animal variation and methodological error. Parameters showing a significantly increased variance by F-test at the $p < 0.05$ level were further examined by Duncan's multiple range test.[7] Measurements which did not show significant differences by analysis of variance, but suggested a definite trend, were also examined by Friedman's non-parametric rank-order method.[21] This was felt to indicate significant differences more reliably when variables were not normally distributed. Correlation coefficients cited are the Pearson product-moment correlation coefficient.

Results

The six control baboons showed no significant variation in any of the physiological or coagulation parameters measured throughout the 4 hours of study. All five baboons survived the shock and resuscitation period, and all were alive 7 days after the conclusion of the study. The average volumes of blood shed to produce shock, and of Ringer's lactate given to maintain pressure and for resuscitation, are shown in Table 1. Hemodynamic measurements, consisting of systolic, diastolic, and mean arterial pressures, central venous pressure, and cardiac output, are shown in Figure 1. Values significantly different from baseline, marked by an asterisk (*) in Figure 1, are the MAP and cardiac output (CO) after 1 hour of shock, and the MAP, CVP, and CO after 2 hours of shock. One hour after resuscitation with Ringer's lactate, both CVP and CO were within the normal range although the CO still showed a low trend ($0.10 > p > 0.05$ in Student t-test). After return of shed blood, MAP also returned to normal, while the CO still tended to be low ($0.10 > p > 0.05$).

The average hematocrit, platelet count, and white blood count are shown in Figure 2 in relation to the various experimental periods. The hematocrit fell during hemorrhage and continued to decline through the period of asanguineous resuscitation. The fall in platelets was in almost direct proportion to that in the hematocrit through the first 3 hours of the experiment. The correlation coefficient between mean hematocrit and mean platelet count for the first four observation times is $r = 0.99$ ($P = .01$). On the other hand, the white blood count rose during shock, from a baseline means of 12,600 to a mean of 35,300 after 2 hours of shock. It then fell during resuscitation.

Observed values of arterial P_{O_2}, P_{CO_2}, and pH are shown in Figure 3. Although there is a tendency for P_{O_2} to rise throughout the experiment, and especially after resuscitation, this trend is just short of significant at the $P = .05$ level by both analysis of variance and Friedman's rank-order test. Similarly, neither P_{CO_2} nor pH change significantly throughout the experimental period. The maximum change in mean pH was from 7.46 in the control period to 7.33 after the second hour of shock.

TABLE 1. *Volumes of Blood Shed during Shock, and of Fluids Given during Resuscitation*

Total blood shed during shock	950 ± 50 ml.
Ringer's lactate given during shock	375 ± 165 ml.
Ringer's lactate given during resuscitation	1945 ± 155 ml.
Total Ringer's lactate : shed blood vol.	= 2.5:1

The prothrombin time (PT) and partial thromboplastin time (PTT) are shown in Figure 4. Analyses of variance show the PT changes significantly ($p < 0.01$) and values significantly different from baseline

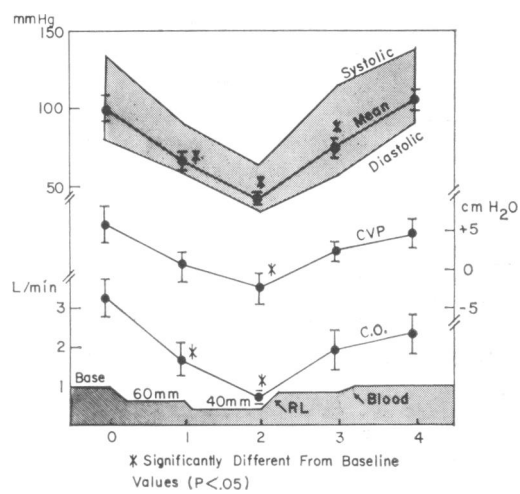

FIG. 1. Hemodynamic changes in hemorrhagic shock. Points plotted for mean arterial pressure, central venous pressure (CVP), and cardiac output (C.O.) are mean ± standard error. The mean systolic and diastolic pressures are indicated by shading. Values significantly different from baseline ($p < 0.05$) are indicated by an asterisk (*). The shading at the bottom of this and subsequent figures is a diagrammatic illustration of the course of the shock and resuscitation, showing points of resuscitation with Ringer's lactate (RL) and heparinized blood, and is not meant to indicate numerical values.

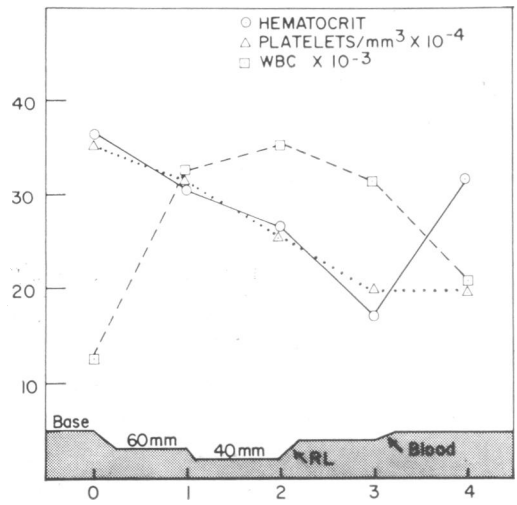

FIG. 2. Mean hematocrit, platelet count, and white blood count in hemorrhagic shock and resuscitation.

are indicated in Figure 4 by an asterisk (*). We note that significant changes are found only in the resuscitation period, and not during the shock period. The PTT varies the same as the PT, but observed differences are not significant by either analysis of variance or the Friedman rank-order test. The PT and PTT on diluted plasma

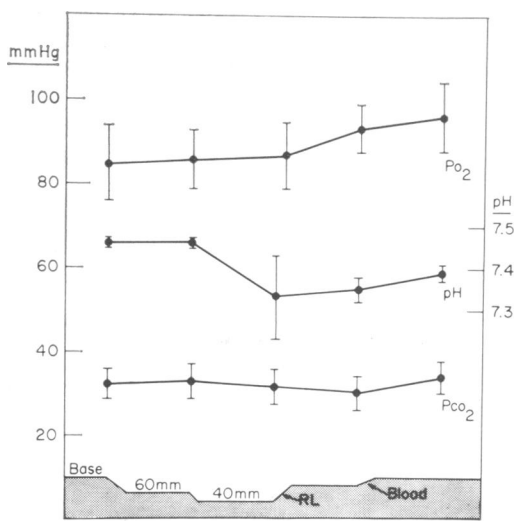

FIG. 3. Arterial blood gases (P_{O_2} and P_{CO_2}) and arterial pH in hemorrhagic shock and resuscitation. Mean values ± standard error are indicated.

were not shorter than the expected values, suggesting absence of hypercoagulability. Fibrinogen and factor VIII, both shown in Figure 5, change significantly in shock and resuscitation ($p < 0.01$). Euglobulin lysis time also tended to decrease. This tendency was significant by rank-order test ($p < 0.05$) but not by analysis of variance. At no sampling time were fibrin split products detected in any of the animals.

Discussion

The hemorrhagic shock model used in these studies has been previously shown to be uniformly fatal if untreated.[17] This particular shock schedule was chosen because earlier investigations had indicated that at least 80% of the animals could be expected to survive through the first 2 hours of shock (although none were long-term survivors), giving ample time for studies to be carried out (unpublished observations by Dr. G. S. Moss). The baboon seems more sensitive to hemorrhagic shock than the African green monkey (*Cercopithecus* sp.) which shows 60% long-term survival after 7 hours at 35 mm.[3] The degree of hypotension produced in these studies cannot be strictly compared with that required to produce "irreversible" shock in other species. Although dogs,[4, 6, 9] cats,[12] and sheep [4] survive through longer hypotensive periods, they all ultimately die in spite of treatment, while our animals survived when treated. Although Hardaway [6] has attributed this delayed death to reinfusion of shed blood, our animals all tolerated reinfusion when it was preceded by large volumes of crystalloid solution. Our shock protocol is similar to that shown by Selkurt [20] to produce renal function changes in the owl monkey *Aotes trivirgatus*.

On the average, our animals were bled to about 35% of their blood volume (of about 1500 cc.). This caused mean arterial pressure to fall to 42% of the baseline value and cardiac output to 20% of normal.

Despite the profoundly compromised circulatory status, arterial pH and blood gases showed only insignificant changes. This is in contrast to studies in the dog, in which metabolic acidosis is common,[1] and in man, in whom arterial hypoxemia is seen.[13]

We did not observe the changes in coagulation parameters reported by Broersma *et al.*,[1] Hardaway,[5] or McKay.[15] During the shock period, we were unable to detect significant *hyper* or *hypo*-coagulability by measurement of PT or PTT. The decreased levels of fibrinogen and Factor VIII could be attributed to either dilution or a consumption coagulopathy. To test for the former, these parameters were correlated with hematocrit, which we assumed to be a measure of intravascular dilution. Although this assumption is not fully justified because of possible red cell sequestration in certain capillary beds, Table 2 shows that good correlations were obtained. The fall in platelet count can also be related to dilution in this manner. We cannot eliminate fibrinolysis as a cause of the decreasing fibrinogen, however, in spite of the absence of fibrin split products in the blood, because the decreasing euglobulin lysis time indicates that there may be fibrinolysis of a degree too small to be detected by our methodology. Alternatively, the ELT may be measuring other non-specific proteases unrelated to the fibrinolytic system. The elevated values of PT and PTT measured after reinfusion may, of course, have been due to the heparin present in the stored blood. This could also have affected the Factor VIII assay.

Of considerable interest is the very elevated white blood cell count observed during hemorrhage. Although moderate leukocytosis is common after acute hemorrhage clinically, to our knowledge, leukocytosis of this degree has not been previously reported in this hemorrhagic shock model.

In conclusion, it would seem that hemorrhagic shock of sufficient severity to produce a state of significantly reduced cardiac output, and hence tissue hypo-perfusion, does not in itself produce significant abnormalities of the clotting system in this

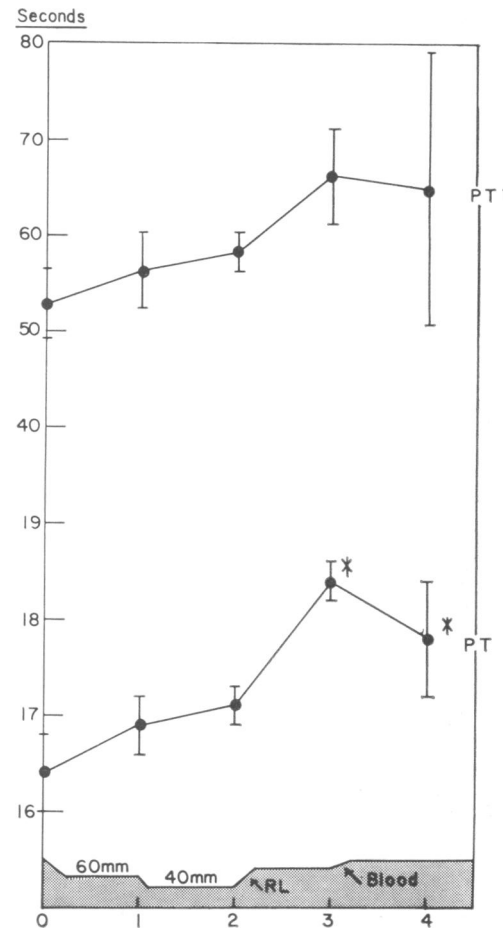

FIG. 4. Prothrombin time (PT) and partial thromboplastin time (PTT) in hemorrhagic shock (mean ± standard error). Asterisks (*) indicate values differing significantly from baseline ($p < 0.05$).

TABLE 2. *Correlation of Blood Components with Hematocrit*

Parameter	*r*	*P*
Platelets[1]	.99	.01
WBC	−.686	n.s.
Fibrinogen	.944	<.05
Factor VIII[1]	.929	<.10

[1] Correlation coefficients for platelets and Factor VIII determined only for time points up to reinfusion of blood.

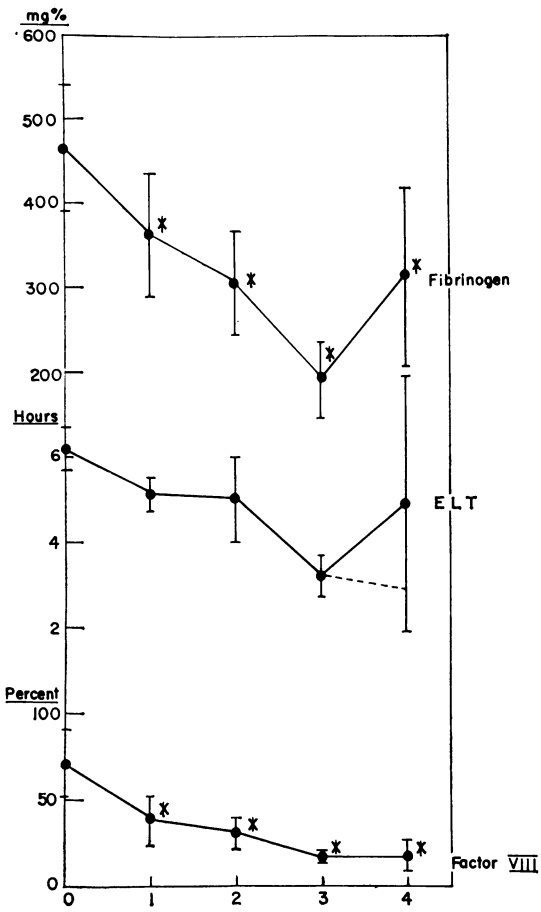

FIG. 5. Changes in fibrinogen, euglobulin lysis time (ELT), and Factor VIII in hemorrhagic shock and resuscitation. Mean values ± standard error are shown, and values significantly different from baseline ($p < 0.05$) are marked by an asterisk (*). The dotted line for the 4-hour ELT curve indicates the trend that would be shown if one abnormally long ELT is excluded from the mean.

primate model. Alterations in fibrinogen, Factor VIII, and platelet count can be adequately accounted for by dilution. Although the arterial pH decrease (0.13 unit) in shock was not significant in this study, a previous study in this laboratory using a larger number of animals (33) found this same change of 0.13 pH units to be statistically significant.[17] That study also noted a significant rise in serum lactate. Thus, we conclude that the shock model does produce inadequate tissue perfusion without concomitant coagulation changes.

A significant prolongation of PT does appear during the resuscitation phase, and this is only partly reversed by a transfusion of fresh, whole, heparinized blood. This is of interest because the resuscitation protocol is similar to that used clinically, where crystalloid solutions are given for initial resuscitation and followed by blood after cross-matching has been completed. Blood given in the clinical situation, however, is usually anticoagulated with ACD rather than heparin.

Summary

Five baboons were subjected to hemorrhagic shock. They were bled to a mean arterial pressure of 60 mm. for an hour followed by an hour at 40 mm. No blood was reinfused during the shock period, but Ringer's lactate solution was given as needed to maintain blood pressure. After 2 hours of shock, the animals were rapidly resuscitated with Ringer's solution and, an hour later, with their shed blood. During shock, cardiac output decreased to 20% of its baseline value, but arterial blood pH decreased only by 0.13 pH unit, and blood gases did not change significantly. Neither *hyper*coagulability nor *hypo*coagulability could be detected by prothrombin or partial thromboplastin times during shock, and decreases in fibrinogen, Factor VIII, and platelets could be explained by dilution. The prothrombin time became prolonged during resuscitation with crystalloid solution, and this was only partly reversed with administration of fresh heparinized blood. Although fibrin split products could not be detected during shock or resuscitation, the euglobulin lysis time decreased during shock. A large, unanticipated rise in the leukocyte count was observed during shock; this returned towards normal during resuscitation. It is concluded that the coagulation changes reported in dogs sub-

jected to hemorrhagic shock do not occur in this primate species. This difference may be attributed to either species difference or to our shock models not employing a bleeding reservoir.

Acknowledgments

The authors wish to express appreciation for the outstanding efforts of Navy hospital corpsmen HM1 J. Ewell, HM2 J. Hartman, HM2 W. Hawker, HM2 H. Shoulders, HM2 W. Spenner, HM2 J. Strohman, HN H. Fuller, HN A. Chester, and Mr. J. Magee.

References

1. Broersma, R. J., Bullemer, G. D., and Mammen, E. F.: Blood Coagulation Changes in Hemorrhagic Shock and Acidosis. Thromb. et Diath. Haemorrh. Suppl., **36**:171, 1969.
2. Buckberg, G. P., Lipman, C. A., Hahn, J. A., Smith, M. J. and Henessen, J. A.: Pulmonary Changes Following Hemorrhagic Shock and Resuscitation in Baboons. J. Thorac Cardiovasc. Surg., **59**:450, 1970.
3. Einheber, A. and Cerilli, G. J.: Hemorrhagic Shock in the Monkey. Am. J. Physiol., **206**: 1183, 1962.
4. Gillett, D. J. and Holmagyi, D. F. J.: Red Cell Circulation in Irreversible Shock in Sheep and Dogs. J. Surg. Res., **10**:443, 1970.
5. Hardaway, R. M. Clinical Management of Shock. Springfield: Charles C Thomas, 1968. pp. 96–106.
6. Hardaway, R. M.: The Fallacy of Hemorrhagic Shock Models in Dogs. Abstracts, VI Conference on Microcirculation, European Society for Microcirculation, 1970, p. 120.
7. Harter, H. L.: Critical Values for Duncan's New Multiple Range Test. Biometrics, **16**: 671, 1960.
8. Hawkey, C. and Symons, C.: A Comparative Study of Blood Coagulation in Human and Non-Human Primates. Proc. 2nd Int. Congr. Primat., **2**:87, 1969.
9. Hirshfeld, T. W., Jr. and Fell, C.: Changes in Regional Blood Content During Hemorrhagic Shock in Dogs. Am. J. Physiol., **216**:380, 1969.
10. Irani, B. B. and Cliffton, E. E.: Coagulation and Metabolic Changes During Hemorrhagic Shock. Angiology, **21**:161, 1970.
11. Lasch, H. G.: Coagulation Disturbances in Shock. Postgrad. Med. J., **45**:539, 1969.
12. Lefer, A. M. and Martin, J.: Origin of Myocardial Depressant Factor in Shock. Am. J. Physiol., **218**:1423, 1970.
13. Lowery, B. O., Cloutier, C. T. and Carey, L. C.: Blood Gas Determinations in the Severely Wounded in Hemorrhagic Shock. Arch. Surg., **99**:330, 1969.
14. Marder, V. J. and Shulman, N. R.: High Molecular Weight Derivatives of Human Fibrinogen Produced by Plasma. II. Mechanism of Their Anti-coagulant Activity. J. Biol. Chem., **244**:2120, 1969.
15. McKay, D. G. Disseminated Intravascular Coagulation. New York: Harper and Row, 1965, pp. 341–343.
16. Moss, G. S., Proctor, H. J., Herman, C. M., Homer, L. D., and Litt, B. D.: Hemorrhagic Shock in the Baboon. I. Circulatory and Metabolic Effects of Dilutional Therapy. J. Trauma, **8**:837, 1968.
17. Moss, G. S., Proctor, H. J., Herman, C. M., Homer, L. D. and Litt, B. D.: A Comparison of Asanguineous Fluids and Whole Blood in the Treatment of Hemorrhagic Shock. Surg. Gynecol. Obstet., **129**:1247, 1969.
18. Parfentjev, I. A., Johnson, M. L., and Cliffton, E. E.: The Determination of Plasma Fibrinogen by Turbidity with Ammonium Sulfate. Arch. Biochem. Biophys., **46**:470, 1953.
19. Proctor, H. J., Moss, G. S., Homer, L. D. and Litt, B. D.: Changes in Lung Compliance in Experimental Hemorrhagic Shock and Resuscitation. Ann. Surg., **169**:82, 1969.
20. Selkurt, E. E.: Primate Kidney Function in Hemorrhagic Shock. Am. J. Physiol., **217**: 955, 1961.
21. Sokal, R. R. and Rohlf, F. J.: Biometry. San Francisco: W. H. Freeman and Company, 1969. pp. 395–399.
22. Tocantins, L. M. and Kazal, L. A.: Blood Coagulation, Hemorrhage, and Thrombosis, New York: Grune and Stratton, 1964. pp. 219, 249, 261.
23. Waldhausen, J. A., Abel, F. L. and Pearce, W. L.: Portal-systemic Venous Shunts in Hemorrhagic Shock in the Dog and Monkey. Ann. Surg., **166**:183, 1967.