"Etudes des réponses à l'hypotension hémorragique chez les oiseaux domestiques (Gallus domesticus)."

David George Wyse
Département de Pharmacologie et Thérapeutique
Ph.D.

SOMMAIRE

Après hémorragie, on aperçoit, chez la volaille ainsi que chez la dinde, des réponses physiologiques étalées d'une façon bien caractéristique et qui diffèrent remarquablement de celles aperçues chez les mammifères (ainsi que chez les oiseaux plongeurs). Généralement, nous trouvons qu'il existe trois différences entre la réaction de ces oiseaux et celle des mammifères durant l'hypotension hémorragique expérimentale. Premièrement, même à la suite d'une petite hémorragie, la pression artérielle moyenne des deux espèces d'oiseaux tombe très rapidement; deuxièmement, chez les oiseaux, l'hémodilution est à la fois plus rapide et plus grande; enfin, les volailles ne semblent développer ni l'hémoconcentration, ni l'irréversibilitée, ces phénomènes étant caractéristiques des chocs hémorragiques chez les mammifères. Les résultats de recherches hémodynamiques obtenus ici ont conduit à la proposition que tous ces phénomènes sont reliés à un manque de vasoconstriction active. L'évidence des faits suggère que cette absence de vasoconstriction active pourrait être partiellement dûe à une tachyphylaxie chronique causée par un niveau élevé de catécholamines dans la circulation.

"Studies of the Response to Hemorrhagic Hypotension in the Domestic Fowl (*Gallus domesticus*)."

David George Wyse

Department of Pharmacology and Therapeutics

Ph.D.

ABSTRACT

Chickens (and turkeys) present a characteristic pattern of response following hemorrhage which is markedly different from that of mammals (and diving birds). Generally, there are three differences between the reaction of these birds and that of mammals during experimental hemorrhagic hypotension. First, the mean arterial pressure of both species of fowl falls very rapidly after even a small hemorrhage; second, hemodilution is both more rapid and larger in the birds; and finally, chickens do not appear to develop hemoconcentration and the "irreversibility" which is characteristic of hemorrhagic shock in mammals. Results of hemodynamic studies on chickens reported here led to the proposal that all these phenomena are related to a lack of active vasoconstriction. The evidence suggests that absence of active vasoconstriction may be partly due to chronic tachyphylaxis resulting from high levels of circulating catecholamines.

HEMORRHAGIC HYPOTENSION IN THE DOMESTIC FOWL.

STUDIES OF THE RESPONSE TO HEMORRHAGIC HYPOTENSION IN THE DOMESTIC FOWL (GALLUS DOMESTICUS).

by

DAVID GEORGE WYSE, B.S.P., M.S.P.

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Department of Pharmacology and Therapeutics,
McGill University,
Montreal.

July, 1969.

ACKNOWLEDGEMENTS

For the opportunities and guidance liberally supplied by Dr. Mark Nickerson, I am forever grateful. His ability to overflow with facility the endless characterizations required in a supervisor will never cease to amaze me.

I acknowledge with pleasure the technical assistance of Mr. Hillel Taub, who worked as if the project were his own.

To my friend, Dr. Harvey Rabin, who wrote the IBM 360/50 computer programs, I drink the ancient cybernetic toast, "May your bytes always have iterations!"

The histological examination of tissue was performed by Dr. J. Dušek and the catecholamine analysis was developed by Dr. J. Jezdinsky and I sincerely thank them both and lament with them for their homeland, Czechoslovakia.

I am extremely grateful to Mr. Royal Raymond, not only for the quality of his excellent photographic work, but also for his eagerness to please.

Kudos are also directed towards Miss Linda Burgess and Miss Tilly Macvean, who typed the final manuscript with minimal critical evaluation of my own dexterity at the keyboard. They spell well!

To all my associates in the Department of Pharmacology and Therapeutics, and especially to my fellow graduate students who often helped unknowingly, I owe a debt of gratitude.

And finally, to Bonnie and Naomi, whose contribution was unique and could have come only from them, thank you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

3. INTRODUCTION

A. The Problem of a Definition of Shock.

It is traditional to introduce any discussion of shock with a consideration of semantics. A scientific term is useful for communication only when it conveys a precise meaning. However, it is well-recognized by physiologists and clinicians that the term "shock" lacks any such precise meaning. It is generally conceded that a reliable definition awaits clearer insight into the pathophysiological processes involved. Nevertheless, it is a term which has wide use and therefore an examination of its connotations is essential.

"Shock", in its current medical usage, describes a number of apparently similar pathophysiological states which constitute a general response to injury by a wide variety of agents and conditions such as trauma, acute blood loss, myocardial insufficiency, bacterial infection, burns and others. The term has also been applied to many unrelated conditions, for example, radiation shock, electric shock, anaphylactic shock, shell shock, diabetic shock, and even cerebrovascular accident. The second group of conditions is not included in this discussion, although some of them possess certain characteristics which are common to "shock" as discussed below. In general, the classical picture of "shock" includes such symptoms as cold skin, pallor, perspiration, weak heart sounds, weak and rapid pulse, hypotension, decreased capillary refilling, decreased pulse pressure, apathy, clouded sensorium, muscular weakness, depressed reflexes, oliguria and hemoconcentration. The classical clinical condition of shock is recognizable in descriptions of two patients given by Cannon (1923). One patient had suffered a compound, comminuted fracture of a limb, the other a severe abdominal

injury. They were cold and perspiring, pale, somewhat cyanosed, listless and apathetic; their pulse rates were rapid and blood pressures low. Injury in these individuals had led to a state of severe physical and mental prostration associated with a marked circulatory disturbance which followed a subacute course to death. Postmortem examination revealed nothing to explain the prostration, except the initial injuries. Although the foregoing symptoms are classically associated with "shock", all or only some may be present or absent, and in fact, the diametrically opposite symptoms may be seen.

The generally nebulous clinical situations which are associated with the term "shock" lead to manifestation of one of the peculiarities of modern man, the compulsion to classify. In the opening paper of a conference on shock, Simeone (1961) concisely states the problem of clinical recognition of shock by noting that with the use of the four clinical criteria of blood pressure, pulse rate, temperature of the extremities, and color of the face, "shock" could be divided into six circulatory patterns. The circulatory patterns are normal, cold tachycardia, warm tachycardia, hypertension, vasovagal, cold hypotension and warm hypotension. Some classifications are even more complex and detailed, such as that of Rushmer et al. (1962), which, even though it is limited to "hypotensive shock", includes eleven major categories and many more sub-groups. This classification example also illustrates the problems of definition which arise from pre-occupation with only one symptom, in this case, hypotension.

Most modern concepts of "shock" do contain one element which seems to be a common denominator for all the clinical situations common-

ly termed "shock". This common theme, as expressed by Simeone (1961), is "The clinical picture which we call shock is a resultant in common of many etiologic situations which ultimately cause inadequate perfusion of tissues and organs". Thus the outstanding feature of the "shock" syndrome is a precarious state of the circulation resulting from an overall insufficiency of effective blood flow. A major initiating factor in most forms of clinical and experimental shock is a decreased effective circulating blood volume.

B. Experimental Shock Model.

The ultimate objective of any study of shock must be a better understanding of its course in man. On this basis, some workers, such as Grant (1961), revive the oft quoted phrase of Pope that, "...the proper study of mankind is man". However, clinical studies, or, experimental studies with human subjects, lead to two major difficulties. First, in the clinical shock situation it is very difficult, if not impossible, to obtain sufficiently uniform conditions for precise comparison of any one particular variable and, in any case, the prime consideration of the researcher must be the welfare of the patient. This requisite of patient welfare not only makes it difficult to control experiments adequately, but also makes the experimental protocol vulnerable to frequent interruptions. Second, in studies on human volunteers, there are certain ethical and practical limits to the degree of experimental trauma which may be inflicted and consequently this type of study should never produce shock. Thus, the saying of Pope is paraphrased by Miles (1961) to become, "The proper study of shock is shock." These

are the two sides of an argument which can be simply resolved by recognition that these statements are not anti-theses, but two sides of the same coin. To reach the objective as stated above, it is necessary to have both animal and human studies complement one another.

Many different methods have been developed for the production of shock in experimental animals (see Selkurt & Rothe, 1961 ; Fine, 1962). The techniques employed often resemble stresses that are known to produce shock in man. These include skeletal muscle trauma, removal of blood, burns, prolonged regional ischemia of limbs by application of tourniquets, exposure and manipulation of the intestines, occlusion of the abdominal aorta or superior mesenteric artery, embolization or ligation of the coronary circulation, bacterial infection or injection of toxins, and prolonged infusion of various drugs, such as noradrenaline and histamine.

Partly because of technical ease, the production of shock by prolonged hemorrhagic hypotension is the method of choice in a number of laboratories. It is highly reproducible, follows a well-known course, and allows control of arterial blood pressure, which facilitates the study of cardiovascular function. As previously mentioned, the common factor in most forms of clinical and experimental shock is a decreased effective circulating volume, and therefore controlled hemorrhage obviously goes directly to the core of the problem. Somewhere between the lethal bleeding volume which results in a rapid death due to cardiorespiratory failure, and a sublethal bleeding volume, lies a critical bleeding volume which leads more slowly to death through induction of hemorrhagic shock. Tolerance factors, rate of bleeding,

intensity and duration of hypotension are important variables. Another important variable, which is too often neglected, is species variation.

C. Species Variation.

As our knowledge of shock increases, it becomes apparent that variations among species, and even within species, make generalizations concerning the shock syndrome dangerous. It must be recognized, however, that species differences in themselves do not invalidate experimental evidence, if they are recognized. In fact, species differences may at times be very useful in emphasizing a particular aspect of the problem. In an excellent review of the more common species used, Zweifach (1961) notes that "...reference is made to from 15 to 20 different means of producing circulatory collapse in as many as 8 to 10 different species." The reader is referred to Zweifach for detailed comparison and multiple references and this discussion will be confined to hemorrhagic shock and the more common species studied. Unless otherwise noted, the following information is contained in the review of Zweifach.

The species most frequently used in the study of hemorrhagic shock is the dog. It is the animal most commonly available for surgical research and, in addition, its size is appropriate for complicated hemodynamic studies. If however, we keep in mind the objective of obtaining information useful in increasing understanding of clinical shock, it is not at all clear that the dog is the best animal for such studies. One characteristic of the dog which is not found in most other species is a relatively large spleen which contracts during hemorr-

hage. This contraction of the spleen results in an auto-transfusion of a relatively large volume with a high erythrocyte concentration (Haddy et al., 1965). Cardiovascular reflexes are highly developed in the dog which can lead to complicated and organized changes in regional and segmental vascular resistances (see Gesell & Moyle, 1922; Freeman et al., 1938; Eckstein et al., 1946; Selkurt et al., 1947; Haddy et al., 1965 and Hollenberg, 1965). Many of the important events during protracted shock are related to changes in the liver. In the dog, a sphincter-like collection of muscle is present in the region of the effluent hepatic vein which is highly sensitive to vasoactive materials. Closure of the sphincter elevates portal pressure and leads to a marked congestion of the liver during hemorrhage. The dog also develops severe edema, congestion, and blood sequestration in the intestine during shock (see Lillehei et al., 1964).

The cat is another species which is widely used in studies of hemorrhagic shock. Like the dog, the cat has a relatively large and active spleen and receives an autotransfusion from it during hemorrhage. The cat has the disadvantage over the dog of being somewhat smaller in size. Like the dog, cardiovascular reflexes, especially those of the carotid body, are highly developed. These reflexes also give the cat the ability to make complicated adjustments in its regional and segmental vascular resistances (see Mellander & Lewis, 1963; Lundgren et al., 1964 and Öberg, 1964).

Smaller animals such as rabbits and rats have an advantage when large numbers of simpler experiments are required, as in studies of survival rate. The rat has a sphincter-like structure in the hepatic

vein but it is not as well-developed as that in the dog. In relation to reflex control of the circulation, both the rabbit and rat (Whigham & Weil, 1966) exhibit atypical depressor reflexes during hemorrhage. These animal species are too small for detailed studies of regional and segmental resistance changes.

Although primates have the obvious advantage of being considerably closer to man on the evolutionary scale, their use requires some caution. Most of the monkeys used for research today have tuberculosis, a factor which may contribute to death in survival studies. Reflex cardiovascular control of the circulation is highly developed in primates and, although segmental changes have not yet been studied, regional changes in most instances are similar to those of dogs and cats (see Selkurt & Rothe, 1961; Einheber & Cerilli, 1962 and Abel et al., 1967). Unlike dogs, primates develop increased vascular resistance in the pulmonary circulation during hemorrhage (Abel et al., 1967). Because primates do not have the hepatic vein "sphincter" of the dog and rat, they therefore do not develop hepatic congestion, portal hypertension and intestinal lesions during hemorrhage. An additional factor which may also help prevent the development of portal hypertension is the presence of portal-systemic venous shunts (see Waldhausen et al., 1967). The monkey does not usually present renal cortical ischemic necrosis as a vasoconstrictive response to hemorrhage whereas other species, especially the rabbit, do. In addition, primates have complicated postural reflexes and specialized venomotor mechanisms relating to filling of the heart which may alter the response to hemorrhage.

Hemodilution during hemorrhage is a universal occurrence in every species of animal which has been studied. Starling (1896), in his classical paper, refers to the fact that after bleeding an animal, presumably a dog, "the remaining blood very shortly afterwards is found to be more dilute than before. It contains less haemoglobin and blood corpuscles and relatively more plasma. The plasma is also more dilute than before, showing that the increase in volume of blood chiefly consists of added water and salts, or at any rate, a fluid which contains less proteid than the plasma." Starling states that this phenomenon had been known since the middle of the 19th century. In the more recent literature, there are many reports of hemodilution following hemorrhage in both anesthetized (Adolph et al., 1933; Deavers et al., 1958; Baker & Remington, 1961; Green, 1961; Baker, 1963 and Haddy et al., 1965) and unanesthetized (Walcott, 1945; Chien, 1958 and Allen et al., 1959) dogs. Hemorrhage has also been found to result in hemodilution to a greater or lesser degree in man (Ebert et al., 1941; Noble & Gregersen, 1946; Lister et al., 1963; Skillman et al., 1967 and Taylor et al., 1967), other primates (Einheber & Cerilli, 1962), cats (Groom et al., 1965),sheep (Halmgyi & Gillett, 1967), rabbits (Courtice & Gunton, 1949 and Critz & Merrick, 1959), rats (Pareira et al., 1962), and birds (Djojosugito et al., 1968). Thus the phenomenon of hemodilution seems to be a universal result of hemorrhage although there may be quantitative differences between species. This hemodilution appears to be part of the compensatory response to hemorrhage which is common to all species.

4. CIRCULATORY COMPENSATION vs DECOMPENSATION DURING HEMORRHAGIC HYPOVOLEMIA: A REVIEW OF THE LITERATURE

A. Irreversibility.

In the section of his book dealing with the nature of shock, Cannon (1923) discusses in some detail what he calls the "sustaining factors" in shock. In summarizing, he states, "...a series of vicious circles may thus be started, which, if not interrupted, lead to a still further aggravation of already existent abnormal state, and which account for the progressive nature of fatal shock." This statement is an early example of recognition of a pernicious aspect of the shock state. A similar concept was expressed by Wiggers (1942) in a definition of shock, "...impairment of the circulation steadily progresses until it eventuates in a state of irreversible circulatory failure." C.J. Wiggers played a very prominent role in the extensive research on shock during and immediately after World War II, and his monograph in 1950 was the definitive work on the subject to that time. Wiggers originally put forth a restrictive definition of shock whose only criterion was irreversible circulatory failure. However, this definition presented practical difficulties and later (see Wiggers, 1950) he recognized this stage as only a part of the picture, to which he applied the term "irreversible shock" while including antecedent reversible states in the overall term "shock". Thus, by Wiggers' definition, the term "irreversible shock" means that stage of shock in which deterioration has progressed to the extent where circulatory failure cannot be reversed by restoration of the original blood volume. The term "normovolemic shock", is also derived from Wiggers and is not exactly synonymous with "irreversible shock". It refers to the state of circulatory inadequacy which exists following the return of

shed blood, when such volume restoration is withheld until after the point of irreversibility is reached (Wiggers, 1950). It is that point of irreversibility upon which much of the recent meaningful research into the problems of shock has been focused. Different hypotheses have been put forward to explain this phenomenon but its exact origin remains unclear. This is attributable in part to the fact that many factors probably contribute to the development of irreversibility. These factors may be connected both in parallel and in series and the tendency to emphasize the primacy of one particular factor has been too difficult to resist for many researchers.

In the method of hemorrhage used in the present study, irreversibility is observed to develop in the following manner. If animals are bled progressively until the arterial pressure falls to some predetermined level where it is maintained by successive bleedings, eventually it becomes necessary to reinfuse blood in order to prevent the animal's pressure from falling further. Reinfusion of all the shed blood after this point is reached often returns the pressure to control levels but it subsequently declines progressively, and the animal usually dies even if additional fluids are given (Green, 1961; Selkurt & Rothe, 1961 and Fine, 1962). The hypotheses put forward to explain this progressive deterioration in hemorrhagic shock fall into two broad categories based upon attempts to localize the failure either in the peripheral circulation or the myocardium.

I. Myocardial Failure. Although a decreased cardiac output is well-recognized as a consequence of hemorrhagic hypovolemia, this is

usually accounted for on the basis of a decrease in venous return to the heart. Earlier reviews of the field of shock (see Cannon, 1923) dismissed cardiac failure as an "initiating" factor in the development of irreversibility. However an actual decrease in cardiac contractility suggestive of cardiac failure has been reported in the later stages of oligemic shock and following retransfusion (Wiggers & Werle, 1942 and Schmidt & Schmier, 1965). It has been assumed that decreased myocardial contractility is more a consequence of a prolonged inadequate supply of blood than the prime factor initiating irreversibility. This view is supported by experiments of Sarnoff et al.(1954) which show that the rise in left ventricular filling pressure that appears after an episode of hypotension could be reversed by increasing the left main coronary flow with a pump. Wiggers (1950) stated that deterioration of myocardial expulsive power contributed to the progressive circulatory failure and its redevelopment following transfusions during the irreversible state. However, a major contribution of decreased contractility to death is usually considered to occur only with extreme degrees of oligemic hypotension or when moderate hypotension is coupled with prior cardiac damage (Rothe & Selkurt, 1964). In fact, in the early stages of hemorrhage, there is probably an increase in contractility presumably due to increased sympathetic activity (Guyton et al., 1951). Nevertheless, at recent conferences on shock the primacy of myocardial deterioration in the development of irreversibility has been revived, chiefly by Guyton (see Guyton & Crowell, 1961 and Howard, 1962).

Guyton (1961) began his argument with the observation that follow-

ing a period of hemorrhagic hypotension, and during the period when it is necessary to reinfuse blood to maintain the arterial pressure, the left atrial pressure rose quite markedly to about 30 mm Hg although systemic arterial pressure remained low. In a similar experiment, in which following hemorrhagic hypotension and development of irreversibility, he infused large volumes of blood from donor dogs to maintain the systemic arterial pressure at 100 mm Hg, the right atrial, pulmonary arterial, and left atrial pressure rose progressively until, at death, they were equal to the systemic arterial pressure. This evidence was taken by Guyton to indicate that death was caused by cardiac deterioration and not by any factor in the peripheral circulation preventing return of blood to the heart. Results of other workers do not support this position. These type of experiments were also reported by Gomez & Hamilton (1964), but with similar results only when the heart was presented with a severe load and they concluded, "Tests made at intervals after the hypotension showed equivocal evidence of cardiac deterioration." Moreover, these authors noted that as shock progressed, extremely large volumes of donor dog blood were required to maintain venous return and central venous pressure, suggesting the presence of a prepotent peripheral component. Others (Henry et al., 1967) reported similar elevation of right atrial and pulmonary arterial pressures but not left atrial pressure following reinfusion, and thus assigned importance to intrinsic failure in the pulmonary system rather than the left heart. The experiments of Sarnoff et al. (1954) which indicated that irreversibility develops in the absence of decreased cardiac contractility have already been mentioned. It has also been demonstrat-

ed that shock differing in no recognizable way from that induced by hemorrhagic hypotension can be produced in the dog in which thoracic aortic blood pressure, and thus presumably coronary blood flow, is maintained during hypotension by a balloon in the thoracic aorta (Smith & Grace, 1957). Weidner et al. (1961) studied the force of myocardial contraction by means of a Walton-Brodie strain gauge in dogs subjected to hemorrhagic hypotension and found that force of contraction fell with hemorrhage, but promptly returned to normal with the reinfusion of shed blood, even late in the decompensatory stage of shock. Thereafter, normal donor dog blood transfused in volumes sufficient to maintain a mean arterial pressure of 80 mm Hg maintained cardiac performance. Bloch et al. (1966) reviewing the various theories of the production of shock recognized a possible role for progressive deterioration of the heart sustaining hemorrhagic shock and irreversibility, but did not assign it the role of the primary initiating factor in irreversibility and this appears to be the current status of myocardial failure in the development of irreversibility.

Guyton (1961) emphasized the role of oxygen debt in reduced cardiac contractility, and, although total oxygen debt was correlated with the survival of the animal and, the time to reach a certain critical oxygen debt and the onset of irreversibility corresponded well (Jones et al., 1968), these observations may merely be related to the general inadequacy of blood flow in shock. It has been pointed out (Nickerson, 1962) that in the measurement of total oxygen debt there is no real distinction between myocardial and peripheral contributions and recent work (Lundsgaard-Hansen et al., 1968) demonstrated a lack of oxygen

debt in the heart during hemorrhagic hypotension. It has also been pointed out (Chien, 1967) that even in the face of adequate filling pressure there are other possible factors, such as, decreases in cardiac sympathetic impulses and myocardial catecholamine content, and a reduction of myocardial reactivity to catecholamine which could operate to reduce myocardial contractility. Of course in any experiment there is an inherent danger of aggravation of myocardial depression by such things as thoracotomy and deep anesthesia.

The suggestion was made by some authors that a cardiotoxic material carried by the blood stream from hypotensive and ischemic regions elsewhere in the body caused functional cardiac damage (Gomez & Hamilton, 1964). The source of this toxic factor was only speculated on, but reference was made to the endotoxin hypothesis of Fine (see Fine, 1962 and Gilbert, 1962) which will be discussed later. Up to this point all the experiments described have been performed in dogs but Lefer et al. (1966), working with cats, showed that papillary muscle isolated in the late stages of postoligemic shock, exhibited depressed excitability and contractility. And further, that papillary muscles isolated from cats in the early stages of postoligemic shock, or from cats subjected to anoxia did not show this depression. Brand & Lefer (1966) have also reported that plasma from cats in the early stage of irreversible postoligemic shock depressed contractility of fresh isolated cat papillary muscle although this effect was reversed upon washing. However, plasma from cats late in postoligemic shock depressed contracility even more and this depression was only partly reversed by washing. Attempts to characterize this depressant factor (Lefer et al.,

1967) yielded evidence mostly of a negative nature. So-called myocardial depressant factor (MDF) was dialyzable, aqueous soluble and heat stable. It was not hemoglobin, hemochromogen, pyruvate or lactate and myocardial depression could not be explained by calcium loss. In addition, it did not appear to be a catecholamine, as was suggested in the case of dogs (Lundsgaard-Hansen et al., 1968), for the depressant effect of cat plasma obtained during postoligemic shock was reversed by noradrenaline (Brand & Lefer, 1967). Thus, the exact cause of myocardial depression late in oligemic shock is still unexplained. That this "toxic factor" was not an initial cause of irreverisibility is shown by the time at which it appears. MDF does not appear in the plasma of cats until late following reinfusion when the irreversible state has already existed for some time.

II. Peripheral Circulatory Failure. Many workers (Crile, 1915; Fine, 1954; Lansing & Stevenson, 1957; Bohr & Goulet, 1962; Rushmer et al., 1962 and Lillehei et al., 1964), have suggested that irreversibility is due to "peripheral circulatory failure." This term has been widely used presumably because it is euphonious, descriptive and directs attention away from the heart and to the peripheral circulation where most investigators in this field over the last 5 decades have believed the primary fault to lie. The exact nature of this "peripheral circulatory failure" is elusive. After the arterial pressure is maintained at a set hypotensive level for a variable period by hemorrhage (standard hemorrhagic shock model, see below), it becomes necessary to reinfuse blood in order to keep the pressure from falling. After rein-

fusion is required, a rapidly increasing percentage of the animals die in spite of reinfusion of all the shed blood, i.e., the shock has become irreversible. In recent reviews (see references above), a "giving out" of some component of the circulation has been cited as the most likely explanation for the uptake of blood, which was required to fill a progressively expanding space. The questions regarding what space expands, and especially what causes it to expand remain unanswered, and many studies of the function of arterioles (Green, 1961) and veins (Mellander & Lewis, 1963; Lundgren et al., 1964 and Hollenberg, 1965) have failed to demonstrate a loss of functional integrity of these components of the circulation in shock, except as a terminal event. The role of "toxins" and disseminated intravascular coagulation are also properly discussed under "peripheral circulatory failure" because their origin and deleterious effects are considered to be peripheral rather than myocardial.

(a) Toxic Factors. The release of toxic substances into the peripheral circulation during shock induced by infection is well recognized as a factor leading to the development of irreversibility. However in the case of uncomplicated blood loss, the role of toxins is not nearly so clear. The term "toxin" can include substances produced by body cells and not properly eliminated or destroyed, as well as substances formed by microorganisms, parasites and so forth. Products of metabolism which are either formed in excessive amounts, or are improperly neutralized or excreted, may act adversely by creating unfavorable conditions such as acidosis, azotemia, and hyperkalemia. For the purpose of this discussion, the term toxin will be limited to a sub-

stance which is not normally found in the circulation, thereby excluding those present merely in increased amounts. Wiggers (1950), in his review, noted that while it is easy to postulate the existence of such toxic agents, it is far more difficult to demonstrate their presence.

The role of toxins in shock has been the subject of discussion in many papers since Aub (1944) suggested a role for toxins produced by various species of Clostridia which are an unavoidable contaminant even during surgery on dogs under conditions which are usually aseptic. Others (Lillehei, 1957) suggested that the gut was a source of a toxic substance, such as an abnormal heme pigment. However, the major toxin hypothesis to date, and the only one to be discussed in relation to hemorrhagic shock, is that of Fine (Fine, 1961 and Gilbert, 1962) which states that bacteria which constitute part of the normal intestinal flora (especially E. coli) produce an endotoxin which is released by the normal lysis of such bacteria and is continuously absorbed from the gut but is subsequently detoxified in the reticuloendothelial system. During hemorrhagic shock (and other forms of shock) the detoxifying mechanism is impaired and a buildup of endotoxin with its concommitant deleterious effects results. Recently (Fine et al., 1968), the hypothesis has been slightly modified as follows: "The refractory state of shock is the result of ischemic damage to the reticuloendothelial system in liver and spleen, and this injury allows a neurotoxin of bacterial origin to produce fatal collapse of the peripheral circulation." This change shifts emphasis from the production of tissue injury to the vascular muscle, through a direct action on that tissue, to the production of such injury via a toxic effect on the sympathetic

nerves. The exact nature of such a toxic effect and how it leads to the observed sequence of events in hemorrhagic shock has never been explained.

Normal presence of endotoxin in portal vein but not systemic blood, and the appearance of endotoxin in the systemic circulation following hemorrhage, has been suggested by the work of Fine and his co-workers but other research does not support this position. Einheber (1961) and Kovách (1961) were unable to induce shock passively by the use of blood from previously irreversibly shocked animals and Sanford and Noyes (1958) using ^{51}Cr-labelled endotoxin, which retained pyrogenicity, lethality, and other typical properties, could not demonstrate absorption from the gastrointestinal tract in either normal dogs or dogs in shock. McNulty & Linares (1960) reported that in germ-free rats as well as rats pretreated with chlortetracycline, lethal hemorrhagic shock had the same course as in normal rats, and, contrary to the claim of Fine, irreversibility could not be consistently prevented in dogs by perfusing the liver (Lillehei, 1957). Mellander & Lewis (1963) have also shown that even if a major portion of the intestine is removed conditions leading to irreversible shock still develop in cats. Other empirical observations, such as the remarkable constancy of reticuloendothelial system activity per gram of tissue in all species coupled with the wide variation in species reactivity to endotoxin discredit this hypothesis (Halpern, 1962).

(b) Disseminated Intravascular Coagulation. It is well-recognized that if flow is sufficiently close to zero, blood will coagulate

within the vessels. An increased tendency for blood to coagulate following hemorrhage was first reported by William Hewson (circa 1770, see McKay, 1965, p341) who noted that in man and animals who were subjected to venesection "the blood which issued last coagulated first." Recently, however, an hypothesis which implicated disseminated intravascular coagulation as a primary cause of irreversibility in shock has been proposed (see monographs of McKay, 1965 and Hardaway, 1966). "Disseminated intravascular coagulation is defined as acute transient coagulation occurring in the flowing blood throughout the vascular tree and which may obstruct the microcirculation." (See Matsumoto et al., 1968.)

Disseminated intravascular coagulation can follow the entrance into the bloodstream of thrombin or products of tissue trauma and can result from intravascular hemolysis and from bacteremia or circulating bacterial endotoxin. It is also known that resultant arterial and venous thromboembolism may impair the function of vital organs, and that clinical shock may result from these processes (see McKay, 1965 and Hardaway, 1966). However, the evidence that disseminated intravascular coagulation may result from shock per se, specifically from peripheral vascular collapse secondary to major hemorrhage is uncertain. The liberal use of anticoagulants in most studies of hemorrhagic shock negates a large proportion of research as evidence for or against this hypothesis.

Crowell & Read (1955) bled dogs into a citrated reservoir to produce irreversible shock and then reinfused the blood after carefully filtering it through gauze to remove any emboli formed while blood was

in the reservoir. They reported high mortaility (6/7), development of lowered clotting time (hypercoagulability) of blood samples, and many postmortem emboli in the lungs. Heparin pretreatment prevented hypercoagulability but this was a consistent finding only with massive doses of heparin (10 mg/kg). Although the syringes and tubes used in this study were siliconized, the authors neglect to say whether the glass reservoir was siliconized, and their work has been criticized (Salzman, 1968) on the basis of the known effects of glass and gauze on blood coagulability. It seems very likely that postmortem emboli might be explained on the basis of reinfusion of blood which had been filtered through gauze.

Similar observations were made by Hardaway et al. (1962) who bled dogs to an arterial pressure of 50 mm Hg by removing blood through a cation-exchange resin and storing the decalcified blood in plastic bags. After a brief period of observation, the shed blood containing no anticoagulant was reinfused to maintain the blood pressure at 100 mm Hg, where it was held for 2 hours by small withdrawals or reinfusions. The balance of the volume bled was then returned to the animal. They reported remarkably improved survival and absence of pathological findings with only moderate doses of heparin (total dose 3 mg/kg). Hardaway et al. (1963 and 1963a) also demonstrated the beneficial effects of fibrinolysin in reducing the incidence of irreversible hemorrhagic shock which followed bleeding through an ion exchange column. These experiments have also been criticized on technical grounds with emphasis on the use of an exchange column (Salzman, 1968) which leads to hemolysis, platelet damage and deterioration of plasma coagulation fac-

tors. Characteristically, hypercoagulability occurs early near the beginning of hemorrhage but is subsequently followed by hypocoagulability. Hypercoagulability can be duplicated simply by restraining animals without bleeding (Hardaway, 1963a) and it has been shown that hypercoagulability also occurs in dogs following sympathetic stimulation or catecholamine injection (Crowell & Read, 1955). Reflex activation of the sympathetic nervous system (see below) during bleeding would seem to be a plausible explanation for the early transient phase of hypercoagulability. Interestingly, treatment with the α-adrenergic blocking agent phenoxybenzamine which improved blood flow and which has no known direct effects on clotting-time has been shown to prevent intravascular coagulation (see Matsumoto et al., 1968). Attar et al. (1966 and 1966a) have also described a trend toward transient shortening of clotting time during the early phases of shock in human patients. Evidence for the occurrence of hypercoagulability is not unanimous and others (Ehrlich et al., 1964) have shown that bleeding animals that have had no anticoagulant pretreatment without returning any of the shed blood leads to no overall hypercoagulability.

It is valid to challenge the occurrence of hypercoagulability following retransfusion simply because of the handling of the shed blood in these experiments and the early hypercoagulability cannot lead to irreversibility because it has disappeared before it becomes necessary to reinfuse blood. Nevertheless, Hardaway has proposed that the evidence cited above indicates disseminated intravascular coagulation, which causes temporary interruption of blood flow and focal tissue necrosis, is the primary cause of irreversible shock in hypovolemia (Hard-

away, 1963b).

In summary, there is evidence for the occurrence of a transient period of hypercoagulability early during hemorrhagic hypotension. That hypercoagulability at a later stage leads to irreversibility in hemorrhagic shock has not been demonstrated. Further research is necessary, especially with regard to the manner of handling withdrawn blood. That intravascular coagulation occurred under the experimental conditions outlined above is not disputed and that this coagulation once it occurs is "irreversible" is also plausible.

(c) Peripheral Resistance. In hemodynamic parlance the adjective "peripheral" refers to distal branches of the systemic arterial tree regardless of anatomical location. Resistance (impedance) to flow includes all factors that impede the flow of blood from the arterial tree and is defined as the ratio between the pressure head, ΔP, between any two sequential points in the vasculature and the resultant flow.

$$R = \frac{\Delta P}{F}$$

The terms regional, or territorial resistance appropriately express the resistance offered to flow through the vascular bed of an organ or territory, such as the kidney, intestine, limbs, or any area supplied by a common artery. The systemic circulation can be visualized as several of these circuits parallel-coupled, each of which contains a number of series-coupled sections of different design and function, e.g.,

arteries, arterioles, true capillaries, small veins, and large veins. On this basis, regional resistances can be further subdivided into segmental resistances; practically, this is done by recording pressures at various points within the particular vascular bed and the regional flow.

Since a decrease in the effective circulating blood volume is generally considered to be the major factor initiating the development of shock, it is not surprising that blood flow to most body tissues is diminished. However, the fractional distribution of the cardiac output among the various organs in shock differs markedly from that observed in the normal state and the reduction of flow in certain organs is substantially greater than can be explained simply on the basis of reduction in pressure. This altered partition of blood flow is due primarily to uneven changes in regional resistance, and is responsible for many of the prominent clinical signs of shock.

Changing vascular tone in the various circuits can suitably redistribute the prevailing cardiac output according to "vital" priorities and this peripheral circulatory control implies adjustments of smooth muscle tone in vascular beds by influences originating from sites within and outside the tissue itself. The remote control systems encompass mechanisms capable of inducing adjustments of peripheral vascular functions which are proportional to the prevailing environmental conditions. During hemorrhage, the ability of these mechanisms to maintain homeostasis is severely tested.

The prime mechanism in most mammals for adjusting vascular tone is the system of vasoconstrictor fibers of the sympathetic nervous sy-

stem. These fibers are not evenly distributed, for example, the mesenteric vessels have a rich supply whereas vasoconstrictor fibers are rather sparse in the cerebral vessels. Therefore uniform activation of the sympathetic nervous system will not exert quantitatively similar vasoconstriction in all vascular beds. For example, stimulation of sympathetic nervous system vasoconstrictor nerves results in a relativsely greater vasoconstriction in mesenteric than in skeletal muscle vessels, although vasoconstriction is more effectively sustained in the latter (see review of Mellander & Johansson, 1968). The distribution of vasoconstrictor fibers within a particular vascular region is also not uniform. The precapillary segment of a particular bed is usually more richly innervated than the postcapillary one. The distribution of constrictor fibers can thus not only affect the overall degree of vasoconstriction in particular vascular beds but also the relative tone of the pre- and postcapillary sections. Sympathetic vasoconstriction is usually more pronounced in the pre- than postcapillary section and may be absent in some larger veins (see Webb-Peploe & Shepherd, 1968).

The degree of sympathetic vasoconstriction throughout the body at any time is determined by the activity of the medullary "vasomotor center". Activity of this vasomotor center is increased by sensory input from "chemoreceptors" in the area of the aortic arch and carotid bifurcation and decreased by sensory input from "baroreceptors" located in the same anatomical regions as the chemoreceptors. Firing of the vasomotor center is also decreased by sensory input from "volume receptors" in the large veins and the heart. These "receptors" and the afferent fibers to the vasomotor center ensure that a fall in blood

pressure or volume reduction will result in increased sympathetic vasoconstriction mediated reflexly through the vasomotor center. Stressful conditions sufficient to result in hypercapnia, acidosis or hypoxia will reinforce vasomotor activity and sympathetic vasoconstriction. The work of Öberg (1964) has demonstrated the reflex nature of the fine adjustments which may be made in pre- or postcapillary segmental tone which aid in fluid shifts during hemorrhagic hypotension.

One of the early hypotheses for the development of irreversible shock was the so-called "vasomotor paralysis" hypothesis. It stated that following vasomotor paralysis a relatively large quantity of blood becomes stagnant in relaxed vessels, chiefly those of the splanchnic area. However, as Cannon (1923) noted, distention of abdominal veins is not present in men in shock while evidence of vasoconstriction is apparent. That veins are not distended is also apparent from the technical difficulty of venipuncture during shock states. An extension of the "vasomotor paralysis" hypothesis was the postulation of Crile (1915) that progressive cardiovascular decompensation in shock was secondary to "exhaustion" of the vasomotor center, resulting in the loss of arteriolar constriction. Cannon (1923) pointed out, however, that the vasomotor center is still responsive to stimulation late in shock and that denervation of vascular tissue during shock still results in an increased flow of blood. Wiggers (1950) noted that during the critical stage leading to the development of irreversibility, the limbs and kidney have high resistances, and mesenteric resistance undergoes a secondary increase after some relaxation of a marked early mesenteric vasoconstriction. Only coronary resistance decreased. Similar find-

ings were also reported by Gregg (1962).

The concept that shock can be initiated and is maintained by virtue of intense vasoconstriction rather than vasodilation received impetus from the experiments of Erlanger & Gasser (1919) who demonstrated that reduction in blood volume, hemoconcentration and circulatory failure can be produced by prolonged administration of adrenaline. Gesell (1918 and 1922) showed that during hemorrhage, blood flow through salivary glands and muscles decreased significantly before any fall of arterial pressure could be detected. In spite of these early observations the vasoconstriction hypothesis was largely abondoned because, as Wiggers (1942) noted, intense vasoconstriction was difficult to reconcile with capillary congestion, and evidence that a continuous infusion of adrenaline could produce shock was not unanimous. However, detailed knowledge of the possible intricate adjustments in vascular resistance was rudimentary at this time. The realization that there were regional differences which were not reflected in total peripheral resistance was only in its early stages and the concept of segmental resistance, and especially the importance of venous resistance in adjustments of capillary hydrostatic pressure had yet to be pointed out.

Recognition that continued severe vasoconstriction incident to hemorrhage may itself be deleterious, reappeared in the literature to stay in 1948, when H.C. Wiggers and associates demonstrated that pretreatment with Dibenamine, and α-adrenergic blocking agent that inhibits sympathetic vasoconstriction, delayed the appearance of irreversibility and markedly decreased mortality from a standardized hemorrhage.

The idea that relaxation of the vasculature can lead to the de-

velopment of irreversibility, however, has not disappeared. Lansing & Stevenson (1957) stated that, "...compensatory vasoconstriction eventually fails and the blood in the reservoir spontaneously reinfuses into the animal." Bohr & Goulet (1962) noted that serial determination of the constrictor potency of plasma from hemorrhaged dogs decreased with time and suggested there may be cardiovascular collapse due to progressive vasodilatation. Vasodilatation leading to irreversibility has also been suggested by Rushmer et al. (1962), and Lillehei et al. (1964) stated that, "...uptake of blood from the reservoir is a sign of failure of the dog to maintain vasoconstriction in the visceral and peripheral vascular beds. Consequently, the size of the vascular bed begins to expand, and blood must be taken up from the reservoir to fill this increasing space."

The most recent and most sophisticated argument suggesting vasodilatation plays a role in the development of irreversibility has come from the Swedish workers (Mellander, 1960; Folkow, 1962; Mellander & Lewis, 1963; Lundgren et al., 1964 and Öberg, 1964) who were the first to emphasize the importance of segmental resistances in fluid shifts. Their hypothesis suggests that vasodilatation and vasoconstriction together lead to reversal from continued bleeding to reinfusion of blood to maintain a constant hypotension. In 1962, Folkow reported that during a period of ischemia in the hindquarters, produced by occlusion of the abdominal aorta, the maximal response to vasoconstrictor fiber stimulation declined much more rapidly in the resistance than in the capacitance vessels. A similar decline in the ability to elicit vasoconstriction in resistance vessels following a period of oligemic hypoten-

sion was reported by Mellander & Lewis (1963) and the following hypothesis was put forward. Late in the course of the hypotensive period, the precapillary resistance response diminishes and nerve stimulation causes a relatively more pronounced development of postcapillary (capacitance) resistance, which increases capillary hydrostatic pressure and net loss of fluid from the circulation; this progressive loss of intravascular fluid leads to irreversibility. This hypothesis has received support from the work of Lundgren et al. (1964), which showed that ultrafiltration of fluid does occur in the later stages of hemorrhagic hypotension. These measurements were carried out at constant inflow and outflow pressures and a change in pre- to postcapillary resistance ratio was inferred from the fluid shifts.

The concept that the pathogenesis of shock involves too much rather than too little vasoconstriction, has become increasingly apparent in the literature (see reviews of Nickerson, 1964; Bloch et al., 1966 and Chien, 1967). Gregg (1962) studied the effect of hemorrhagic shock on regional blood flow using an electromagnetic flowmeter and concluded, "...the pathogenesis of shock in unanesthetized dogs cannot be attributed to 'peripheral vascular collapse' at the arteriolar site. The hemodynamic distrubance is post-arteriolar in location." Hollenberg (1965) reported that precapillary resistances, judged by constant inflow and arterial pressure, in the gastrointestinal tract, skeletal muscle and skin did not decrease during decompensation whereas postcapillary resistance, judged by small vein wedge pressure, increased progressively in both the intestine and skeletal muscle. These observations led to suggestion of a mechanism leading to irreversibility in

shock which is similar in principle to that proposed by the Swedish workers, i.e., that increased capillary hydrostatic pressure results in a loss of vascular fluid by ultrafiltration, but this hypothesis proposes an increased postcapillary resistance and unaltered precapillary vasoconstriction and, although the net effect is the same, the resistance change emphasized is postcapillary rather than precapillary and it is an increase rather than a decrease. The hypothesis that vasoconstriction results in decreased flow and eventually ultrafiltration and even sequestration of vascular fluid and thus leads to irreversibility is attractive. Progressively decreased flow would result not only from vasoconstriction itself, but also from further reduction of effective circulating volume. It should be noted that in contrast to an increase in postcapillary resistance, a progressive decrease in precapillary resistance would not provide a common mechanism for the sequential processes of ultrafiltration and sequestration. If different mechanisms were responsible for net ultrafiltration (early decompensation) and sequestration (late decompensation), one might expect that the change from one process to the other would produce some discontinuity in the uptake curve during sustained hypotension and such a change in rate has not been reported. Finally, it is interesting that in all the other hypothesis discussed, i.e., myocardial failure, toxic factor, and disseminated intravascular coagulation, decreased flow preceded the factor central to the hypothesis.

The main point of difference between the hypothesis of the Swedish workers and that of Hollenberg is whether or not precapillary resistance is diminished during the stages of hemorrhagic shock leading

to irreversibility, when fluid is lost from the vascular compartment. The evidence that precapillary resistance declines has been provided from observations in cats which indicate that first, sympathetic vasoconstrictor fiber rates of firing (indirect evidence) do not increase during this stage and second, sympathetic stimulation does not decrease flow to the same extent as it did earlier, whereas, vasoconstriction in the capacitance vessels is undiminished (see Mellander & Lewis, 1963; Lundgren et al., 1964 and Öberg, 1964). Hollenberg (1965) monitored flow in the terminal abdominal aorta and superior mesenteric artery of dogs with an electromagnetic flowmeter and found that during the constant pressure period leading up to irreversibility, there was no tendency for flow to increase in either of these arteries. The work of others (see Gregg, 1962 and Fell, 1966), also in dogs and also using electromagnetic flowmeters, supports the observations of Hollenberg. Both Gregg and Fell noted a slight decrease of hindlimb resistance after the end of the initial bleedout period following which both hindlimb and mesenteric resistance remained elevated and were undiminished until reinfusion when they fell, only to rise progressively until death. Identical results to those of Hollenberg, Gregg and Fell were also obtained in perfused dog gracilis muscle with its nervous supply to the rest of the animal intact (see Rothe et al., 1963). A rapid decrease in precapillary resistance comparable to that reported by the Swedish workers in cats has also not been found in primates (Selkurt & Rothe, 1961). In an extensive review in 1961, Green concluded, "...it has not been demonstrated that disturbances in the behavior of the resistance vessels are responsible for the decompensatory phenomenon in irrev-

ersible shock."

It is important to emphasize that the precapillary resistance under consideration is that during the period leading up to the irreversible stage. If an animal is bled a fixed small percentage of its initial blood volume followed by no further hemorrhage, compensation by the animal will gradually reduce reflex sympathetic vasoconstriction, and, thus, resistance. Thus, the "waning" of vascular resistance reported by many authors is not necessarily because the efferent limb of reflex vasoconstriction becomes ineffective, but because vasoconstriction is "turned off" by a gradual return of homeostasis (see review of Haddy et al., 1968).

The difference between the response of dogs and other species and that of cats might be resolved by a species difference. If the dog is able to increase its rate of sympathetic discharge during the period leading to irreversibility to maintain the elevated precapillary resistance, the increased sympathetic firing could be manifest in an increased postcapillary resistance. Mellander & Lewis (1963) were able to show that increasing the rate of sympathetic stimulation in cats resulted in an increase in precapillary resistance and a further increase in postcapillary resistance.

B. Hemodilution vs Hemoconcentration.

Hemodilution is defined as a decrease in large vessel hematocrit and plasma protein concentration. Hemoconcentration is the opposite phenomenon. Hemodilution results from the mobilization of relatively erythrocyte- and protein-poor fluid from pools which may exist in either

the vascular or extravascular space. An example of an erythrocyte-poor pool within the vascular space is the microcirculation which characteristically has a lower hematocrit than large vessels partly because of the phenomenon of "plasma skimming". A source of fluid which has no erythrocytes and is relatively protein-poor is the extravascular fluid, especially that in the interstitial space. Hemoconcentration, on the other hand, can result from mobilization of erythrocyte- and protein-rich fluid from a pool such as the spleen. It can also result from the loss of protein-poor fluid from the vascular space, i.e., ultrafiltration.

Animals bled somewhat less than the acute lethal bleeding volume, and then held at the resultant arterial blood pressure by the removal or reinfusion of blood, go through two characteristic stages. There is an initial stage which has been described as "compensatory" (Green, 1961) because if hemorrhage is stopped at this time, the blood pressure rises, and if the shed blood is returned, the majority of animals survive. If the blood pressure is held constant during this stage, the compensatory process is expressed by a continued loss of blood. Compensation is followed, after a variable but relatively short interval, by the development of a "decompensatory" stage in which the continuous reinfusion of blood is required to prevent the animal's blood pressure from falling. If reinfusion is withheld during the decompensatory stage, the blood pressure falls rapidly and the animal soon dies. During this period a progressively increasing percentage will die despite the reinfusion of all the shed blood. Since initially it is necessary to remove blood from the animal to prevent arterial pressure from rising,

the change to a tendency for the pressure to fall unless blood is returned has also been called "reversal".

Because he thought pure hemorrhage results only in hemodilution, whereas, in shock there characteristically is evidence of hemoconcentration, Moon (1932) argued that hemorrhage and shock are distinct entities, despite obvious similarities. Blalock (1934) pointed out that in hemorrhaged dogs, if the resultant hypotension is maintained for a sufficient time by small hemorrhages or infusions as required, a major proportion of the animals hemoconcentrate and develop the pathological changes characteristic of shock. However, Moon's opinion concerning the absence of hemoconcentration in this preparation prevailed, for Wiggers (1950) in his influential monograph states that shock induced by hemorrhage is not associated with hemoconcentration. More recently (see Lillehei et al., 1962; Hollenberg, 1965 and Chien, 1967 for other references), hemoconcentration in dogs subjected to hemorrhage has clearly been established as one of the characteristic signs of the irreversible state. Thus, it appears that these two phenomena, hemodilution and hemoconcentration, are characteristically associated with compensatory and decompensatory processes respectively.

C. Compensation Characterized by Hemodilution.

Older works on physiology attribute the dilution of blood to an increased flow of lymph from the thoracic duct into the blood, although Starling (1896) disputed this argument on the grounds that bleeding diminishes the flow of lymph. However, a number of more recent studies indicate that hemorrhage does result in an initial increased flow

of lymph from the thoracic duct (Wessley, 1958; Cope & Litwin, 1962; Pearl et al., 1963; Hopkins et al., 1964 and Smith et al., 1965). When oligemic hypotension is sustained, however, the thoracic duct lymph flow decreases progressively, after the initial rise, to below the control level but rises again following retransfusion and declines in the preterminal state (Wessley, 1958 and Pearl et al., 1963). The initial increase is explained by a reduction in capacity of the thoracic duct and an increase of vasomotion in the small lymphatics (Wessley, 1958). The secondary increase following retransfusion, on the other hand, is probably the result of increased lymph formation (Wessley, 1958 and Pearl et al., 1963). Finally, although increased lymph flow through the thoracic duct may very well participate in the early stages of hemodilution following hemorrhage, it is not the only mechanism, for cannulation of the thoracic duct and diversion of lymph away from the blood does not prevent hemodilution (Starling, 1896).

Another cause of apparent hemodilution could be the preferential trapping or sequestration in the peripheral circulation of erythrocytes rather than whole blood or plasma. In fact, the red cell volume measured by dilution techniques is found to decrease in the post-transfusion period after severe hemorrhage (Deavers et al., 1958; Baker & Remington, 1961; Shoemaker, 1962; Baker, 1963). Analyzing the time-concentration curve of ^{51}Cr-labelled erythrocytes, Shoemaker (1962) found a marked slowing of equilibration with a significant portion of the red cell volume. This slowed mixing is apparently specific for the red cells since the equilibration of plasma labels is not delayed. Preferential trapping of erythrocytes in the peripheral circulation

results in an increase in the F-cells ratio (ratio of whole body to large vessel hematocrit) which gradually approaches unity as the period of oligemic hypotension increases (Baker, 1963). Conversely, however, it may be postulated that such a change in the F-cells ratio is the result of a shift of plasma from the microcirculation into the larger vessels. This possibility has not been investigated. Finally, although there is an initial increase in lymph flow through the thoracic duct and a later preferential trapping or sequestration of erythrocytes in the periphery, these mechanisms contribute a relatively small amount to the observed overall hemodilution.

The maximal mobilization of fluid following hemorrhage in splenectomized dogs, has been estimated to be about 10% of the initial blood volume (Allen et al., 1959). Quantitatively, this replacement in dogs is faster but less complete than in man (Lister et al., 1963), but both slower and less complete than occurs in other species such as rats (Pareira et al., 1962), rabbits (Courtice & Gunton, 1949) and birds (Djojosugito et al., 1968). The major proportion of this hemodilution is the result of absorption of fluid from the interstitial space according to the principles of the Starling Hypothesis.

The Starling Hypothesis (Starling, 1896) states that the direction and rate of fluid transfer between plasma and tissue fluids are determined by three factors; a) the hydrostatic pressures on each side of the capillary membranes, b) the protein osmotic pressures of plasma and tissue fluids acting across the capillary membranes, and c) the physical properties of the capillary membranes considered as mechanical filters. The various forces involved in such fluid shifts have

been quantitated and expressed mathematically through the classical work of Pappenheimer and Soto-Rivera (1948), who studied isolated perfused hindlimbs of dogs and cats.

The capillary membrane has a very low permeability to proteins and, thus, acts as an ultrafilter of plasma and tissue fluid. The direction of movement of protein-free fluid is determined by the balance of hydrostatic and osmotic pressures on either side of this ultrafilter. Tissue fluid usually has less protein (often close to zero) than plasma and, thus, the osmotic pressure difference across the membrane favors absorption of fluid into the vascular space. Counterbalancing this force acting to move fluid inward, is the capillary hydrostatic pressure. Determination of tissue hydrostatic pressure usually gives small positive values close to zero (except for the perforated capsule method, see Guyton, 1963) and these pressures are usually negligible. Capillary hydrostatic pressure is dependent on a number of variables and can be quantitatively expressed (Pappenheimer & Soto-Rivera, 1948) as follows:

$$pC = \frac{\frac{r_v}{r_a} pA + pV}{1 + \frac{r_v}{r_a}}$$

where pC is capillary hydrostatic pressure, pA and pV are arterial and venous pressures, and r_a and r_v are arterial and venous resistances, respectively.

At the capillary level, the direction of fluid movement is effec-

tively determined by the balance of capillary hydrostatic and plasma osmotic pressures. However, the amount of fluid absorbed or filtered is also determined by the total capillary surface area usually expressed as the capillary filtration coefficient (CFC). CFC has been determined by volumetric or gravimetric recording of the rate of net fluid movement produced by a known rise in mean capillary hydrostatic pressure and it is expressed in ml fluid filtered/min x 100 g tissue x mm Hg transcapillary pressure gradient (Mellander & Johansson, 1968). Thus, the rate of net transcapillary fluid movement, F, can be written:

$$F = CFC \times (P_c - \pi_{pl} - P_{if} + \pi_{if})$$

where P_c and P_{if} are capillary and interstitial fluid hydrostatic pressures, and π_{pl} and π_{if} are plasma and interstitial fluid osmotic pressures, respectively.

It is necessary to know what changes in these values take place following hemorrhage to be able to interpret fluid shifts on a proper hemodynamic basis. An additional important theoretical point is whether or not the properties of the membrane are altered following hemorrhage. It is of some substantial consequence, for example, if the membrane becomes permeable to protein following hemorrhage. The release of vasoactive polypeptides may alter capillary permeability, but in hemorrhage there is no evidence that this plays a part in affecting transcapillary fluid transfer. It is only in the very terminal stages that there is any evidence for the loss of whole blood or plasma, and this could be due to other factors.

It is obvious from the formula for the determination of capillary hydrostatic pressure that, if hemorrhage is large enough to result in a fall in arterial inflow pressure in a particular vascular bed, the capillary hydrostatic pressure will fall and result in an influx of interstitial fluid even if there is no change in the pre- and postcapillary resistances. Since following hemorrhage there initially is no substantial change in the total amount of protein inside or outside the vascular system (Fine & Seligmen, 1943 and Fine & Seligman, 1944), for a given fall in capillary hydrostatic pressure this influx theoretically should gradually diminish and eventually stop because of equilibration of protein osmotic pressure on either side of the membrane. However, this would appear to require movement of a much larger volume of fluid than has been measured.

In addition to the overall fall in pressure across a regional vascular bed, there can be changes in pre- and postcapillary resistance and these changes can have a profound effect on the movement of fluid across the capillary membrane (see reviews of Haddy et al., 1968; Mellander, 1968; Mellander & Johansson, 1968 and Wiederhielm, 1968). Theoretically it is possible for fluid absorption to occur solely because of resistance changes with all other variables remaining unchanged. Chien (1958) had earlier demonstrated that moderate hemorrhage does not necessarily cause a significant fall in arterial and venous pressures but nevertheless substantial fluid reabsorption occurs. It appears rather clear now that pre- and postcapillary resistance changes are involved in this observation and Mellander (1960) and Öberg (1964) have conclusively demonstrated that fluid shifts can result from this type

of resistance change in a particular regional bed, even if the arterial inflow and venous outflow pressures remain constant.

There are basically three ways to assess pre- and postcapillary resistance changes and thus their influence on fluid shifts. These are: a) microscopic observation, b) regional volume or weight, and, c) small vessel pressures. No one method is completely satisfactory in itself and any complete method must combine a technique for measurement of resistance with a technique for measuring fluid flux. The major work in direct observation of the microvasculature has been done by Zweifach and his co-workers (Zweifach et al., 1944; Zweifach et al., 1948 and Zweifach & Hershey, 1949). They observe that the immediate adjustment to hemorrhage in the microcirculation of dog omentum is a widespread constriction of the arteries and veins. Compensatory vasoconstriction in the precapillary section, augmented by a fall in arterial and venous pressure, would facilitate fluid influx into the circulation. Later during prolonged hemorrhage, decompensatory alterations are also observed, as will be discussed in a subsequent section. Fluid flux was not measured in these studies but the observations of the microcirculation are supportive evidence for some of the observations from the experiments described below.

The Swedish workers use a method that involves monitoring regional tissue volume (see Mellander, 1960 for details) while subjecting the remainder of the animal to hemorrhage (Mellander & Lewis, 1963; Lundgren et al., 1964 and Öberg, 1964). An initial rapid decrease in volume represents contraction of capacitance vessels and a later, slower volume reduction is attributed to transcapillary fluid shifts.

In this method, which employs constant inflow and outflow pressures, fluid shifts reflect the relative condition of pre- and postcapillary resistance vessels. This preparation has one further advantage in that one can calculate the CFC, which reflects the capillary area available for filtration and exchange and hence the condition of the precapillary sphincters (Cobbold et al., 1963). The effects of hemorrhage on the hindlimbs are a rise in the pre- to postcapillary resistance ratio and an increase in capillary surface area. These responses, which would favor fluid influx, are more marked in muscle than in skin and could be elicited with hemorrhages as small as 2 ml/kg. In both these tissues the fluid influx becomes more pronounced with increasing amounts of hemorrhage. In view of total skeletal muscle mass, these authors argue that this tissue probably makes the largest contribution to the overall replacement of plasma volume. The pre- and postcapillary resistance ratio in intestinal vessels remained essentially unchanged and this was attributed to local autoregulation.

It is important to recognize, however, that the regional volume technique only measures a ratio and at constant inflow and outflow pressures changes in pre- and postcapillary resistances are merely inferred. The technique does not determine, for example, whether an increased pre- to postcapillary resistance ratio is due to: a) an increase in precapillary resistance with postcapillary resistance unchanged, b) a decrease in postcapillary resistance with precapillary resistance relatively unchanged, or c) an increase in both pre- and postcapillary resistance but a relatively greater change in the precapillary section.

The third method for assessing changes in pre- and postcapillary

resistance is the small vessel pressure technique developed by Haddy et al. (1954). Although this technique provides more direct evidence regarding pre- and postcapillary resistance changes as individual components of the overall ratio, it is not able to subdivide a vascular bed precisely and some venule resistance is inherently included in the precapillary section. Haddy et al. (1965) used this technique in conjunction with indicator-dilution techniques and found that, following hemorrhage, both pre- and postcapillary resistances increased, but the precapillary increase was greater than that postcapillary. This favors fluid absorption and is in agreement with observations of regional volumes and direct microscopic observations.

In conclusion, hemodilution is a well-recognized consequence of hemorrhage in all species studied and appears to be one of the major compensatory mechanisms following blood loss. It is primarily the result of a fall in capillary hydrostatic pressure resulting both from the overall fall in pressure due to hypovolemia and a relatively greater increase in pre- than postcapillary resistance. Small contributions to hemodilution may be made by an initial, transient increase in lymph flow through the thoracic duct and a later preferential trapping or sequestration of erythrocytes in the periphery.

D. Decompensation Characterized by Hemoconcentration.

Hemoconcentration is a phenomenon which is usually associated with the later stages of shock. In the hemorrhagic shock model it occurs frequently enough to be judged one of the signs of impending irreversibility (Lillehei et al., 1962). However, statements still

appear to the effect that hemoconcentration is not found in man even after severe hemorrhages (Chien, 1967). Eventually, this will probably be revised in a manner analgous to the revision of the similar opinion of Moon (1932) concerning hemorrhage in dogs. Most references quoted to support a lack of hemoconcentration in man following hemorrhage suffer from too many inadequacies to be given serious consideration. Either they have a complete lack of control (Noble & Gregersen, 1946) and/or use cases of shock which have already been subjected to vigorous fluid therapy (Beecher et al., 1947 and Artz et al., 1955). It must be remembered that without therapy, the irreversible stage of shock is very short indeed, and, in the clinical situation where patient welfare requires immediate fluid therapy, hemoconcentration naturally would be difficult to find. Experiments on other primates have not focused on this particular aspect of hemorrhagic shock, but are mostly concerned with the absence of portal hypertension in monkeys (see Selkurt & Rothe, 1961 and Einheber & Cerilli, 1962). One recent paper reports a significant decrease in ^{131}I-measured blood volume during the later portions of the postinfusion phase but does not provide data on hematocrit or plasma protein concentration (see Abel et al., 1967).

Although hemoconcentration is seen predominantly late in shock, in some species such as dogs and cats, there is a phase of hemoconcentration immediately following the initiation of hemorrhage. This is the result of contraction of the spleen and autotransfusion of relatively erythrocyte-rich fluid. This hemoconcentration does not occur in splenectomized animals (Haddy et al., 1965). Later, a small contribu-

tion to hemoconcentration is made by addition to the circulation of a certain amount of protein, probably from the liver (Chien, 1958; Deavers et al., 1963 and Smith et al., 1965). However, the major portion of late hemoconcentration is the result of other mechanisms.

On the basis of recent studies, decompensation has been divided into two phases (Hollenberg, 1965). In the first, an ultrafiltrate of plasma leaves the vascular space and this is associated with progressive parallel increases in plasma protein concentration and hematocrit. In the second, the fluid loss appears to represent sequestration of whole blood, and plasma protein concentration and hematocrit remain relatively constant, although measured blood volume continues to decrease. In agreement with the concept of increased transcapillary fluid loss, the intestinal mucosa of the dog is usually congested and edematous and the gut lumen may be filled with fluid. The fluid in the gut lumen is often blood and a bloody diarrhea may be seen (see Lillehei et al., 1964). Such gross amounts of bloody fluid are not commonly found in the gut of monkeys and man (Zweifach, 1961). Although the mechanisms of fluid influx early in shock have been studied in some detail, relatively little attention has been given to the mechanism of ultrafiltration and sequestration during the decompensatory stage.

In their microscopic observations of dog omentum during shock, Zweifach (Zweifach et al., 1944; Zweifach et al., 1948 and Zweifach & Hershey, 1949) reported changes in the microcirculation which support the occurrence of a rise in capillary hydrostatic pressure and a concomittant loss of fluid from the circulation. After several hours at

40-50 mm Hg, vasomotion in the terminal arterioles and precapillary sphincters is reduced and finally ceases. This opening of the precapillary sphincters permits increasing amounts of blood to enter capillary channels, and although no significant venular vasoconstriction is evident, the outflow from the collecting venules into the larger veins is markedly curtailed and the venules and capillaries are engorged with blood suggesting some impairment of venous outflow. A decreased venous outflow could cause a rise in capillary hydrostatic pressure by increasing back pressure. The constriction of the larger blood vessels remains relatively unaffected throughout indicating that overall precapillary resistance and large vein resistance are unchanged while the crucial change occurs in the small veins. Retransfusion at this point results in an immediate acceleration of blood flow. In dogs that survive, the vasomotion and reactivity of the microcirculation also recover, whereas in the nonsurvivors, the changes in the microcirculation seen prior to retransfusion remain.

Although the regional volume technique of the Swedish workers effectively demonstrated the pre- to postcapillary resistance ratio changes involved in hemodilution early in shock (Oberg, 1964), they paid much less attention to hemoconcentration during the decompensatory stage. Experiments of Mellander & Lewis (1963) demonstrated that after lowering the inflow pressure to 40-50 mm Hg by hemorrhage and keeping it lowered for more than 2 hours, short periods of stimulation of the sympathetic vasoconstrictor fibers caused progressively less fluid absorption until, finally, stimulation resulted in ultrafiltration. This was attributed to a gradual loss of the ability of the

precapillary section to develop tone upon stimulation, while postcapillary sections were still reactive. A further conclusion was that this difference in reactivity to nerve stimulation was the result of a more dominant effect of local metabolic factors in the precapillary section. In these experiments, CFC was observed to increase very shortly after the start of hemorrhage but not to increase further as hypotension progressed and this led to the conclusion that precapillary sphincters were even more under the influence of local metabolic factors than were the precapillary resistance vessels. Thus, the postcapillary vessels appeared to be dominated more by extrinsic nerve influences while the precapillary section was primarily under metabolic control. Lundgren et al. (1964) obtained similar results during a much larger hemorrhage (30-40% of blood volume). Their data support the earlier suggestion that following hemorrhage in cats skeletal muscle resistance vessels lose tone at a faster rate than postcapillary (capacitance) vessels. The differences between these results and those of Hollenberg (see below, 1965) remain to be reconciled.

Using the technique of small vessel pressures, Hollenberg (1965) showed that in the dog during prolonged oligemic hypotension, precapillary resistance is elevated and constant (arterial pressure and flow constant), whereas postcapillary resistance, judged by small vein wedge pressure, continues to rise as the period of hypotension progresses. The rise in small vein pressure was seen in both the intestinal and hindlimb vascular beds but it appeared sooner and increased more quickly in the intestinal small veins. Pressure did not increase in the mesenteric vein and therefore the increase in small vein wedge pressure

in the intestine is not due to back pressure resulting from closure of the hepatic sphincter. Increased small vein wedge pressure would also result in the elevation of capillary hydrostatic pressure, but by a mechanism quite different from that suggested by Mellander & Lewis (1963) and Lundgren et al. (1964) (see earlier detailed discussion in Peripheral Circulatory Failure).

In summary, the later hemoconcentration seen following hemorrhagic shock appears to be predominantly the result of an increased capillary hydrostatic pressure. The increased capillary hydrostatic pressure appears to be due to a progressive increase in postcapillary resistance with precapillary resistance maintained at an elevated level (see Peripheral Circulatory Failure). Some authors, however, favor loss of precapillary resistance with postcapillary resistance remaining elevated as the cause of the increased capillary hydrostatic pressure. The reconciliation of these two different loci of change in segmental resistance may be a species difference between cats and dogs, effects of different anesthetics, or, inherent differences in technique. Although skeletal muscle may play a dominant role in hemodilution as is suggested in the previous section, both the intestine and skeletal muscle seem to be involved in hemoconcentration, and, at least in the dog, the intestine may play a dominant role.

E. Role of the Sympathetic Nervous System.

The role of the sympathetic nervous system in the hemodynamic adjustment to blood loss is of considerable importance. Since sympathetic activity causes cardiac stimulation, reduction of the size of veins,

and arteriolar constriction, it serves to support the arterial pressure in the face of reduction in blood volume. Because the vasoconstriction does not involve the coronary and cerebral circulations, the maintenance of arterial pressure favors the perfusion of these two "immediately vital" organs, and sympathetic activity constitutes a compensatory mechanism for immediate survival in hemorrhage. The blood flow through the regions under sympathetic vasoconstriction, however, is reduced by a greater proportion than the arterial pressure. The overall role of the sympathetic nervous system in hemorrhage has been recently reviewed in some detail (see Chien, 1967) and this material will not be repeated here. That increased sympathetic activity eventually has a deleterious effect has also been reviewed (see Nickerson, 1955; Nickerson, 1964 and Nickerson & Gourzis, 1962), but merits some further comment.

The administration of adrenaline or noradrenaline can result in both extreme vasoconstriction and the development of irreversible shock which is indistinguishable from that induced by trauma or hemorrhage (Erlanger & Gasser, 1919 and Yard & Nickerson, 1956). Similarly, cerebral decortication produces extreme activity of the sympathetic nervous system, vasoconstriction and shock (Freeman, 1933). These procedures also produce hemoconcentration and diminished blood volume. Furthermore the circulatory failure engendered by hemorrhage is potentiated by the administration of relatively small amounts of adrenaline (Remington et al., 1950), noradrenaline (Hollenberg, 1965), or by the increased sympathetic activity induced by buffer nerve section (Remington et al., 1950). Similarly, in shock induced by limb trauma, section

of the dorsal roots to reduce sensory input decreases both reflex vasoconstriction and lethality (Wang et al., 1947), and conversely, reflex vasoconstriction induced by sciatic nerve stimualtion potentiates the shock process, despite a significant increase in arterial pressure (Overman & Wang, 1947).

It has frequently been noted in both patients and experimental animals that hypotension associated with vasodilatation is remarkably well tolerated (Phemister et al., 1945 and Page, 1961). Animals in which vasoconstriction mediated by the sympathetic nervous system has been reduced or abolished by sympathectomy, ganglionic or adrenergic blockade or depletion of noradrenaline stores are considerably more resistant than normal controls to shock induced by many different procedures. However, there are many studies in the literature which claim reduced resistance (see Chien, 1967 for references) following a reduction in sympathetic activity. Obviously then, there is a great deal of confusion on this subject. The volume of literature alone is a substantial obstacle to a clearer understanding of this point. In addition, many of these studies suffer from poor experimental design, a paucity of clear, objective thinking, or both. For example, it has been argued that pretreatment by α-adrenergic blockade reduces the maximal bleeding volume, or at least delays its arrival when the constant pressure technique is used, and therefore the severity of stress is not comparable to untreated controls. Also, it has been noted that administration of agents such as phenoxybenzamine during the shock period without fluid replacement has disasterous effects (see Chien, 1967 for references). Based on this type of evidence,

some authors have concluded the α-adrenergic blocking agents have not been demonstrated to be beneficial in shock therapy. What this illustrates however is a superficial concept of shock itself, its therapy, and the purpose of pretreatment with α-adrenergic blocking agents. Even proponents of the use of phenoxybenzamine recognize the value of adequate sympathetic nervous system activation early in shock and certainly do not advocate the use of such drugs without adequate fluid replacement therapy (see Nickerson & Gourzis, 1962). The purpose of phenoxybenzamine pretreatment in most experiments is not to evaluate a therapeutic regimen in shock, but to illustrate the role of excessive sympathetic stimulation in the development of irreversibility. (See Hollenberg, 1965.)

The point to be considered here is the role of sympathetic nervous system activity in the development of irreversibility. It is interesting to note that increased sympathetic activity and/or excess catecholamines can be implicated in every major hypothesis for the development of irreversibility. Production of myocardial damage (Szakacs & Cannon, 1958; Maling et al., 1960 and Szakacs & Melhlman, 1960), induction of disseminated intravascular coagulation (Crowell & Read, 1955), and terminal elevation of capillary hydrostatic pressure (Hollenberg, 1965) have all been associated with excess sympathetic nervous system stimulation or catecholamines, and, endotoxin is known to potentiate vasoconstriction due to catecholamines or sympathetic nervous system stimulation (Raškova & Vaněček, 1964). Thus, at the end of his extensive review of the role of the sympathetic nervous system in shock, Chien (1967) was led to the conservative conclusion, "With pro-

longed hemorrhagic hypotension, the continued sympathetic vasoconstriction in the abdominal viscera results in a progressive aggravation of anaerobic metabolism and acidosis. Eventually the cardiovascular and other body systems may be adversely affected."

5. THE PURPOSE AND SCOPE OF THE PRESENT STUDY

Preliminary studies established that chickens tolerate hemorrhagic hypotension very well, and the studies were extended to investigate the basis for the observed ability to survive prolonged hemorrhagic hypovolemia, the massive and rapid replenishment of blood volume with extravascular fluid, and the apparent lack of irreversibility to retransfusion following hemorrhage in this species. As the work progressed it became necessary to investigate the following: (1) resistance to hemorrhage and extent of hemodilution in chickens under various experimental conditions, (2) resistance to the development of irreversibility in chickens, (3) comparative aspects of hemorrhagic hypotension in chickens and turkeys, (4) total and regional blood flows and total, regional and segmental peripheral resistances during hemorrhagic hypotension in chickens, (5) blood flows and peripheral resistances during hemorrhagic hypotension coupled with noradrenaline infusion, and (6) plasma levels of catecholamines during hemorrhagic hypotension in chickens.

6. GENERAL METHODS AND DEFINITIONS OF TERMS

A. Method of Hemorrhage.

Hemorrhage was selected to produce shock in the present study because of the factors of simplicity and reproducibility. Procedures for induction of shock by hemorrhage fall into two general categories: those in which a fixed volume is withdrawn, regardless of changes in blood pressure, and those in which enough blood is withdrawn to reduce mean arterial pressure to some definite stabilized level. There are however many modifications of both of these general methods (see Selkurt & Rothe, 1961 and Gourzis, 1962). A modification of the latter method was chosen for this study. Because of practical difficulties in constructing an automatic bleeding apparatus which would function efficiently for small volumes (Gourzis, 1962), a manual bleeding method was used. Blood was removed until a fixed arterial pressure was reached and then this pressure was maintained by the removal of additional blood as was necessary. The major feature of this type of hemorrhage, i.e., blood pressure held constant, is that compensatory processes express themselves as a continued removal of blood and decompensatory processes as a fall in arterial pressure or the necessity of reinfusion of blood to maintain a constant pressure.

Experiments were carried out on White Leghorn hens obtained from a local commercial supplier, which weighed from 1.00 to 2.50 kg and were free of obvious disease. Upon arrival, they were housed on wire mesh in a constant environment at a temperature of approximately 70° F for at least one week prior to use. Mature hens were defined as ranging in age from 12 to 18 months while pullets were 6 to 8 months. A diet of a standard commercial feed and water was supplied

ad libitum. No special effort was made to fast the animals. The daily schedule of experiments coupled with the animal house routine usually ensured overnight fasting. Experiments began at the same time each morning and invariably the crop was empty at the time the animal was anesthetized. The laboratory was approximately the same temperature as the animal house.

Chickens were anesthetized with paraldehyde, B.P. (1.25 ml/kg) administered intramuscularly into the large breast muscle mass. Approximately one-half hour following paraldehyde, the sites of cannulation were infiltered subcutaneously with lidocaine, U.S.P. (1.0 ml of a 2% solution). At this stage, the chickens were frequently still responsive to loud noises or to nociceptive stimuli at loci outside the influence of the lidocaine. No additional anesthetic was required for the duration of the experiment.

Surgical exposure of the ischiatic (sciatic) artery was performed by a combination of cautery and blunt dissection. Cautery was necessary to avoid excessive bleeding while passing through the skin and tensor fascia latae muscle (see Bradley, p. 29, 1960) to reach the ischiatic artery, external iliac vein, and the ischiatic nerve which run together. The incision was made parallel, and approximately 1 inch posterior to the femur. The ischiatic artery lies immediately parallel to, and slightly beneath the anterior margin of the biceps femoris muscle. Catheters were made of thin-walled polyethylene tubing (PE 160) fitted at one end with a three-way stopcock to allow irrigation with 0.9% NaCl as required to maintain patency. The tip was slightly lubricated with silicone grease and there was no un-

due difficulty in advancing the arterial catheter to the abdominal aorta (approximately 4 inches).

Following administration of heparin (2.5 mg/kg), arterial bleeding was accomplished by withdrawing blood with a glass syringe at 4 minute intervals (0.5 ml/kg/min) until the mean arterial blood pressure reached 50 mm Hg. Each portion of blood was immediately placed into a polyethylene reservoir and simultaneously oxygenated and agitated by a stream of 95% O_2 and 5% CO_2. Samples of blood for the measurement of hematocrit, plasma protein concentration or activity of RISA (see below) were drawn through the same catheter used for bleeding after the dead space in the cannula was cleared. The volumes of samples were always included in the bleeding volume.

The rate of hemorrhage employed usually lowered the mean arterial blood pressure to 50 mm Hg within approximately 20 minutes. The arterial pressure was then maintained at this level by additional small bleedings as necessary at a rate which never exceeded the initial bleeding rate. Late in the experiment, if it appeared that obtaining a blood sample from the animal might precipitate cardiovascular collapse, the volume removed for such a sample was immediately replaced by an equal volume of blood from the reservoir. Stepwise reinfusion was accomplished in a manner exactly the reverse of the bleeding but at a faster rate (1 ml/kg/min).

B. Measurement of Cardiovascular Variables.

I. Plasma and Blood Volumes. Plasma volume determinations were carried out by the indicator-dilution method using ^{125}I-tagged

human serum albumin (RISA). After taking an arterial blood sample to determine the background activity, a suitable quantity of a RISA solution (10 microcuries/ml) was placed in a cannula in either an external jugular or external iliac vein and then flushed into the animal with a small amount of saline. When doing serial determinations of plasma volume, the amount of RISA for the first measurement was approximately 2 microcuries and this was increased arithmetically by 1-2 microcuries for each subsequent determination to ensure a favorable ratio of background/total activity. The exact volume of RISA solution injected was determined from the change in weight of the syringe and the activity of the solution was determined daily by counting.

Arterial blood samples were obtained from the ischiatic artery after clearing the dead space in the cannula. In earlier experiments, arterial samples were taken 4, 8, 16 and 32 minutes after the RISA injection. The activity in these samples and in the RISA standards was determined by counting duplicates in a well-type scintillation counter. After correction for background, the activities of the serial arterial samples were plotted on semi-logarithmic graph paper and a line of best fit was extrapolated to zero time. However, occasionally the 4 minute sample did not fit the overall regression line (see B17, Fig. 1) and, in later experiments, sample times of 8, 16, 24 and 32 minutes were used. The zero time indicator-dilution was used to calculate the plasma volume. When plasma volume was measured during the period of hemorrhagic hypotension, bleeding was suspended until after the 16 minute sample had been taken.

The slopes of the regression lines of the activities of arter-

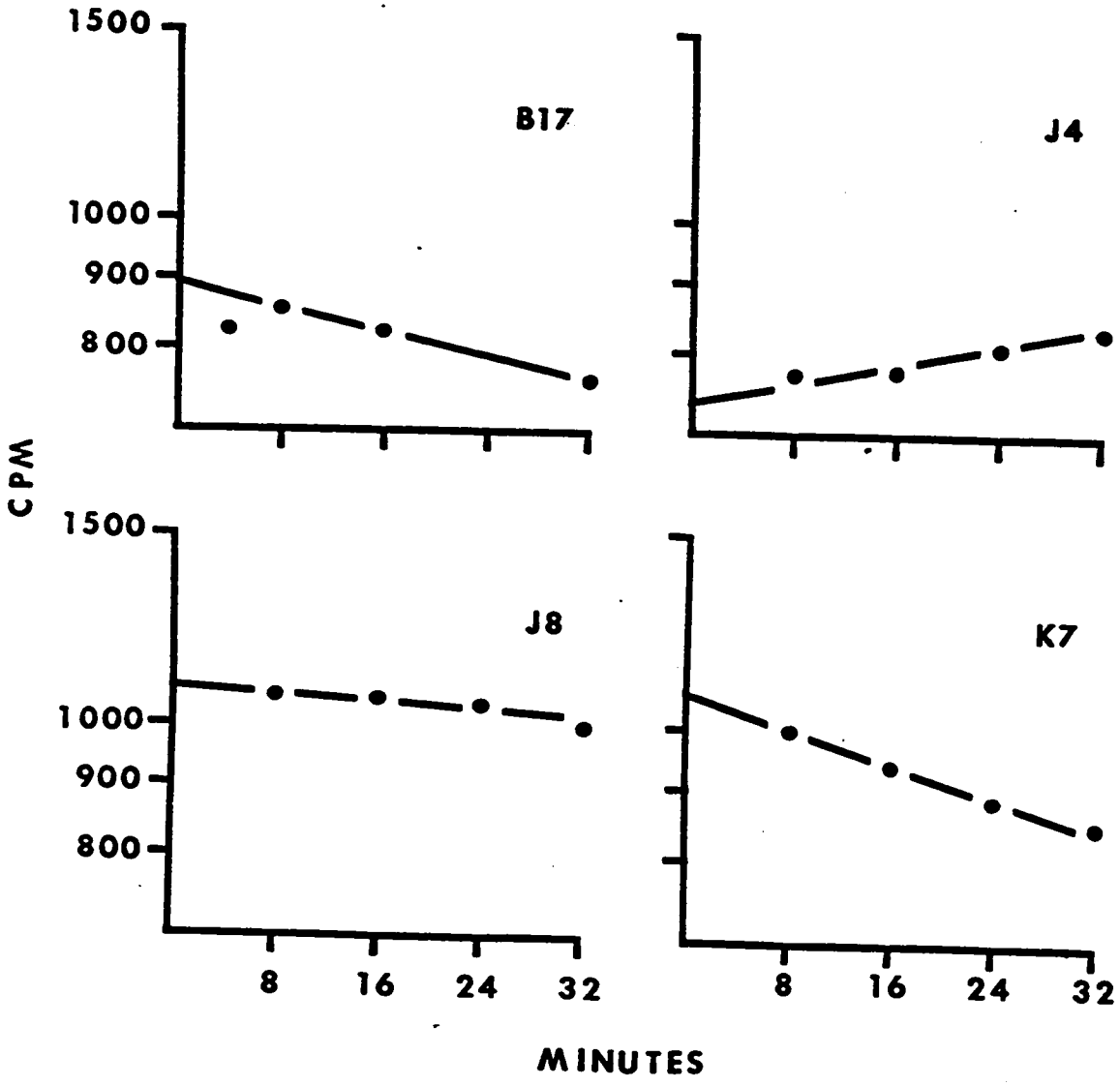

Figure 1. Four Sample Regression-Line Plots of Activity of Radio-Iodinated Serum Albumin (RISA) versus Time obtained in Plasma Volume Determinations in Chickens. The number shown with each plot is the identification number for that experiment. B17 illustrates a problem which arises when the first sample is removed too soon after the injection of RISA. J4 illustrates an unusual course associated with a rise in blood pressure (see text).

ial samples varied somewhat from experiment to experiment even during the control period. A sample of the variety of slopes obtained is illustrated by J4, J8 and K7 of Fig. 1, all of which were obtained during control periods. This illustrates the necessity of constructing a regression-line for each blood volume determination. The line in J4 illustrates a special case which occurs as blood pressure rises (see General Discussion).

Blood volumes were estimated from the plasma volume and the large vessel microcapillary hematocrit. They were uncorrected for uneven distribution of plasma and erythrocytes. The F-cells ratio has not been determined in chickens, but in ducks (Cohen, 1967) it has been reported to be 0.88, which is approximately the same as in most mammals. If, as in dogs (Baker, 1963), the F-cells ratio increased towards unity during hemorrhage, failure to correct for large vessel/whole body hematocrit may have caused an overestimation of the blood volume by approximately 10% before bleeding, and somewhat less after bleeding.

II. Hematocrit and Plasma Protein Concentration. After clearing the dead space in the catheter, arterial blood samples for determination of hematocrit and plasma protein concentration were obtained from an ischiatic artery. Approximately 1 ml of blood was sufficient for duplicate determinations of both hematocrit and plasma protein concentration.

Hematocrits were determined from duplicate samples of well-mixed arterial blood in unheparinized microhematocrit tubes sealed by flame.

The tubes were centrifuged for 5 minutes in an International Model MB Microhematocrit Centrifuge at full speed (approximately 12,500 g). Hematocrits were not corrected for trapped plasma. This led to no substantial error as Cohen (1967b) has reported a value of 2.12% for the total plasma trapped in the cellular pellet of avian blood.

Plasma protein concentrations were usually determined on the same day but occasionally were measured the next morning. A twelve hour delay in analysis did not change the determined values provided the initial dilution step was performed immediately. The assay method was the standard clinical Biuret Method (see Annino, 1960), with a minor volumetric modification to accommodate the slightly more dilute avian blood. The developed blue color was read at 550 mμ in a Bausch & Lomb "Spectronic 20" spectrophotometer. A standard curve was constructed using commercially available standard protein solutions (Protein Standard, Harleco). Determinations on six separate standard protein solutions (total of 24 samples) were performed to determine this curve. The equation of best fit for these 24 points calculated by the Method of Least Squares was: $y = 0.0604x + 0.0050$, $r = 0.9992$, where y is the absorbance, x is the total protein in Gm%, and r is the correlation coefficient.

Experience in this laboratory has confirmed the accuracy and reproducibility of the Biuret Method for plasma protein determination. A single-blind technique for analysis of samples of known protein content was carried out approximately every six months and deviations between calculated and known protein concentrations were never more than 5%. A total of 27 known samples tested in this manner gave an error

estimate of $-1.1 \pm 0.6\%$ (vectored mean $\pm$ S.E.).

III. Arterial and Venous Pressures. All cannulations for pressure measurement were performed using catheters of thin-walled polyethylene tubing, usually slightly lubricated at the tip with silicone lubricating grease, and fitted at one end with a three-way stopcock to allow easy access for irrigation with 0.9% NaCl solution. A Statham P23BC transducer was used for venous and Statham P23AC or P23AA pressure transducers for arterial pressures. Periodic calibration checks of the pressure transducers were made using a saline manometer for P23BC transducers and a mercury manometer for P23AC and P23AA transducers. With the animal lying on its side, the zero reference point for all measurements of pressure was the sternum (approximately 1 inch above the table). All pressures were recorded on a Grass Model 5A Polygraph.

Mean arterial blood pressure (MAP) was measured near the abdominal aorta through the same catheter used for bleeding. Large vein pressure (LVP) was measured in the ipsilateral external iliac vein. The vein, which lies immediately ventral to the ischiatic artery, was cannulated with the same size tubing as the artery (PE 160) and the tip was advanced a distance of about 2 inches toward the renal portal system and the inferior vena cava (see Akester, 1967). Central venous pressure (CVP) was measured in the thoracic venous system through an external jugular vein which was cannulated with somewhat larger tubing (PE 205). Venous wedge pressure (VWP) was measured in a small side-branch of the external iliac vein which predominantly drained

skeletal muscle (determined by postmortem dissection). The catheter was placed ipsilateral to the electromagnetic flowmeter probe (see below) and contralateral to the MAP cannula. Retrograde cannulation was done with very small tubing (o.d. 0.5 mm, PE 10), and the wedge position was judged by resistance to further advancement of the catheter, a sudden increase of approximately 5 mm Hg in the recorded pressure, and appearance of spontaneous fluctuation in the recorded pressure which closely followed changes in flow. Insertion of the VWP catheter was usually 1-2 inches.

IV. Regional Flow. Regional flow was measured by means of a Statham M-4000 electromagnetic flowmeter and Statham Flo-Probes^{R} (type MDS or S) of the appropriate lumen diameter. These probes were calibrated in vitro on lengths of mesenteric or ischiatic artery bathed in saline and artifically perfused. Constant flow was maintained by either a fixed height of perfusate in a column or a motor-driven infusion pump and measured by timed collection of perfusate in a graduated cylinder. The rate of perfusion was varied by a screw-clamp placed downstream from the probe. Three types of perfusate were used: a) 0.9% NaCl, b) dog plasma, or c) dog blood. The hematocrit of the blood was varied over the range of 5-55%. The wide range of hematocrits encountered during the experiments necessitated using these relationships to calculate the "true flow" from the flowmeter output. During the experiments, "zero-flow" obtained electrically did not always coincide exactly with "zero-flow" obtained by mechanical occlusion of the artery. Therefore, for each flow recorded, mechanical zero (true

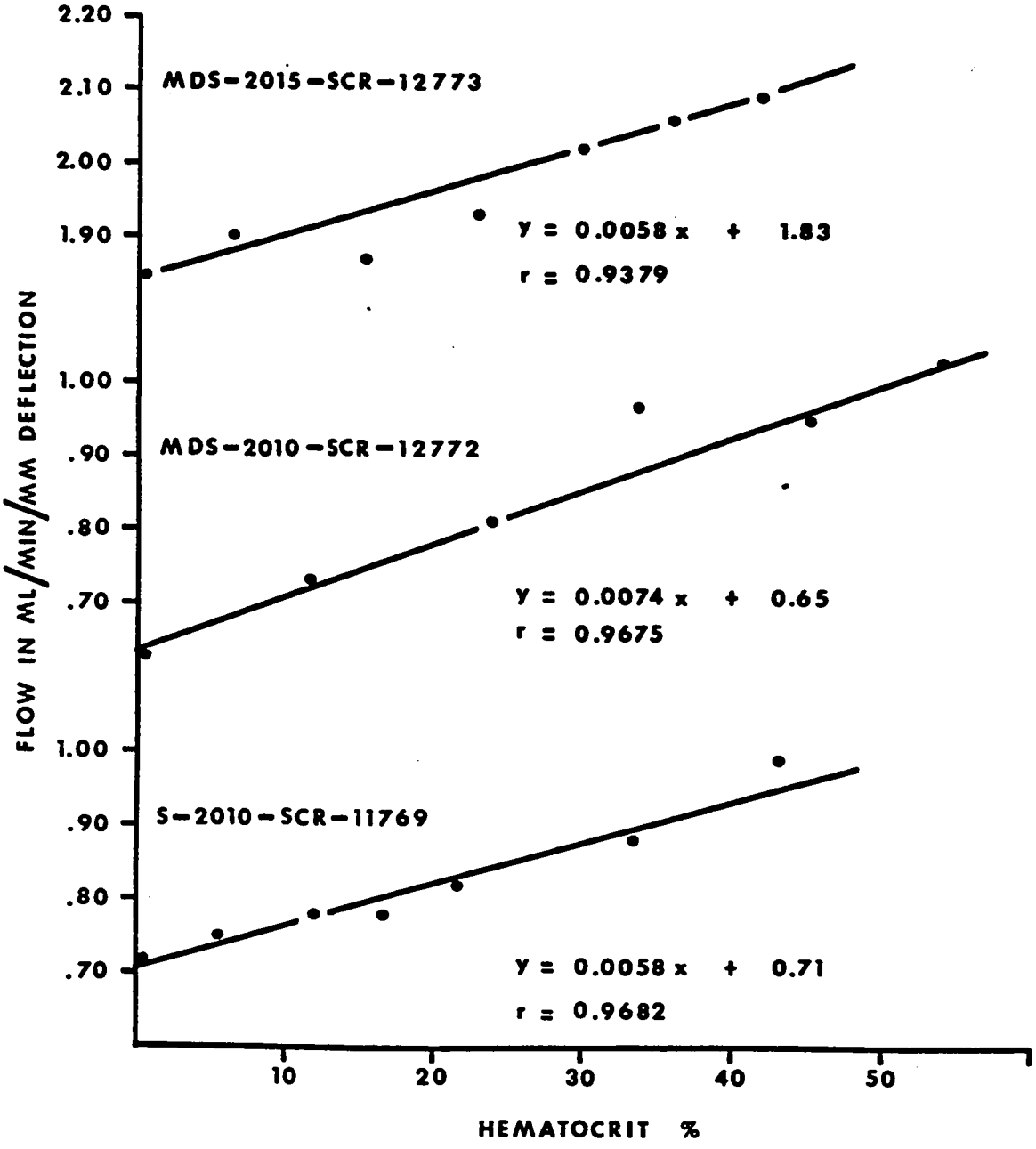

Figure 2. Calibration of Three Electromagnetic Flowmeter Probes for Variation of Output with Changes in Hematocrit. The serial number of each probe is shown above its regression-line. The equation for the line of best fit (Method of Least Squares) and the correlation coefficient are shown below each line. These equations are used to correct the flowmeter output for changes in hematocrit.

zero flow) was obtained by brief (5 sec) clamping of the artery downstream from the probe. Flow was recorded on a Grass Model 5A Polygraph and mean flow was taken to be the arithmetic mean of the systolic and diastolic flow.

When measuring ischiatic flow, the ischiatic artery contralateral to the catheter for pressure measurement was exposed as described above. The single cranial mesenteric artery (see Bradley, 1960 for all anatomical references) was exposed through a flank incision on the right side after infiltrating subcutaneously with 2% lidocaine (5.0 ml). Surgery through the layers into the peritoneum was performed by cautery. The incision originated at the large ilial bone mass and proceeded parallel to the femur for 5-6 inches ending near the sternum. Care had to be taken near the ilium to avoid the kidney and near the sternum to avoid the liver. The incision was at least 1 inch caudal to the last rib in order to avoid pneumothorax. The sartorius muscle was held back, and successive cuts were made in the obliquus abdominis externus and internus muscles, the peritoneal wall, and the abdominal air sac. The intestine was then gently pushed out of the way with saline-moistened gauze to expose the cranial mesenteric artery near the ovary.

The arteries were carefully freed from their sheaths by blunt dissection so as to damage the perivascular nerves as little as possible. A section of artery about 3-4 times the width of the probe was exposed to ensure ample room for clamping and to avoid any undue traction on the artery by the probe. Loose ligatures of umbilical tape were placed on either side of the desired probe position. Gentle trac-

tion was applied, first to the upstream, then to the downstream ligature, and the probe was gently slipped onto the artery between the ligatures which were then released and removed. One hour was allowed for attainment of relative cardiovascular stability before proceeding with the experiment.

V. Cardiac Output. Cardiac output was determined by the indicator-dilution method of Hamilton (Hamilton et al., 1932). In shock, however, recirculation of dye during a substantial proportion of the important semi-logarithmic part of the dye curve results in overestimation of the area under the curve when conventional methods are used (see Oriol et al., 1967 and Oriol & McGregor, 1967). These workers have demonstrated the applicability of the Dow formula (Dow, 1955) to determine the area under curves obtained during shock. This formula utilizes empirical values measured from only the first part of the curve.

Cardio-Green ^{R} (indocyanine green) was placed into the CVP catheter (0.05 mg in 0.10 ml) and rapidly flushed into the animal as a single bolus with saline (1.0 ml). Arterial blood was drawn from the catheter in an ischiatic artery through a Gilson Medical Electronics Dye Tracer (Model DTL) cuvette by a Harvard Infusion-Withdrawal Pump (Model 600-900) at a constant rate of 10.3 ml/min. The resulting dye curve was monitored on a Texas Instrument Servo-Riter II Recorder.

The volume of blood required for a complete curve was approximately 4 ml and was returned to the animal at the same rate immediately following completion of the curve. At the end of each day, duplicate

blood samples containing known concentrations of indocyanine green were drawn through the densitometer at the same rate to construct a standard curve. From a total of 61 samples, the line of best fit calculated by the Method of Least Squares was: $y = 1.299x + 0.092$, $r = 0.996$, where y is the deflection on the recorder in inches, x the concentration of dye in mg/l, and r the correlation coefficient.

This rate of withdrawal coupled with the small volume from the tip of the cannula to the middle of the cuvette (approximately 0.8 ml) made it possible to avoid the cumbersome corrections for curve distortion (see Milnor & Jose, 1960) which are sometimes necessary when applying the Dow formula to data from an external sampling system. The area under the curve was obtained directly from Dow's formula (Sekelj et al., 1966):

$$\text{Area} = \frac{PC \times PCT}{K_1 - (K_2\,[PCT/AT])}$$

where PCT is the peak concentration time, PC the peak concentration, and AT the appearance time. PCT is the sum of the appearance time and buildup time. K_1 and K_2 are constants which have previously been determined for man and for dogs (see Dow, 1955; Sekelj et al., 1966 and Oriol et al., 1967), but which had to be determined for chickens.

To determine K_1 and K_2, the areas (with the dimensions mg/l sec) of 28 curves obtained from chickens before bleeding were determined from the absolute area (planimetry), the standard curve for indocyanine green, and the chart speed of the recorder. Using the PC, PCT, AT

(dimensions of mg/l and seconds) and arbitrary values for K_1 and K_2, the areas of these same curves were determined on an IBM 360/50 computer. All possible combinations of K_1 and K_2, in small increments of 0.01, between 2.40 and 3.60 inclusive for K_1, and between 0.60 and 1.30 inclusive for K_2 (8,591 iterations) were used to compute these areas. The computer was further instructed to save only those pairs of K_1 and K_2 which gave the highest linear correlation between the calculated and measured areas.

The highest correlation coefficient was 0.9518, obtained with $K_1 = 2.58$ and $K_2 = 0.84$. This high correlation coefficient indicated an excellent linear relationship between the calculated and measured areas of these 28 curves, but the values of K_1 and K_2 differed somewhat from those previously reported for other species (see Dow, 1955; Sekelj et al., 1966 and Oriol et al., 1967). The equation of the regression-line, calculated by the Method of Least Squares, which related calculated to measured area was: $y = 1.324x + 1.081$, where y is the calculated and x is the measured area. This line indicated that the one-to-one relationship between the measured and calculated areas previously reported for other species (see Sekelj et al., 1966) does not apply to the chicken. However, when the calculated area was converted by means of this equation to a "corrected" area, the relationship between measured and "corrected" areas and the scatter of individual areas could be estimated for each individual set of areas. The mean was 1.018, which showed excellent agreement between measured and "corrected" areas, and the standard deviation was 0.136, a scatter of approximately 14%.

Because of the circuitous nature of the above procedure, i.e., using one set of data to construct a mathematical relationship and then using the same set of data to test the mathematical relationship, an additional check was performed. Ten curves obtained during hypotension which appeared to have no visible distortion were randomly selected and the measured and "corrected" areas were obtained as described above. The measured/"corrected" area ratio for each pair was then determined and the mean and standard deviation of all these ratios was calculated. Three ratios were possible: a) a ratio substantially greater than one, which would indicate recirculation had occurred during hypotension and had been corrected for, b) a ratio close to one, this type of ratio would indicate that the curves selected did not contain significant recirculation, and c) a ratio substantially less than one, i.e., the Dow formula had overestimated the area. For our purpose, either a) or b) was considered an acceptable result. The actual result was a measured/"corrected" area ratio of 1.081 with a standard deviation of 0.245. Thus, it appeared that these ten curves probably exhibited a mixture of a) and b). This result was considered to validate use of the Dow formula in these experiments.

The cardiac output in l/min was calculated from the formula:

$$CO = \frac{I \times 60}{Area}$$

Where I is the mg of dye injected and area is the "corrected area" obtained from the Dow formula. All cardiac outputs were subsequently converted to ml/kg/min.

C. Estimation of Plasma Catecholamines.

The technique for estimation of catecholamines was a modification of the aluminum oxide-trihydroxyindole method of Anton and Sayre (1962). Blood from the ischiatic arterial cannula was allowed to freely flow for 105 seconds (approximately 30 ml) into a chilled polycarbonate centrifuge tube containing heparin and metabisulphite. After separation in a refrigerated centrifuge, plasma was deproteinized with perchloric acid and the catecholamines in the supernatant were adsorbed onto aluminum oxide. Catecholamines were eluted from the aluminum oxide by perchloric acid after several washings. Fluorescence was measured in an Aminco-Bowman Spectrophoto-fluorimeter at excitation and emission wavelengths respectively of 396 and 500 mμ for noradrenaline and 410 and 518 mμ for adrenaline. Adrenaline and noradrenaline were differentiated on the basis of differences in fluorescence at pH 2 and 7. Recovery of known standards averaged 85%.

D. Other Calculations, Terminology, and Statistics.

All peripheral resistances were calculated in Peripheral Resistance Units (PRU) by dividing the pressure gradient (mm Hg) by flow (ml/min). These gradients and flows were as follows: TPR, from MAP to CVP and the cardiac output (ml/kg/min); regional resistance, from MAP to CVP and the regional flow; arterial resistance (r_a), from MAP to VWP and the regional flow; and venous resistance (r_v), from VWP to CVP and the regional flow.

Capillary hydrostatic pressure (CP) was calculated from the formula of Pappenheimer and Soto-Rivera (1948):

$$CP = \frac{\frac{r_v}{r_a} MAP + VWP}{1 + \frac{r_v}{r_a}}$$

where the symbols have the same definitions as above.

The volume of blood removed from the animal at any time during an experiment is referred to as the "bleeding volume". The volume of blood removed to lower the MAP to the predetermined hypotensive level is the "initial bleeding volume" (IBV), the largest volume of blood removed during the course of a procedure is the "maximal bleeding volume" (MBV), and the difference between the IBV and MBV is the "secondary bleeding volume" (SBV), all expressed in ml/kg. When necessary, blood from the reservoir was returned to the animal through the arterial catheter. Any such "reinfusion" of blood was measured and deducted from the bleeding volume.

Serial estimates of plasma volume were calculated by the formula of Chien (1958). Provided the amount and duration of hemorrhage had not surpassed certain limits, this calculation agreed well with the measured plasma volume. The formula is given below and its solution is referred to as the "estimated plasma volume":

$$\text{estimated plasma volume} = \frac{(PV_1 \times PP_1) - \text{total plasma protein removed}}{PP_2}$$

where PV_1 is the initial measured plasma volume (ml/kg), PP_1 is the plasma protein concentration at the time of measurement of PV_1, and PP_2 is the plasma protein concentration at the time of the estimate.

"Relative" changes in the various cardiovascular parameters are expressed as a percentage of the value recorded at the initiation of bleeding. The "relative bleeding volume" is the bleeding volume expressed as a percentage of the initial measured blood volume.

Means are usually reported with the standard error (S.E.). For comparison of variables using data from the same set of experiments, all p values reported were calculated from the *t*-test for paired data. For comparison of variables using data from two different sets of experiments, all p values reported were calculated from the *t*-test for unpaired data. Statistical analysis of data from more than two different sets of experiments was carried out in one of several ways. The data were first tested by One-Way Analysis of Variance (ANVAR). If ANVAR indicated significant differences at $p < 0.05$, the location of the significant differences was sought by Duncan's Multiple Range Test (DMRT). Often however, this test could detect no significant differences in spite of the fact that ANVAR had already predicted their existence. Under these circumstances, the *t*-test for unpaired data was used to test each pair of means. In some instances, when it was necessary to compare more than two means under experimental conditions for which ANVAR was not a valid test of significance, the *t*-test for unpaired data was used alone. Differences were considered to be significant if $p < 0.05$ and the notation "n.s." indicates the differences were not significant at this level.

Most calculations except for ANVAR and DMRT were done on an Olivetti Programma 101 Desk Computer using appropriate programs. ANVAR and DMRT were performed on an IBM 360/50 Computer. The IBM 360/50 was

instructed to perform ANVAR, and, if ANVAR indicated a significance at $p < 0.05$, to follow up with DMRT at the appropriate p level for every possible combination of two means.

7. SPECIFIC EXPERIMENTS, RESULTS AND COMMENTS

A. Development of a Model for the Study of Hemorrhagic Hypotension in the Chicken.

I. Survey of Seasonal Variations in Selected Cardiovascular Parameters in Chickens. Cardiovascular studies carried out on mammals are often complicated by seasonal variation in the cardiovascular status of the animals. Seasonal variations in some cardiovascular parameters have also been reported in chickens (see Vogel & Sturkie, 1963 and Sturkie, 1967). These workers reported that blood volumes and cardiac outputs measured during the summer months were significantly lower than those measured in winter.

They also reported that cold-acclimatized birds (0-2° C for 12 hours and 10-12° C for 12 hours) showed little or no change from control (23-25° C for 24 hours), whereas heat-acclimatized birds (32° C for 12 hours and 24-25° C for 12 hours) had lower cardiac outputs and plasma volumes. In these studies, the length of day was standardized by artificial light and the differences were solely attributed to different ambient temperatures. These changes do not correspond to those reported for man and it was suggested that the circulatory adaptations to heat were different. The increase in cardiac output in man at high ambient temperatures presumably results from peripheral vasodilation, an increased vascular volume and a greater cardiac output to facilitate heat loss through the skin. In contrast, chickens lose heat predominantly through the respiratory system instead of through the skin and a decreased cardiac output and plasma volume would not adversely effect the chicken's ability to adjust to high ambient temperatures.

Although environmental conditions were rigidly controlled in the present study, it was sometimes necessary to compare experiments done at different times during the year and the following analysis of selected cardiovascular variables was carried out to test the validity of such comparison.

Methods. All experimental records obtained over a two year period were used as a source of data. The six cardiovascular parameters most consistently recorded were: plasma volume, blood volume, MAP, heart rate, hematocrit, and plasma protein concentration. Only the first measurement of each parameter following anesthesia and surgery in mature hens anesthetized with paraldehyde was used. Methods of measurement and statistics were as described under general methods.

The chickens were housed on wire mesh in a constant environment at a temperature of approximately 70° F for at least one week before use. Variation in length of daylight was minimized by the use of artificial lighting during the winter.

The experiments performed in any particular calendar month were considered to be a group, and groups of less than 8 animals were rejected. The result was a continuous spectrum of groups from November through April inclusive, but there were several gaps during the summer months and only July contained a sufficient number of animals for valid comparison.

Results. The results are shown in Fig. 3 and Table I. The data in Fig. 3 was analyzed by ANVAR followed by DMRT and the only

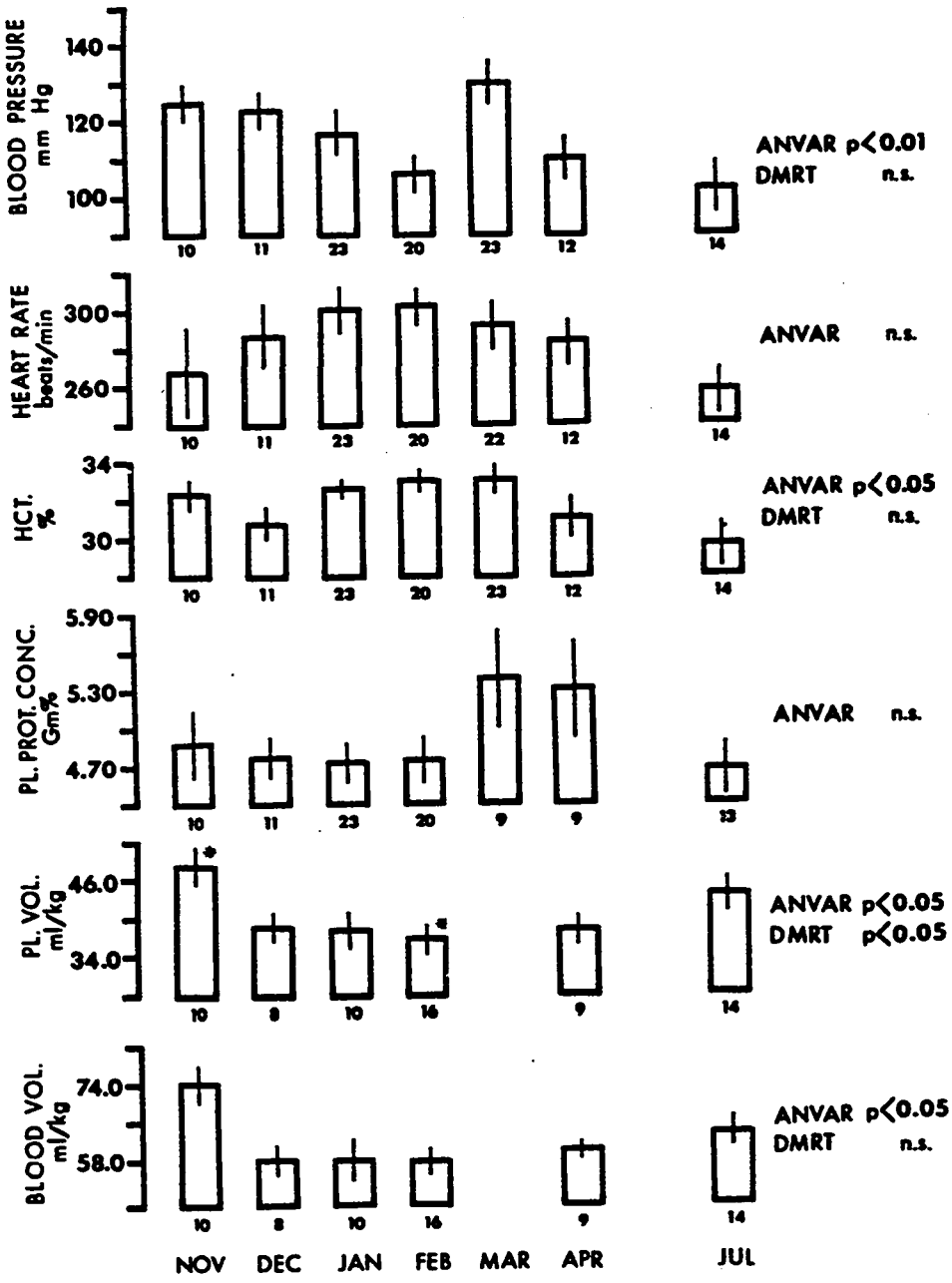

Figure 3. Seasonal Variation of Six Selected Cardiovascular Variables in Chickens. Bars represent the mean for each month (± S.E.). The number beneath each bar indicates the number of observations for that month. ANVAR is One-Way Analysis of Variance. DMRT is Duncan's Multiple Range Test. Asterisks indicate two means are significantly different when tested by DMRT.

Table I. Comparison of Six Cardiovascular Variables of Chickens Measured during the Months of January and July.

	n	January	n	July	p
MAP (mm Hg)	23	117 ± 6	14	102 ± 7	n.s.
Heart Rate (beats/min)	23	301 ± 13	14	257 ± 13	<0.05
Hematocrit (%)	23	32.6 ± 0.7	14	29.6 ± 1.2	<0.05
Plasma Protein Conc. (Gm%)	23	4.74 ± 0.15	14	4.66 ± 0.23	n.s.
Plasma Volume (ml/kg)	10	38.0 ± 3.1	14	43.2 ± 2.2	n.s.
Blood Volume (ml/kg)	10	55.7 ± 4.8	14	61.4 ± 3.2	n.s.

clearly statistically significant difference was between the plasma volumes determined in November and February. This was an unexpected result and was suspected to be an artifact, as will be commented upon later. No other clear significant differences were revealed by this test. However, ANVAR indicated significant differences in MAP, hematocrit and blood volume, although they could not be located by DMRT. Also, although ANVAR showed no significant difference in heart rate, this seemed to vary with time, reaching a peak during the months of January and February and falling off smoothly on either side. Because ANVAR cannot detect the significance of smooth variations in the plane xy, an additional analysis by the t-test for unpaired data was performed (Table I). The month of January was selected to compare with July because this represented the maximum time separation (6 months) between any two groups. This analysis showed both heart rate and hematocrit to be significantly lower in July.

Comment. The one completely unexpected result was the definite statistical difference between the plasma volumes measured in November and February. This difference also appeared in the blood volume, but was not significant in this case. As was previously mentioned, this apparent difference was believed to be an artifact. The probable explanation is based upon a chance difference between the two groups. It has been reported in the literature (Kotula & Helbacka, 1966) that blood or plasma volumes expressed in ml/kg do not remain constant with variation of size of chickens. A larger chicken has a substantially smaller blood or plasma volume when expressed in ml/kg. Thus the

fact that the November group of chickens had a significantly ($p < 0.05$) lower weight (1.64 ± 0.07 kg) than the February group (1.83 ± 0.06 kg) may account for the apparent difference in plasma volumes. In addition, the difference may have been accentuated by the smaller sample size of the November group.

It appeared that one week of acclimatization to the stable animal house environment was sufficient to minimize any seasonal variation in these six cardiovascular variables. However, there may still be some differences between winter and summer chickens under these conditions. As previously reported (Vogel & Sturkie, 1963 and Sturkie, 1967), the blood pressure had a tendency to be lower during the summer months. However, contrary to these previous reports, a lowered plasma volume was not seen. In fact, if anything, the plasma volume was increased during the summer months.

The observation of an increased plasma volume in the month of July was supported by a statistically significant decrease in hematocrit. The lowered plasma volume reported following prolonged exposure to high temperatures (see Vogel & Sturkie, 1963) has been attributed to dehydration (see Sturkie, 1967) and herein may lie the explanation of the apparent difference between previous studies and this one. The question of whether or not the water supply was critically short in their experiments resulting in dehydration is important. A lowered blood pressure during the summer months has been a consistent finding in most previous studies and also in this laboratory. However, a lowered blood pressure has been associated with an increased plasma volume in this laboratory rather than a decrease as Vogel & Sturkie (1963) have

reported in summer chickens.

The decreased heart rate during summer months which was found in this study has not previously been reported. Whether these differences were pre-existing seasonal variation or the result of a seasonal difference in sensitivity to paraldehyde cannot be said.

II. Ability of Chickens to Withstand a Period of Hemorrhagic Hypotension. These experiments were the initial ones of this investigation. Their purpose was to investigate the ability of chickens to survive a prolonged period of hemorrhagic hypotension and to obtain some measure of the effect of such a period of hypotension on a limited number of cardiovascular variables.

Methods. The animals in this group had not been rigorously selected to minimize variation in age and according to the definitions under general methods, the group was heterogeneous with respect to age, containing both pullets and mature hens. The methods for the measurement of MAP, hematocrit, plasma protein concentration, and plasma and blood volumes were given under general methods.

Plasma and blood volumes were measured in only about half the experiments. "Residual plasma volume" was calculated by subtracting the volume of plasma removed from the initial measured plasma volume and represented the amount of plasma which would remain in the vascular space if there were no fluid influx. "Estimated plasma volume" was calculated as outlined under general methods.

Only one ischiatic artery and the ipsilateral external iliac

vein were cannulated and cautery was kept to a minimum. MAP was lowered to 50 mm Hg and maintained at this level as described under general methods. After a period of 3-4 hours at this pressure, all the blood remaining in the reservoir was reinfused. The cannulas were removed, the ischiatic artery and external iliac vein were ligated, and, the wound was closed by sutures. Clean but not aseptic technique was used and no antibiotics were administered. All birds were maintained for 45 hours or until death to observe survival.

Results. The effect of this prolonged period of hemorrhagic hypotension on the selected cardiovascular variables in the whole series of experiments is seen in Table II. Fig. 4 and Fig. 5 are graphical summaries incorporating data from only the 7 experiments in which plasma and blood volumes were measured. Table II shows that all the variables measured which were common to the two groups were essentially identical in both the total group and the 7 experiments in which plasma and blood volumes were measured. Therefore, using only data from the 7 experiments in which plasma and blood volumes were measured resulted in no loss of information. Because determinations of hematocrit and plasma protein concentration were not performed according to a rigid protocol in these early experiments, only the final point shown during the latter half of the experiment included every animal. Thus the plot from 60 to 210 minutes in Fig. 5 and in the lower two curves in Fig. 4 lacked a certain amount of smoothness.

For these 7 experiments the IBV was 16.4 $\pm$ 1.6 ml/kg and the MBV was 37.8 $\pm$ 2.6 ml/kg. Thus, much more blood was removed after

Table II. Effects of a Period of Hemorrhagic Hypotension on Some Cardiovascular Variables of Chickens.

	Pre-Hemorrhage	After 3-4 Hr. Hypotension
	Whole Series n=17	
MAP (mm Hg)	96 ± 6	47 ± 1
Hematocrit (%)	29.1 ± 0.6	15.0 ± 0.8
Plasma Protein Conc. (Gm%)	4.02 ± 0.16	2.41 ± 0.09
Bleeding Volume (ml/kg)	---	38.6 ± 1.9
	Plasma and Blood Volumes Measured n=7 (included in Whole Series)	
MAP (mm Hg)	104 ± 10	49 ± 1
Hematocrit (%)	30.6 ± 0.9	15.2 ± 1.4
Plasma Protein Conc. (Gm%)	4.48 ± 0.16	2.46 ± 0.17
Blood Volume (ml/kg)	70.6 ± 5.0	58.9 ± 4.6
Bleeding Volume (ml/kg)	---	37.8 ± 2.5

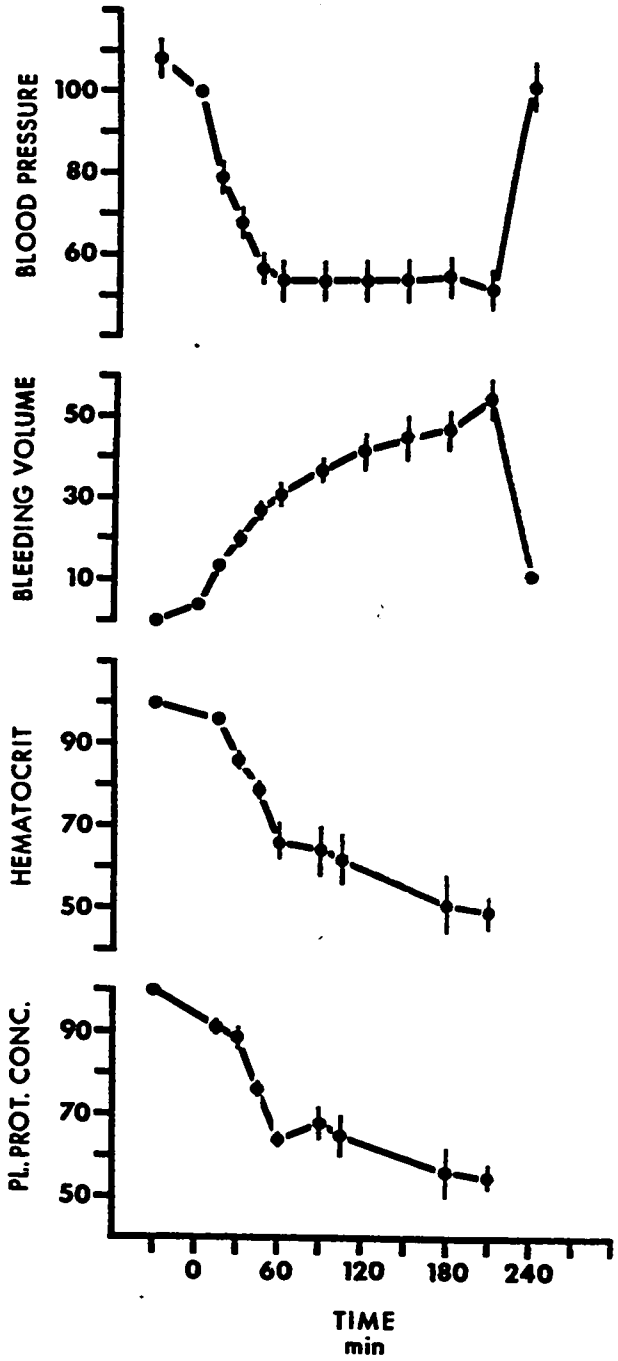

Figure 4. Course of Hemorrhagic Hypotension in White Leghorn Hens. Zero minutes is the start of systematic, graded hemorrhage. All numbers on the ordinate are percentages. Blood pressure at zero minutes and initial measurements of hematocrit and plasma protein concentration are 100%. Bleeding volume is expressed as a percentage of initial measured blood volume. Where no S.E.'s are shown, they are included in the limits of the symbols.

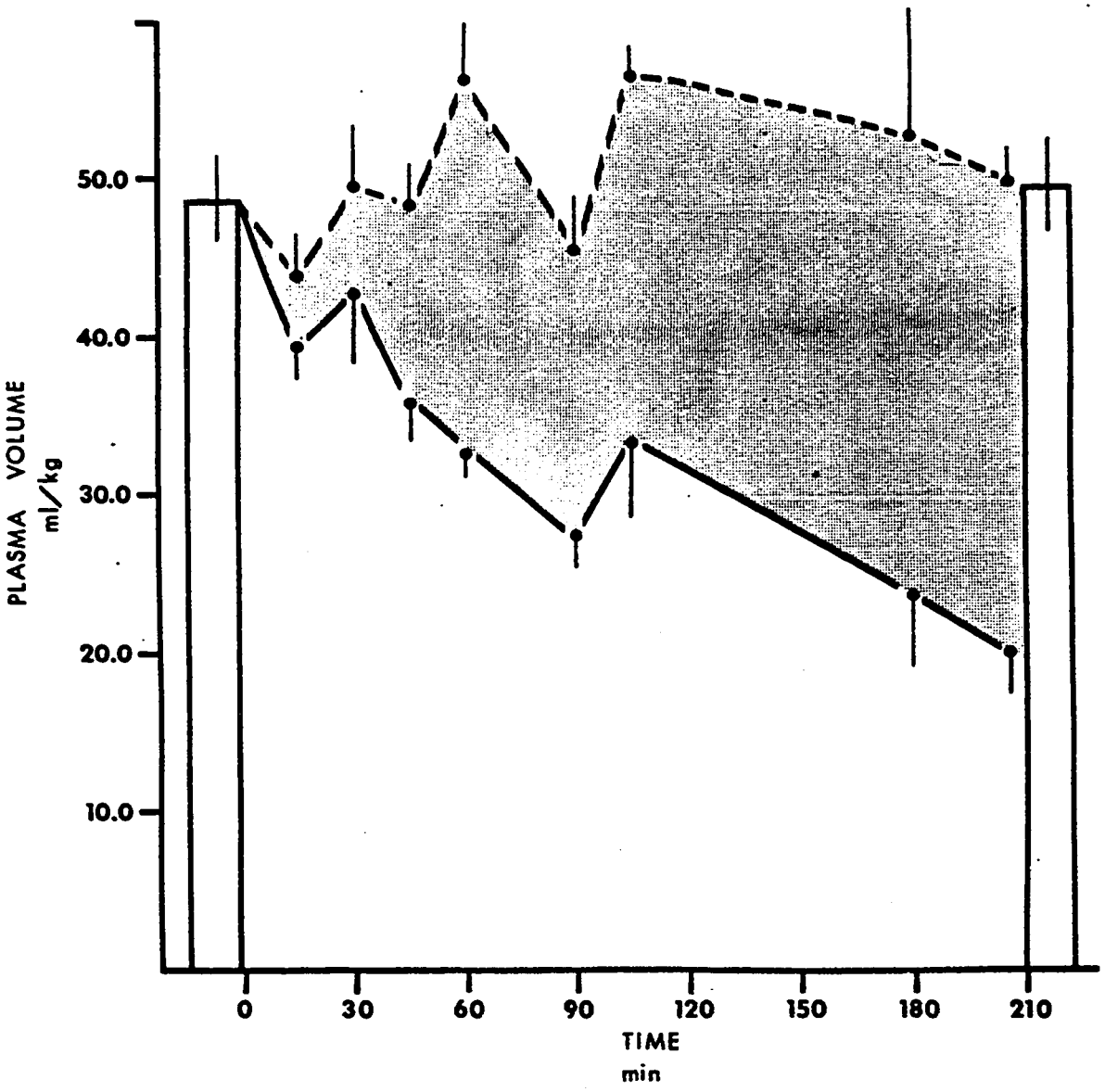

Figure 5. Hemodilution in Chickens during a Period of Hemorrhagic Hypotension. Bars indicate the measured plasma volume. The lower, solid line is the initial plasma volume minus the volume of plasma removed. The upper, broken line is the plasma volume estimated on the basis of protein dilution. Vertical lines indicate the S.E. of each point.

reaching 50 mm Hg than was necessary to lower MAP to this level in the first place. The volume reinfused following the period of hemorrhagic hypotension was 30.2 ± 3.0 ml/kg and the net volume deficit at the end of the experiment was 8 ml/kg, the amount of blood used in determining hematocrit, plasma protein concentration, and plasma and blood volumes.

The total duration of hypotension and continuous bleeding was 3-4 hours and the time taken for the MAP to fall to 50 mm Hg was approximately 20 minutes. The average time from reaching 50 mm Hg to reinfusion in these 7 experiments was 200 ± 15 minutes.

Fig. 4 shows the "relative" changes in blood pressure, hematocrit and plasma protein concentration during hemorrhage. During the blood volume determination (portion of Fig. 4 to the left of 0 min), it was necessary to remove a small amount of blood (approximately 2 ml/kg) and Fig. 4 shows that this resulted in a small but definite fall in MAP, hematocrit and plasma protein concentration. When systematic, graded hemorrhage was started, these variables fell even more markedly until, after 3-4 hours of hypotension, when the volume removed was 55.4 ± 5.1% of the initial measured blood volume, hematocrit and plasma protein concentration had fallen to 49.5 ± 3.8 and 54.9 ± 2.8% respectively.

Table II shows that even after the removal of 37.8 ml/kg, the measured blood volume had fallen only 11.7 ml/kg. This observation, supported by the marked decreases in hematocrit and plasma protein concentration, were indicative of the massive hemodilution which is illustrated diagrammatically in Fig. 5. Plasma volume measured be-

fore the start of hemorrhage and that just prior to reinfusion were essentially identical, indicating that the volume of plasma removed over the 3-4 hours of hemorrhagic hypotension was quantitatively replaced. The shaded area between the two lines in Fig. 5 represents the amount of fluid mobilized into the vascular space.

From a quantitative viewpoint, the volume of plasma remaining in the vascular space just before reinfusion which was part of the initial plasma volume was 19.8 $\pm$ 2.6 ml/kg. Estimation of the plasma volume from the protein dilution indicated a plasma volume of 49.4 $\pm$ 2.5 ml/kg after 3-4 hours of hemorrhagic hypotension, a figure which was in excellent agreement with the measured volume at this time. Thus, the total volume of fluid mobilized was 29.6 ml/kg (61% of the initial plasma volume) and represents a remarkable 7.4 ml/kg/hr.

Of the 17 hens subjected to this experimental procedure, all survived the experiment itself and only 3 died within a 48-hour postoperative observation period, 1 at 12-24 and 2 at 24-48 hours after reinfusion. A few of the 48-hour survivors were observed over longer periods (up to 96 hours) and these appeared capable of continued survival at that time.

Comment. The plasma and blood volumes reported here were higher than those encountered in homogeneous mature hens and this was believed to be because of the smaller size of the present series of chickens (1.37 $\pm$ 0.06 kg) resulting in larger volumes on a ml/kg basis in a similar manner to that discussed earlier with respect to seasonal variation (Section I). Otherwise, the selected cardiovascular parameters

fit well with the previously reported values.

One of the most remarkable differences between these experiments in chickens and experiments in dogs was the relative ease with which the MAP in chickens can be lowered by removal of even a small amount of blood. Chien (1958) has reported that in splenectomized dogs, the bleeding volume must be greater than 10% of the initial blood volume before there was any fall in MAP. In these experiments on chickens, removal of only 2 ml/kg (less than 3% of the initial blood volume) lowered the MAP by 8% (9 mm Hg).

Further, although the time to reach 50 mm Hg in these experiments was very similar to that to reach 35 mm Hg in dogs (Hollenberg, 1965), the bleeding volumes were substantially different. Hollenberg reported an IBV and MBV of 45.1 and 52.1 ml/kg respectively while these experiments on chickens gave corresponding values of 16.4 and 37.8 ml/kg, both substantially lower than those found in dogs. It must be noted however, that the MBV reported for chickens in these experiments was not truly a <u>maximum</u>. The experiments were arbitrarily terminated after 3-4 hours of hypotension and possibly the <u>maximum</u> bleeding volume had not been reached. The plasma volume reported by Hollenberg (49.2 ml/kg) was essentially the same as that found in the chickens, and, assuming a somewhat larger blood volume in the dogs because the red cell volume (hematocrit = 41.5%) was larger than chickens, the differences, especially between IBV's were still remarkable.

There were also obvious differences in the relationship between IBV and MBV in the two species. In dogs Hollenberg reported a time from IBV to MBV of about 42 minutes while in the present experiments a

period of about 200 minutes intervened between IBV and MBV. Not only was the time-relationship different, but the volumes themselves differed markedly. The ratio of SBV to IBV (relationship between the volume removed after reaching the set hypotensive level and the volume removed to reach that level) was 0.16 in dogs and the ratio for chickens was 1.9.

In the experiments on dogs (Hollenberg, 1965), approximately 3 hours after MBV, during which time the dogs spontaneously took up 47 ml/kg from the reservoir, death resulted. In these experiments on chickens it was never necessary to reinfuse blood and apparently a point of irreversibility had not been reached because reinfusion of the shed blood gave an excellent survival rate.

The massive hemodilution and fluid mobilization seen in the chickens is also very interesting. It has been reported (Allen et al., 1959) that following removal of 57% of the initial blood volume of splenectomized dogs, a percentage removal similar to the present observation in chickens, the fluid mobilization was approximately 10% of the initial blood volume. The present experiments in chickens resulted in fluid mobilization of 36.9% of the initial blood volume (29.6 ml/kg), a figure nearly 4 times as great as that seen in the splenectomized dog. Hollenberg (1965) has reported that the maximum plasma volume replacement in dogs with an intact spleen was 12.4 ml/kg (8.2 ml/kg of low-protein fluid). Thus the maximum plasma volume replacement in intact dogs was less than half that seen in the present experiments in chickens.

During a period of 4 hours, protein-poor fluid was mobilized

into the vascular space of the chickens at an average rate of 7.4 ml/kg/hr. The data of Hollenberg (1965) indicated mobilization of protein-poor fluid by the dog at a rate (6.1 ml/kg/hr) similar to the present experiments in chickens but after slightly more than one hour, this mobilization not only stopped, but began to reverse. However, the rate of fluid mobilization is always more rapid initially. Therefore, a better comparison of rates of mobilization in dogs and chickens was seen when similar time intervals were compared. The rate of fluid mobilization during the first 90 minutes in chickens in these experiments averaged 12.1 ml/kg/hr which was approximately double the rate for dogs calculated from the data of Hollenberg. The experiments of Skillman et al. (1967) showed that in the first six hours following a hemorrhage of approximately 11 ml/kg in man, the rate of fluid mobilization averaged only 1 ml/kg/hr.

Thus, the chicken's response to hemorrhage showed two marked differences when compared to mammals: a) a marked inability to maintain MAP in the face of even a small hemorrhage, and, b) a massive extravascular fluid mobilization which quantitatively was 2.5 to 4 times greater than that reported for dogs bled to a similar extent (depending upon whether or not the dogs were splenectomized), faster than that in dogs and much faster than that in man.

III. Effects of Age and Artificial Ventilation on the Course of Hemorrhagic Hypotension in Chickens. Preliminary observations had suggested that some chickens subjected to a period of hemorrhagic hypotension died from respiratory arrest rather than cardiovascular

failure of the type usually associated with hemorrhagic shock. There also seemed to be some differences in the response to a period of hemorrhagic hypotension in chickens of different ages. The experiments reported in this section were carried out to assess any effects of age and of artificial ventilation on the response of chickens to a period of hemorrhagic hypotension. In addition, it was desirable to obtain answers to some other questions which had arisen during the first series of experiments. How much blood could be removed before death occurred and what period of time would this take? Further, to what extent could the hemodilution seen in the previous experiments be extended? Finally, was there any evidence for hemoconcentration and decompensation in the terminal stages of the hypotensive period?

Methods. Chickens were selected for age and housed as described under general methods. Four different groups were used: a) Unrespired Pullets, b) Respired Pullets, c) Unrespired Hens, and, d) Respired Hens. Following anesthetization and cannulation of an ischiatic artery and the ipsilateral external iliac vein, the following measurements were recorded or calculated initially, every 15 minutes for the first hour of bleeding, and every 30 minutes thereafter: MAP, large vein pressure (LVP), bleeding volume, heart rate, hematocrit, plasma protein concentration, initial plasma volume minus the volume of plasma removed, and plasma volume estimated on the basis of protein dilution. Plasma and blood volumes were measured initially, and at 90, 210 and 360 minutes after the start of bleeding. All techniques for these measurements and calculations are described under general methods.

The animals were bled to a MAP of 50 mm Hg as described under general methods and maintained at this level until death or for ten hours. During the later stages of the experiments, the small amounts of blood removed for determinations of plasma volume, hematocrit and plasma protein concentration were quantitatively replaced by blood from the reservoir to avoid cardiovascular collapse which might have been precipitated by taking the samples, otherwise no blood was reinfused. A lack of an open connection between the animal and the reservoir ensured that any changes in volumes, hematocrit, and plasma protein concentration were the result of hemodynamic adjustments within the animal. In longer experiments a supplementary dose of heparin (2.5 mg/kg) was given after two hours of bleeding.

All birds had a plastic catheter inserted into their trachea to ensure a free airway. Artificial ventilation was accomplished by means of intermittant positive pressure with room air using a Harvard Respiration Pump (Model No. 607). The rate was set at 30/min and the volume was judged visually from thoracic expansion. Adequate oxygenation was evidenced by bright red arterial blood, but because of the relatively small tidal volume in chickens (see Burton & Smith, 1968), there was probably a tendency to hyperventilation.

Results. The bleeding volumes and time intervals from reaching 50 mm Hg MAP until death for the four groups are shown in Table III. Only two chickens survived the 10 hour limit and both these were in the Respired Hens group. For purposes of computing the mean time interval from reaching 50 mm Hg MAP until death, both these experi-

Table III. Effects of Age and Artificial Ventilation on Bleeding Volumes and Duration of Hypotension Leading to Death.

	n	Weight (kg)	IBV (ml/kg)	MBV (ml/kg)	SBV (ml/kg)	Minutes at 50 mm Hg
Unrespired Pullets	14	1.78 ± 0.05	13.9 ± 1.4	32.9 ± 2.6	18.9 ± 2.4	179 ± 32*
Respired Pullets	14	1.69 ± 0.04	10.7 ± 1.0	37.2 ± 2.4	26.5 ± 2.3	319 ± 28
Unrespired Hens	13	1.73 ± 0.08	12.6 ± 1.7	37.4 ± 3.1	24.8 ± 3.2	302 ± 44
Respired Hens	12	1.80 ± 0.05	9.8 ± 1.1	38.1 ± 2.3	28.3 ± 2.1	384 ± 62

* Significantly less than the other three groups $p<0.05$

ments were considered to be 600 minutes. The bleeding volumes were remarkably similar in all groups although the SBV for Unrespired Pullets appeared somewhat lower than the other SBV's. The time interval from reaching 50 mm Hg MAP until death was also significantly shorter ($p < 0.05$) for Unrespired Pullets than for other groups when tested by *t*-test for unpaired data.

The lower SBV of Unrespired Pullets was not detected as significant by ANVAR although it was close at $p < 0.05$. Therefore, this difference was considered borderline even though the *t*-test for unpaired data showed the SBV of Unrespired Pullets to be significantly less than that of Respired Pullets ($p < 0.05$).

Because of the difficulty of demonstrating a significant difference in the time interval from reaching 50 mm Hg MAP until death in Unrespired Pullets, an alternative method of illustration was also used. Fig. 6 is a plot of the percentage deaths versus the minutes at 50 mm Hg. Except for the last value in each case, the points in each group seemed to follow relatively straight lines. The lines shown in Fig. 6 are the lines of best fit calculated by the Method of Least Squares. Proof that these are straight-line relationships was given by the correlation coefficients which in all four groups was never less than 0.95. The effect of artificial ventilation in pullets appeared as a shift of the curve to the right as shown in Fig. 6. The curves for Unrespired and Respired Hens are not shown, but were very similar to the curve for Respired Pullets shown in Fig. 6 (broken line).

Accepting the validity of these straight-line relationships,

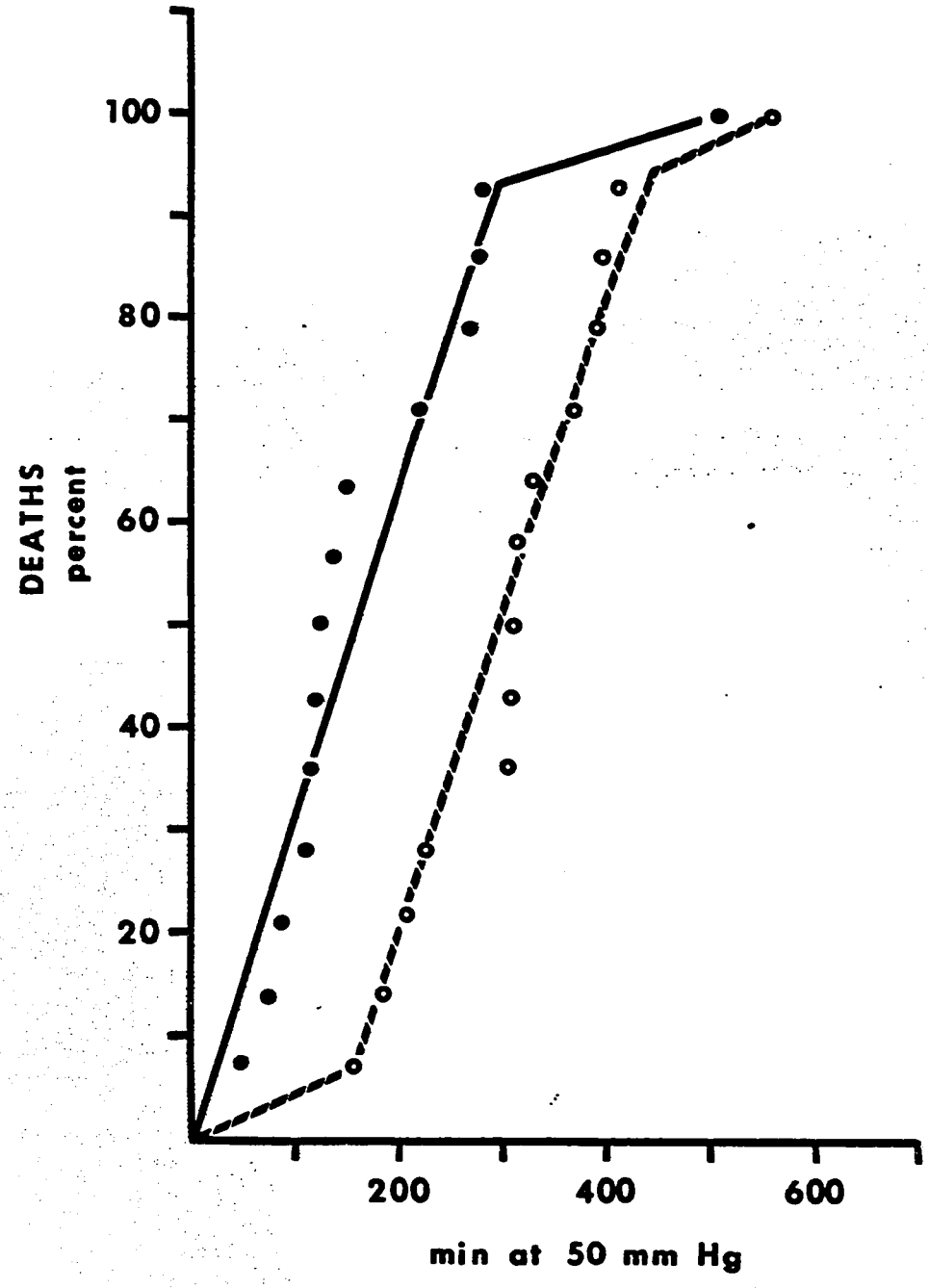

Figure 6. Plot of Percentage Deaths versus Duration of Hypotension in Unrespired and Respired Pullets. Closed circles and solid line are Unrespired Pullets. Open circles and broken line are Respired Pullets.

the time coordinate at which the regression line for each group intercepted the coordinate corresponding to 50% deaths became an estimate of the duration at 50 mm Hg MAP necessary to produce death in half the animals. The equations for the lines of best fit shown in Fig. 6 were solved to give these "LT_{50}" values (Lethal Time for 50% of the animals) which were 154 minutes for Unrespired Pullets and 301 minutes for Respired Pullets. Corresponding values for the Unrespired and Respired Hens were 283 and 302 minutes respectively.

The measurements of cardiovascular parameters at selected time intervals during the experiments are summarized in Tables IV to VII inclusive. In these tables, 0 minutes was taken to be the initiation of the systematic, graded hemorrhage. The negative times represent the control period preceding bleeding during which the first measurements of plasma and blood volumes were made. For the sake of brevity, only values recorded at the selected time intervals were shown in these tables.

In Table IV it can be seen that MAP was identical in all groups at all the selected times. LVP in respired chickens seemed to be lower than that in the corresponding unrespired group and lower in hens than in pullets. However, these differences were not detected as significant by DMRT. Analysis of LVP at 90 minutes (ANVAR, $p < 0.05$) by t-test for unpaired data detected no significant difference between nonrespired and the corresponding respired groups but Respired Hens had lower LVP's than Unrespired Pullets. At 210 minutes, the Respired Hens did show a significantly lower LVP than the Unrespired Hens. Terminal measurements of LVP followed the general trend of higher LVP's

Table IV. Effects of a Period of Hemorrhagic Hypotension on Mean Arterial and Large Vein Pressures.

	-30 Minutes	0 Minutes	90 Minutes	210 Minutes	Terminal
			MAP (mm Hg)		
Unrespired Pullets	111 ± 5	118 ± 5	50 ± 0	---	38 ± 3
Respired Pullets	104 ± 5	106 ± 3	50 ± 0	50 ± 0	37 ± 3
Unrespired Hens	107 ± 5	106 ± 5	50 ± 0	50 ± 0	34 ± 3
Respired Hens	112 ± 7	106 ± 6	50 ± 0	50 ± 0	36 ± 5
			LVP (mm Hg)		
Unrespired Pullets	16.7 ± 1.5	17.0 ± 1.9	13.7 ± 1.4	---	10.2 ± 0.9
Respired Pullets	16.0 ± 2.1	14.6 ± 1.8	9.9 ± 1.2	6.9 ± 1.0	5.9 ± 0.9**
Unrespired Hens	15.3 ± 1.9	13.4 ± 1.5	10.0 ± 1.1	9.8 ± 1.2	8.9 ± 1.3
Respired Hens	14.4 ± 2.1	11.6 ± 1.5	7.7 ± 0.9**	6.4 ± 0.7*	5.9 ± 1.0**

* Significantly different from Unrespired Hens $p<0.05$

** Significantly different from Unrespired Pullets $p<0.01$

Table V. Effects of a Period of Hemorrhagic Hypotension on Hematocrit and Plasma Protein Concentration.

	-30 Minutes	0 Minutes	90 Minutes	210 Minutes	Terminal
			Hematocrit (%)		
Unrespired Pullets	31.4 ± 1.2	30.1 ± 1.0	21.3 ± 0.9	---	19.3 ± 1.0
Respired Pullets	31.0 ± 0.8	30.1 ± 0.8	22.3 ± 0.5	17.8 ± 0.8	18.3 ± 0.7
Unrespired Hens	33.3 ± 1.8	32.9 ± 1.1	24.5 ± 1.1	20.2 ± 1.1	18.3 ± 1.2
Respired Hens	29.5 ± 0.8	28.8 ± 0.8**	21.9 ± 0.9	19.8 ± 0.9	16.2 ± 0.8
			Plasma Protein Concentration (Gm%)		
Unrespired Pullets	5.46 ± 0.25	5.29 ± 0.19	3.91 ± 0.20	---	3.60 ± 0.14
Respired Pullets	5.06 ± 0.26	4.86 ± 0.19	3.71 ± 0.21	3.20 ± 0.25	3.47 ± 0.27
Unrespired Hens	4.97 ± 0.19	4.64 ± 0.18	3.41 ± 0.17	2.83 ± 0.21	2.93 ± 0.21
Respired Hens	5.95 ± 0.39	5.41 ± 0.25*	4.22 ± 0.35	4.21 ± 0.43**	3.55 ± 0.15

* Significantly different than Unrespired Hens $p<0.05$

** Significantly different than Unrespired Hens $p<0.01$

Table VI. Bleeding Volume and Measured Plasma Volume during a Period of Hemorrhagic Hypotension.

	90 Minutes	210 Minutes	Terminal
	Bleeding Volume (ml/kg)		
Unrespired Pullets	25.6 ± 1.8	---	32.9 ± 2.6
Respired Pullets	22.2 ± 1.1	31.2 ± 2.1	37.1 ± 2.4
Unrespired Hens	21.5 ± 1.2	32.4 ± 1.6	37.3 ± 3.4
Respired Hens	21.6 ± 2.0	25.8 ± 2.7	37.6 ± 2.1
	0 Minutes	90 Minutes	210 Minutes
	Plasma Volume (ml/kg)		
Unrespired Pullets	37.3 ± 1.5	31.9 ± 1.2	---
Respired Pullets	36.4 ± 1.6	33.0 ± 1.2	34.0 ± 1.5
Unrespired Hens	38.1 ± 2.0	33.7 ± 1.7	35.4 ± 1.9
Respired Hens	40.8 ± 1.7	36.0 ± 1.8	34.5 ± 2.4

Table VII. Effect of a Period of Hemorrhagic Hypotension on Heart Rate.

	-30 Minutes	0 Minutes	90 Minutes	210 Minutes	Terminal
	Heart Rate (beats/min)				
Unrespired Pullets	319 ± 13	326 ± 12	395 ± 15	---	335 ± 18
Respired Pullets	239 ± 11*	227 ± 12*	321 ± 12*	331 ± 7*	230 ± 16*
Unrespired Hens	314 ± 18	304 ± 14	393 ± 8	392 ± 9	354 ± 14
Respired Hens	250 ± 12*	229 ± 12*	307 ± 14*	313 ± 9*	241 ± 23*

* Significantly different from both Unrespired Hens and Pullets; ANVAR, $p<0.01$

DMRT, $p<0.05$

in the unrespired than in the corresponding respired group. However, DMRT did not detect these differences as significant (ANVAR, $p < 0.01$) and therefore the *t*-test for unpaired data was once again applied. Both Respired Hens and Respired Pullets had significantly lower LVP's than Unrespired Pullets but not Unrespired Hens. In general, the effect of artificial ventilation was to lower LVP. The exact reason for this lowered LVP was unknown. Perhaps it resulted from a mechanical effect of ventilation on the large veins of the thorax and abdomen which in turn could have affected LVP in the iliac region.

In Table V, mobilization of protein-poor fluid was apparent from the magnitude of decrease in hematocrit and plasma protein concentration. The degree and rate of decrease were remarkably constant in all four groups. ANVAR unexpectedly predicted significant differences between hematocrits and plasma protein concentrations at zero minutes. What these differences were could not be detected by DMRT and no definite pattern on the basis of age and artificial ventilation could be seen on visual inspection. The *t*-test for unpaired data detected the differences to be between Unrespired and Respired Hens. Unrespired Hens had higher hematocrits ($p < 0.01$) and lower plasma protein concentrations ($p < 0.05$) than the Respired Hens. There was no apparent reason for these differences as the experiments were done at the same time of the year. In the Respired Hens, hematocrit remained lower and plasma protein concentration higher than in the Unrespired Hens for the duration of the experiments but was only statistically significant in the case of plasma protein concentration at 210 minutes.

The extent of the decreases in hematocrit and plasma protein

concentration was very similar in all groups. It did seem, however, that within an individual group the hematocrit always fell to a relatively greater extent than the plasma protein concentration. Although the hematocrit and plasma protein concentrations both fell during the initial blood volume determination, the plasma protein concentration always fell to a greater extent than the hematocrit during this period. Thus although the hematocrit appeared to fall more (approximately 45%) than plasma protein concentration (approximately 40%) from 0 minutes to death, the "relative" decrease from -30 minutes to death was the same in both cases (approximately 47%).

After an initial slight fall, the measured plasma volume shown in Table VI tended to remain constant in spite of continued hemorrhage.

In Table VII, the increased heart rate which levelled off and finally declined in the terminal stages was a consistent finding for all groups. Of all the cardiovascular variables measured in these experiments this was the only one where a definite, unquestionable statistical difference between the various groups was seen. A comparison of heart rates between mature hens and pullets showed no differences which could be attributed to age, but artifical ventilation resulted in a lowered heart rate in both pullets and mature hens. This difference was consistently shown to be significant by ANVAR ($p < 0.01$) and DMRT ($p < 0.05$) in every case.

Further comparison of these four different groups over the entire duration of the experiment is given graphically in Figs. 7 to 11 inclusive. The obvious difference between Unrespired and Respired Pullets in Fig. 7 was that the respired chickens could be bled for a

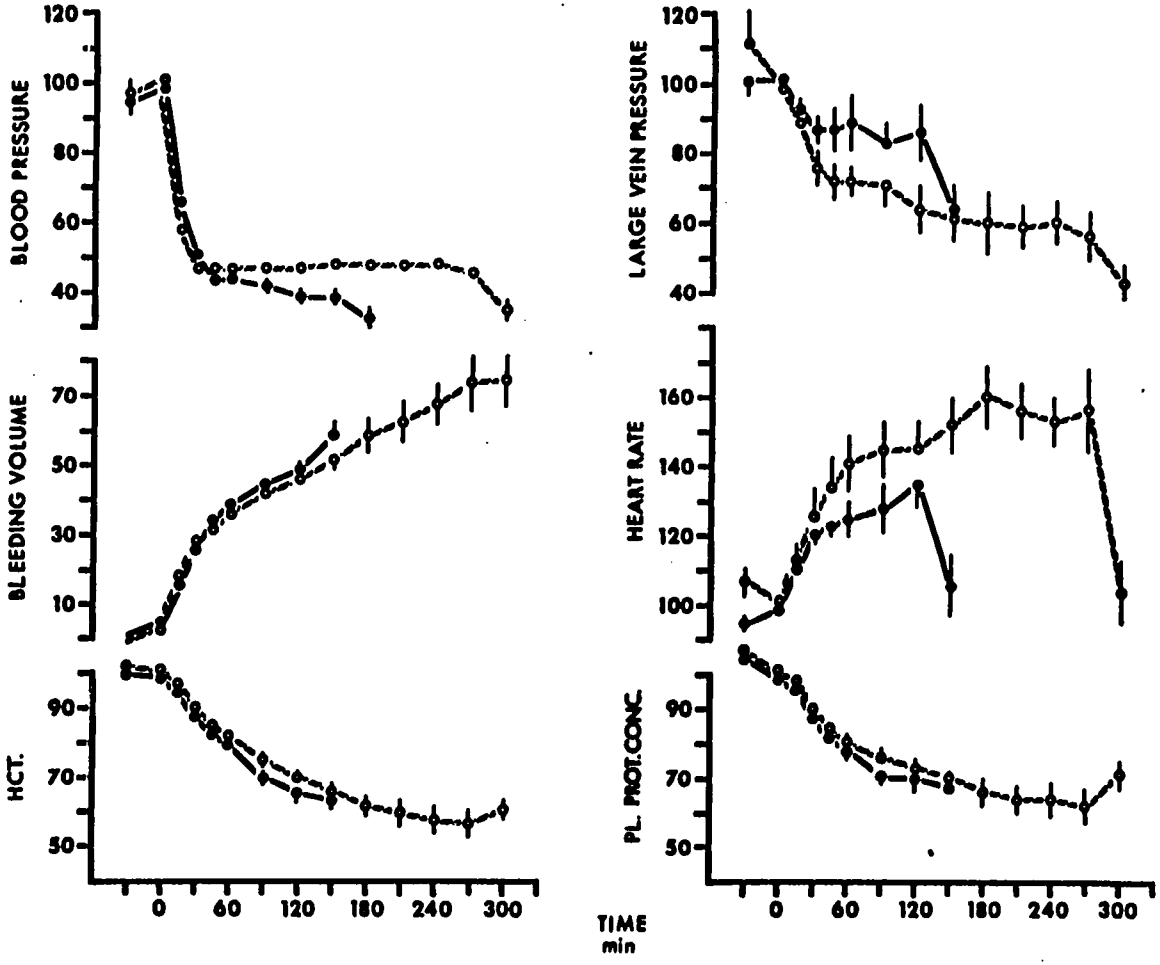

Figure 7. Comparison of the Course of Hemorrhagic Hypotension in Unrespired and Respired Pullets. Zero minutes is the start of systematic, graded hemorrhage. All numbers on the ordinate are percentages. Zero minute measurements of blood pressure, large vein pressure, heart rate, hematocrit and plasma protein concentration are 100%. Bleeding volume is expressed as a percentage of initial measured blood volume. Closed circles and solid lines are Unrespired Pullets. Open circles and broken lines are Respired Pullets. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s of two points overlap, they are shown in only one direction.

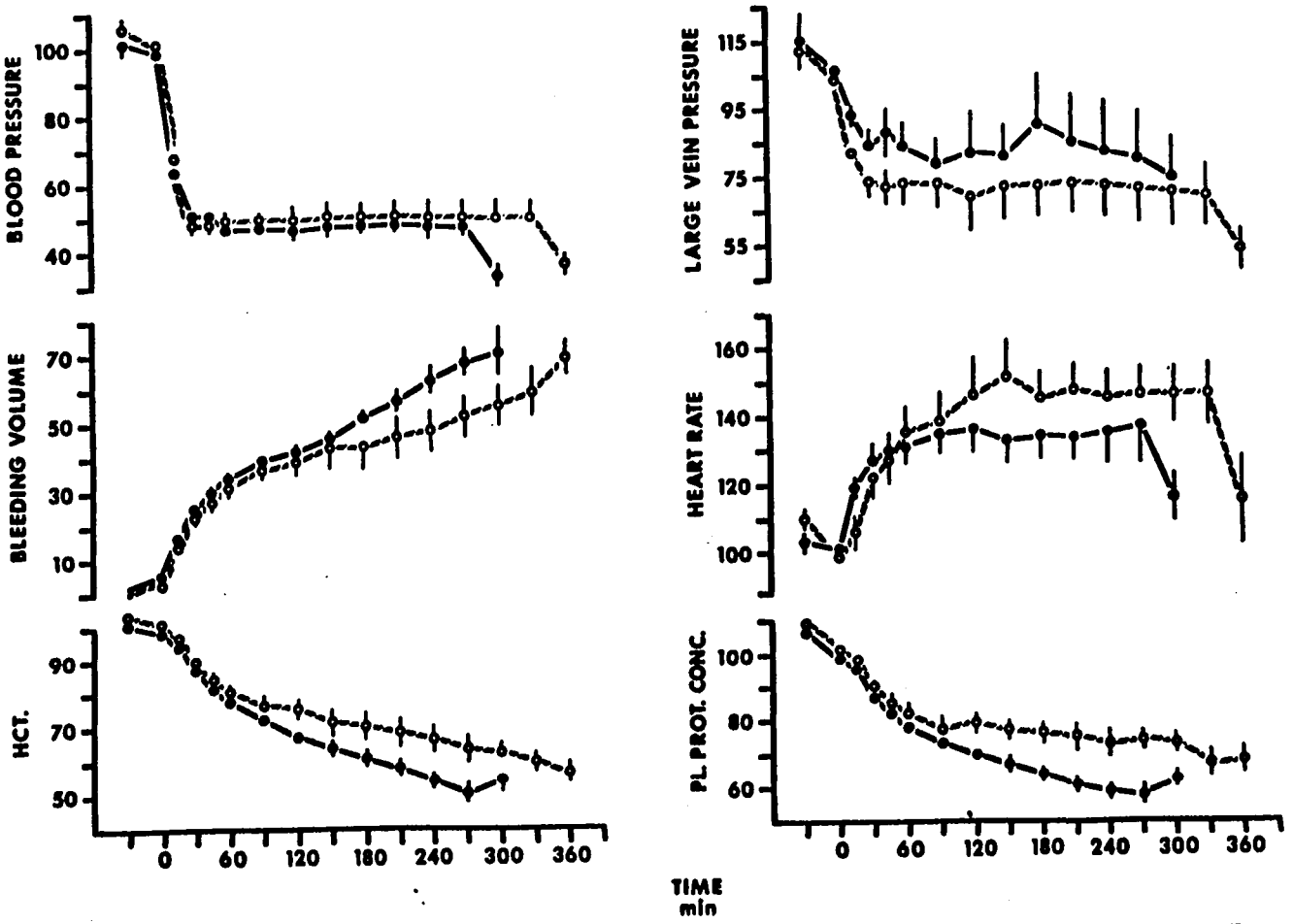

Figure 8. Comparison of the Course of Hemorrhagic Hypotension in Unrespired and Respired Hens. Zero minutes is the start of systematic graded hemorrhage. All numbers on the ordinates are percentages. Zero minute measurements of blood pressure, large vein pressure, heart rate, hematocrit and plasma protein concentration are 100%. Bleeding volume is expressed as a percentage of initial measured blood volume. Closed circles and solid lines are Unrespired Hens. Open circles and broken lines are Respired Hens. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s of two points overlap, they are shown in only one direction.

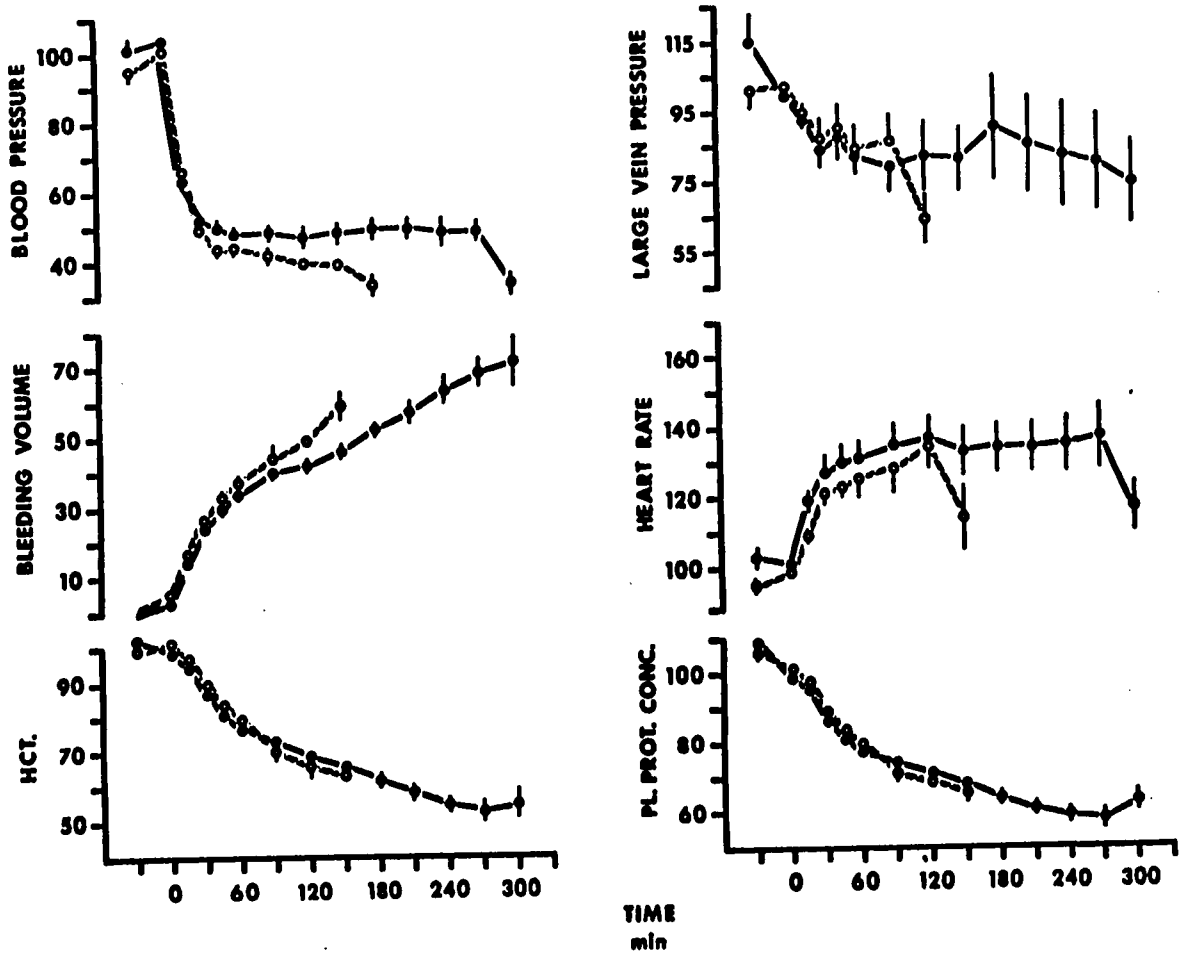

Figure 9. Comparison of the Course of Hemorrhagic Hypotension in Unrespired Pullets and Hens. Zero minutes is the start of systematic, graded hemorrhage. All numbers on the ordinate are percentages. Zero minute measurements of blood pressure, large vein pressure, heart rate, hematocrit and plasma protein concentration are 100%. Bleeding volume is expressed as a percentage of initial measured blood volume. Closed circles and solid lines are Unrespired Hens. Open circles and broken lines are Unrespired Pullets. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s of two points overlap, they are shown in only one direction.

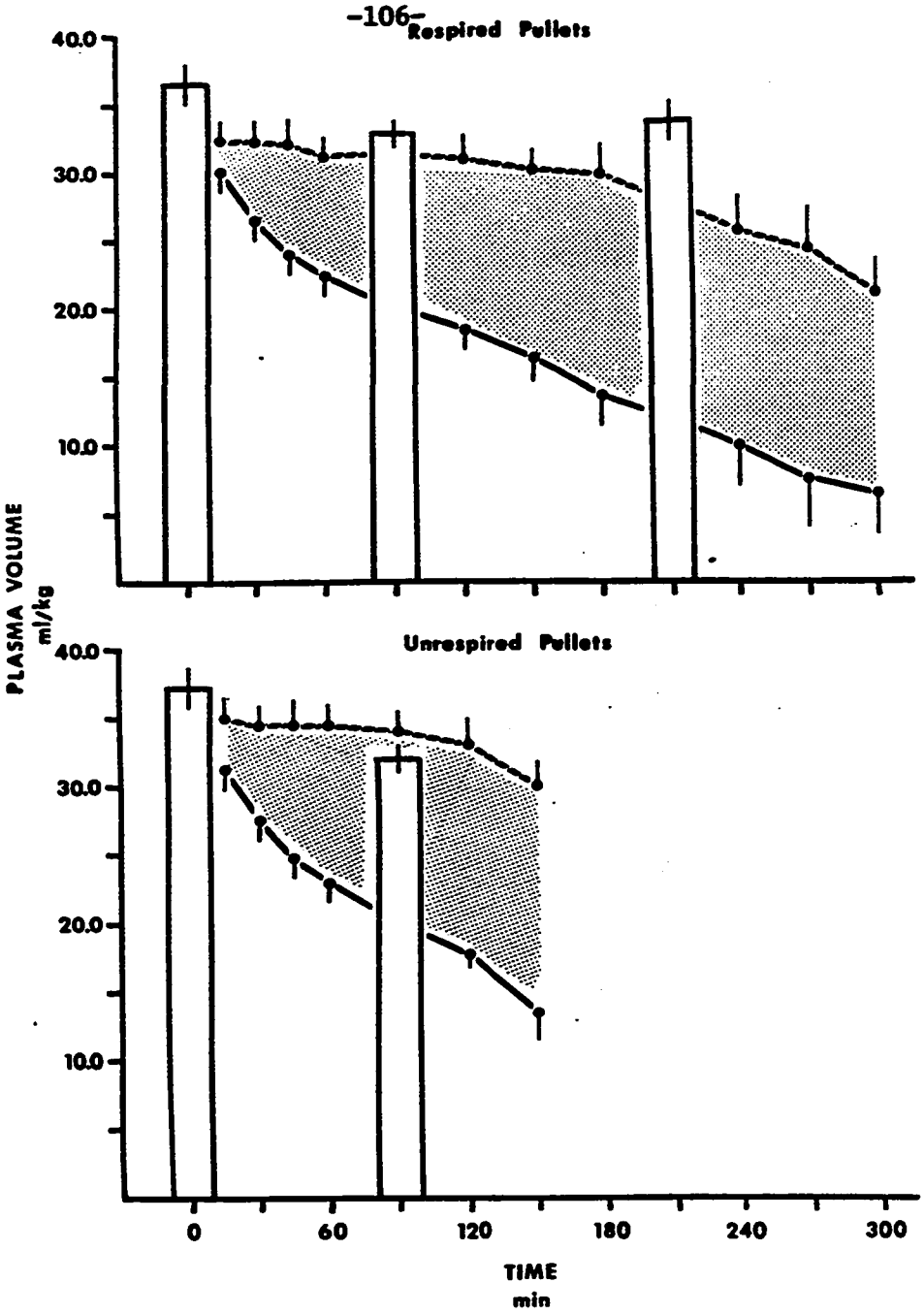

Figure 10. Comparison of Hemodilution in Unrespired and Respired Pullets. Bars indicate the measured plasma volume. The lower, solid line in each diagram is the initial plasma volume minus the volume of plasma removed. The upper, broken line in each diagram is the plasma volume estimated on the basis of protein dilution. Vertical lines indicate the S.E. of each point.

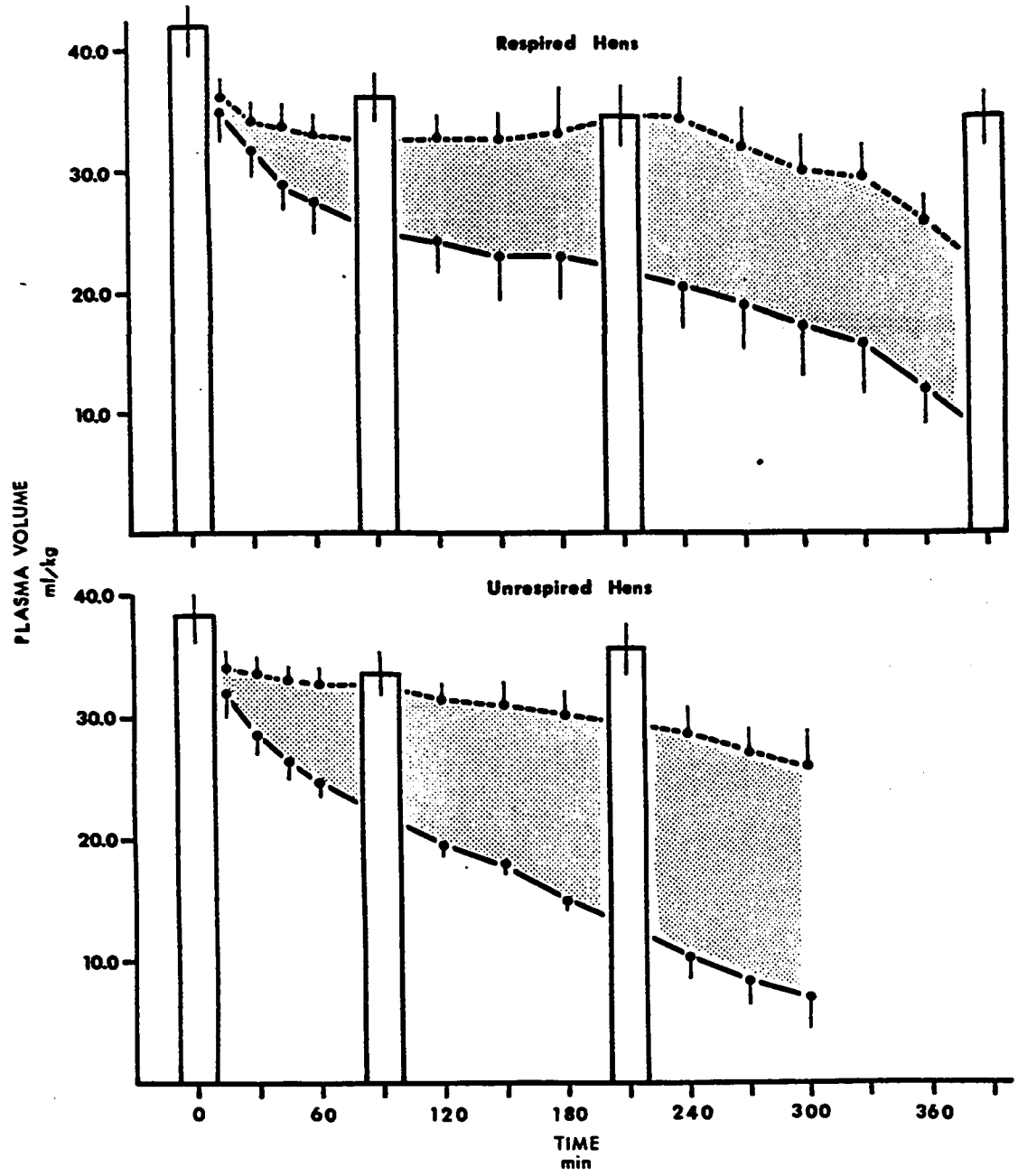

Figure 11. Comparison of Hemodilution in Unrespired and Respired Hens. Bars indicate the measured plasma volume. The lower, solid line in each diagram is the initial plasma volume minus the volume of plasma removed. The upper, broken line in each diagram is the plasma volume estimated on the basis of protein dilution. Vertical lines indicate the S.E. of each point.

longer period. In both groups the "relative bleeding volume" was greater than 50% of the initial measured blood volume. In the case of Respired Pullets, a volume equivalent to about 75% of the initial measured blood volume was removed and somewhat less blood (60%) was removed from Unrespired Pullets. The falls in MAP and LVP and the marked decrease in hematocrit and plasma protein concentration are obvious in this diagram.

In view of the differences between absolute heart rates seen in Table VII, it was interesting to note that the "relative" changes in heart rate were very similar. During the later stages the heart rate appeared to have increased more in the Respired than Unrespired Pullets but the differences were not significant. The effect of artificial ventilation on the heart rate was evident during the period from -30 to 0 minutes where "relative heart rate" was falling in Respired Pullets while it rose slightly in Unrespired Pullets during the same time period. With the initiation of hemorrhage the "relative heart rate" showed the same tendency as the "absolute heart rate" shown in Table VII, i.e., an increase which then levelled off and a terminal decrease.

Everything which was said concerning pullets in Fig. 7 is also true for mature hens in Fig. 8. However, in the case of mature hens, the non-respired lived almost as long as the respired ones. Also, there was an interesting divergence of the "relative bleeding volumes" between Unrespired and Respired Hens starting at approximately 150 minutes. From this point onward, the rate of bleeding was slower in Respired Hens than in Unrespired Hens although, eventually, the MBV

was the same in both groups (70%). This slower bleeding resulted in a slower hemodilution, reflected in the changes in both hematocrit and plasma protein concentration.

In Fig. 9, Unrespired Pullets behaved in an identical manner to the Unrespired Hens following hemorrhagic hypotension up until their death, approximately 150 minutes sooner than the Unrespired Hens.

In Figs. 10 and 11, the shaded area between the solid and broken lines indicated mobilized extravascular fluid (see legends for detailed explanation of figures). In every group the measured plasma volumes initially fell somewhat but subsequently remained relatively constant even though plasma continued to be removed. The "estimated plasma volume" (see general methods) agreed well with the measured plasma volume only during the initial part of the experiments and later, it too began to fall. Agreement between "estimated" and measured plasma volumes indicated that early mobilization was almost entirely protein-poor fluid. Because the calculation of "estimated plasma volume" was dependent on the assumption that no protein was added or lost during the experiment, it appeared that in the later stages of prolonged bleeding, protein or protein-containing fluid may have been added.

If the final measured plasma volume and the terminal "residual plasma volumes (solid lines) were compared, the total amount of fluid mobilized was similar in all groups although slightly higher in mature hens than pullets. Total fluid mobilization in pullets was 20.3 ml/kg for non-respired and 19.4 ml/kg for respired chickens. For Unrespired and Respired Hens, the corresponding figures were 23.1 and 26.4

ml/kg respectively. The average rates of fluid mobilization for the different groups reflected the duration of the experiments. The average rate of fluid mobilization in the Unrespired Pullets was 5.8 ml/kg/hr while the other groups had rates of approximately 4 ml/kg/hr. This was a reflection of the fact that mobilization of fluid was more rapid during the first part of an experiment and thus, because the total amounts of fluid mobilized were similar in all groups, the group with the shorter duration must have a faster rate. The rates reported for these experiments were less than those in the preceding experiments for this same reason. However, when the rate of fluid mobilization during the first 90 minutes was calculated for each group, it was similar for all groups. On the basis of the initial 90 minutes, Unrespired and Respired Pullets mobilized fluid at average rates of 7.9 and 8.5 ml/kg/hr respectively, while Unrespired and Respired Hens had average rates of fluid mobilization of 7.7 and 7.4 ml/kg/hr.

There was some suggestion of slight hemoconcentration occurring terminally in these experiments (Figs. 7, 8 and 9). To examine this phenomenon further, Fig. 12 was constructed. Fig. 12 used a directionally opposite time ordinate to that used in Fig. 7, 8 and 9. Instead of starting at 0 minutes and proceeding toward death, the "relative" hematocrit and plasma protein concentrations were plotted versus time by starting with the death of the animal and proceeding back towards 0 minutes. The curves obtained by this procedure and which are shown in Fig. 12 were suggestive of the occurrence of terminal hemoconcentration but none of the differences was even close to being statistically significant.

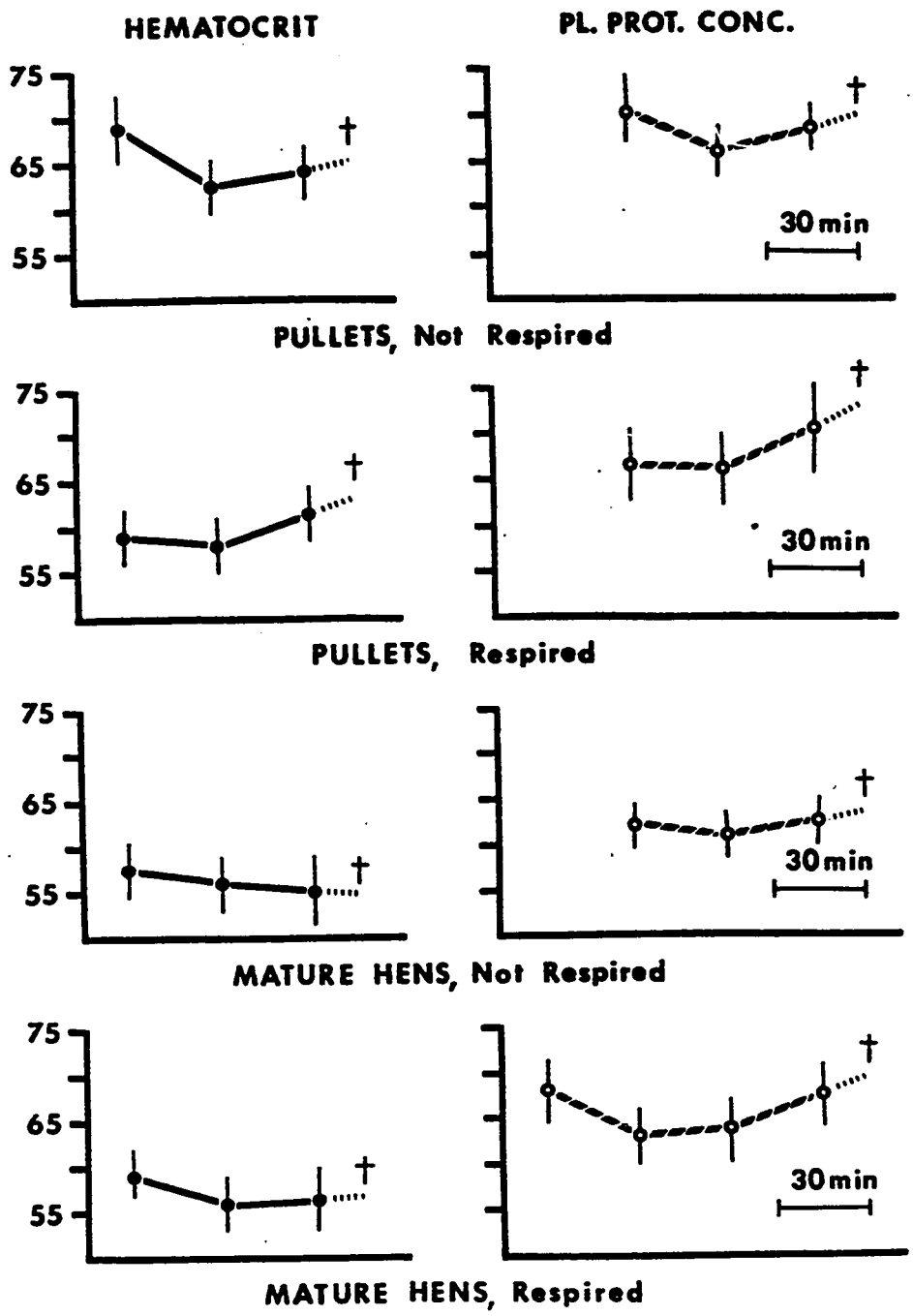

Figure 12. Terminal Effects of a Period of Hemorrhagic Hypotension on Hematocrit and Plasma Protein Concentration in Pullets and Hens. All numbers on the ordinate are percentages of the value at zero time. Corresponding times in individual experiments were related to time of death. The small crosses indicate the death of the animals and the last, next to last, and 3rd from last measurements are plotted for each group. The dotted lines are extrapolations from the final measurement to death. The vertical lines indicate the S.E. of each point.

Comment. These experiments demonstrated that, during a period of hemorrhagic hypotension pullets did not live for nearly as long as older chickens did without artificial ventilation. Minor differences between the various groups in this study were merely a reflection of a longer period of bleeding. Accordingly, a longer duration of hemorrhagic hypotension yielded a larger SBV in mature hens than in pullets. A larger "relative bleeding volume" (70%) in mature hens compared to that in pullets (60%) resulted in a lower terminal LVP in mature hens. A lower terminal LVP can be explained simply on the basis of removal of a larger portion of the initial blood volume. A lower LVP in both groups of respired chickens could be the result of a mechanical effect of artificial ventilation on the large veins. Early deaths in the pullets may have been the result of respiratory arrest because artificial ventilation of pullets yielded results more similar to those in mature hens.

However, artificial ventilation as was accomplished in these experiments, was not without other effects which resulted in some further variation among the groups. One major difference was that hens which were artifically ventilated had a lowered heart rate which was statistically highly significant. The reason for this lowered heart rate in respired hens was not known. Some possible explanations are: a) hyperventilation may have removed a normal chemoreceptor stimulus which was mediated to the heart through sympathetic nerves and removal of this sympathetic tone lowered the heart rate, or, b) because paraldehyde is largely excreted through the lungs (see Lang & Borgstedt, 1968), a relatively large "dead space" in the arti-

ficial ventilation apparatus may have interfered with the excretion of paraldehyde enough to result in a buildup in its blood levels which may have caused cardiac depression, or, c) a tendency to over-inflate the lungs may have caused a feedback to a medullary respiratory center of sufficient strength to result in an overflow of medullary activity of respiratory areas into cardiovascular areas, resulting in an increased firing of the vagus, or, d) artificial ventilation could have mechanically compressed the large veins of the thorax increasing venous return and cardiac contractility, resulting in more effective "pumping" at a lower rate. None of these possibilities can be excluded as a cause of lowered heart rate in the respired chickens.

In the later stages of experiments in mature hens, artificial ventilation also slowed the rate of hemodilution and thus the bleeding rate. A lowered LVP in respired chickens has already been mentioned and finally, in the respired mature hens, there was also an unexplained lower hematocrit and higher plasma concentration than there was in the other groups.

Thus to avoid deaths from respiratory failure and to obtain longer experiments where death was more likely to be a cardiovascular event, and also, to avoid the variable effects of rather crude artificial ventilation, it was decided to do all further experiments on spontaneously breathing mature hens. It is necessary, then, to examine spontaneously breathing mature hens in a little more detail especially from a quantitative point of view.

A period of approximately 5 hours at 50 mm Hg MAP was necessary to result in death for 50% of mature White Leghorn hens. Before death

occurred, however, a volume of blood equivalent to about 70% of the initial blood volume was removed. This amount of bleeding resulted in an overall fall of 50% in both hematocrit and plasma protein concentration. However, the rates of fall of these two meters of fluid mobilization were not identical. Initially, the plasma protein concentration fell more rapidly than the hematocrit, perhaps because of the addition of a small volume of erythrocyte-rich blood from the spleen. Later, roughly in the last half of the period of hemorrhagic hypotension, the plasma protein concentration fell at a slower rate than the hematocrit. This may be explained by the addition of protein to the vascular fluid, or, by the loss of erythrocytes from the vascular space. An explanation of this phenomenon on the basis of protein being added to the vascular fluid was supported by the fact that the estimates of the plasma volume based on protein dilution were lower than the measured plasma volume during the last half of the period of hemorrhagic hypotension. Such estimates were accurate only when no protein other than that removed by bleeding was lost from the vascular space and no new protein was added. And, underestimation of plasma volume using this calculation which occurred after severe or prolonged hemorrhage in other species was explained on the basis of protein being added to the vascular fluid (see Chien, 1958).

The massive fluid mobilization in chickens was illustrated by the fact that in mature hens after 3 hours at 50 mm Hg MAP when 2/3 of the original plasma volume had been removed, the measured plasma volume had fallen by a mere 7%. Over a period of approximately 5

hours at 50 mm Hg MAP more than 80% of the initial plasma volume was removed and the plasma volume never fell much more than this original small amount (7%). The chicken's ability to replace a plasma volume which has been diminished by 2/3 of its original volume in 3 hours represents a fluid mobilization of 8.4 ml/kg/hr. Finally, in mature White Leghorn hens, there was a slight indication of hemoconcentration and decompensation in the terminal stages of a period of hemorrhagic hypotension similar to that reported to occur in dogs (see Hollenberg, 1965), but it was not shown to be statistically significant.

IV. The Course of Hemorrhagic Hypotension in Chickens: A Comparison of Barbiturate and Paraldehyde Anesthesia. General anesthesia can complicate interpretation of the results of cardiovascular experiments, but it often cannot be avoided. Consequently, it is desirable to know the contribution of anesthesia to the results obtained. Because paraldehyde is a somewhat unusual agent for general anesthesia, it is important to know that the characteristic response of chickens to a period of hemorrhagic hypotension reported in the preceding sections cannot be attributed to an effect of paraldehyde itself. Some general anesthetic is essential in experiments of this type, and a comparison was made of paraldehyde and a barbiturate, the type of anesthetic most commonly used in cardiovascular experiments on mammals.

Method. Only mature hens were used in these experiments. Preliminary experiments indicated that pentobarbital sodium, the most

commonly used barbiturate for general anesthesia in experimental animal research, had a very short duration in chickens and the supplemental dosages required were sufficiently undesirable to reject this agent in favor of a longer acting barbiturate. The assumption was that cardiovascular effects of barbiturates are very similar. Phenobarbital sodium, in a dose (130 mg/kg, intramuscular), which had been previously used in cardiovascular studies in chickens (see McGinnis & Ringer, 1966), was the barbiturate selected.

Surgical preparation was as described under general methods for the cannulation of a single ischiatic artery and the ipsilateral external iliac vein. All birds had a plastic catheter in their trachea to ensure a free airway.

Hens were bled to 50 mm Hg MAP as described under general methods and maintained at this pressure until death. Cardiovascular and other variables measured or calculated every 15 minutes during the first hour, and, every 30 minutes thereafter were: MAP, LVP, heart rate, respiration rate, hematocrit, plasma protein concentration, "estimated plasma volume", and the initial measured plasma volume minus the volume of plasma removed. In addition, plasma and blood volumes were measured initially, at 90 and 210 minutes. All techniques are described under general methods.

Results. Results obtained in these experiments were compared to mature hens from the preceding section which were anesthetized with paraldehyde. Two groups of chickens had almost identical bleeding volumes, although barbiturate anesthesia resulted in shorter (not

statistically significant) experiments (Table VIII). The duration of hypotension in each group was examined further by a plot of percentage deaths versus the time from IBV to death (Fig. 13). The linear relationship between percentage deaths and duration of hypotension was shown by correlation coefficients of 0.9908 for chickens anesthetized with paraldehyde and 0.9884 for those anesthetized with phenobarbital (only linear from 20-80% deaths). The linear equations were solved to find the duration at 50 mm Hg MAP necessary to produce 50% deaths. This "LT_{50}" (Lethal Time for 50% of the animals) was 283 minutes for paraldehyde anesthesia and 195 minutes for phenobarbital anesthesia. The regression lines were non-homogeneous ($p < 0.01$) and therefore this difference is statistically significant.

Tables IX and X and Fig. 14 summarize the effects of a period of hemorrhagic hypotension leading to death in hens anesthetized with paraldehyde or phenobarbital. Results were remarkably similar in the two groups as was especially evident in the "relative" changes in cardiovascular variables shown in Fig. 14. The hematocrits (Table X) of hens anesthetized with phenobarbital was lower than those of paraldehyde anesthetized hens. The explanation of this difference may lie in a seasonal variation because phenobarbital anesthesia experiments were performed in the month of June whereas paraldehyde anesthesia experiments were performed in the months of January, February and March (see Section I). Significant differences between the two groups in MAP and heart rate (Table IX) at 210 minutes were merely a reflection of the fact that 210 minutes was nearly "terminal" in the group anesthetized with barbiturate.

Table VIII. Comparison of Weights, Bleeding Volumes and Duration of Hypotension in Mature White Leghorn Hens Anesthetized with Intramuscular Paraldehyde (1.25 ml/kg) or Phenobarbital Sodium (130 mg/kg).

	Paraldehyde n=13	Phenobarbital n=10	p
Weight (kg)	1.73 ± 0.08	1.77 ± 0.06	n.s.
IBV (ml/kg)	12.6 ± 1.7	14.9 ± 2.7	n.s.
MBV (ml/kg)	37.4 ± 3.1	36.9 ± 2.4	n.s.
SBV (ml/kg)	24.8 ± 3.2	21.9 ± 3.3	n.s.
Minutes at 50 mm Hg	302 ± 44	233 ± 48	n.s.

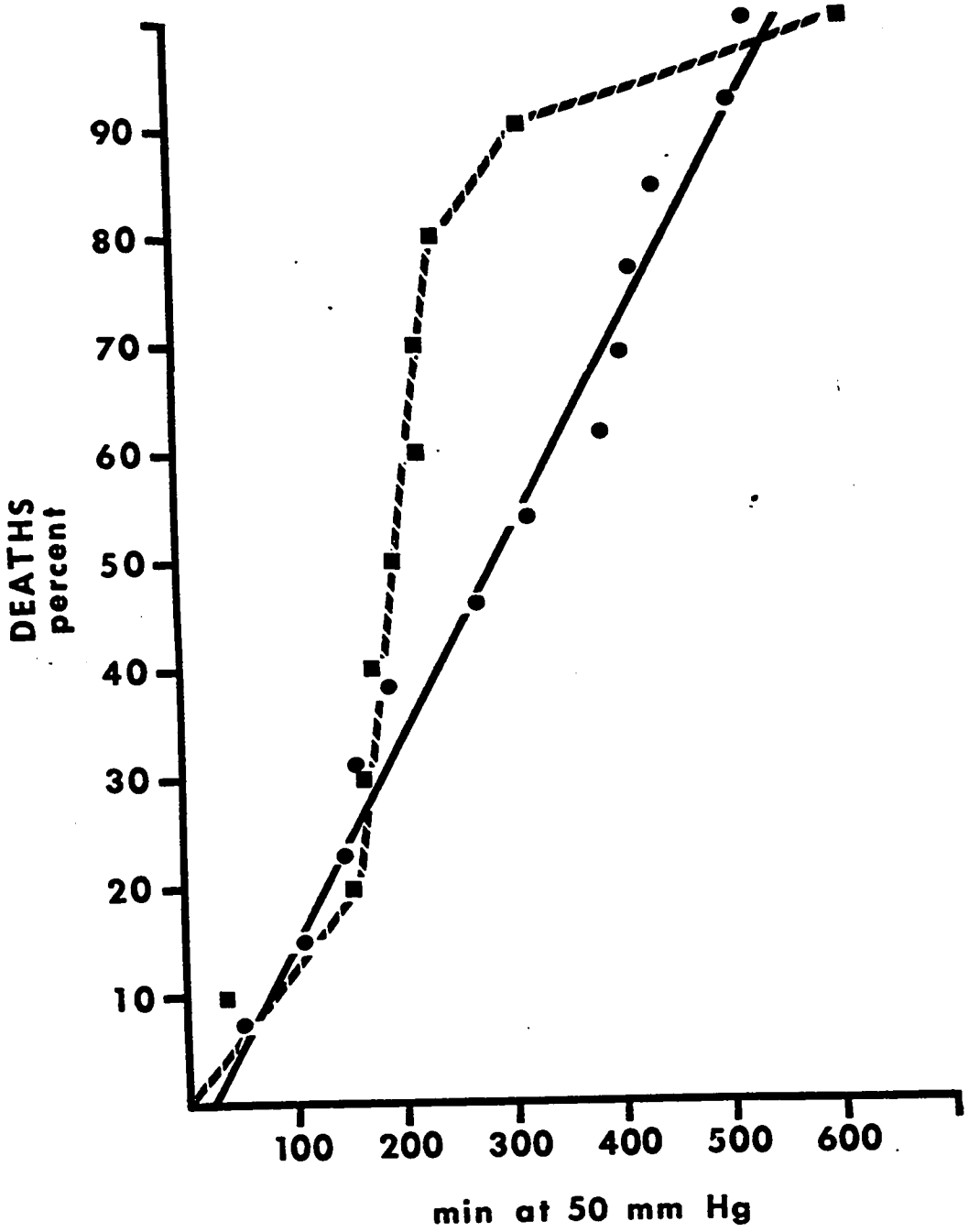

Figure 13. Plot of Percentage Deaths versus Duration of Hypotension in Chickens Anesthetized with Barbiturate or Paraldehyde. Closed squares and broken line are chickens anesthetized with phenobarbital sodium (130 mg/kg, im) and closed circles and solid line are chickens anesthetized with paraldehyde (1.25 ml/kg, im). The lines of best fit calculated by the Method of Least Squares are shown.

Table IX. Comparison of MAP and heart rate during a Period of Hemorrhagic Hypotension in Mature White Leghorn Hens Anesthetized with Intramuscular Paraldehyde (1.25 ml/kg) or Phenobarbital Sodium (130 mg/kg).

	Paraldehyde	Phenobarbital	p
	MAP (mm Hg)		
-30 Minutes	107 ± 5 (13)	106 ± 4 (10)	n.s.
0 Minutes	106 ± 5 (13)	117 ± 3 (10)	n.s.
90 Minutes	50 ± 0 (11)	50 ± 0 (9)	n.s.
210 Minutes	50 ± 0 (8)	37 ± 4 (8)	<0.05
Terminal	34 ± 3 (12)	33 ± 3 (10)	n.s.
	Heart Rate (beats/min)		
-30 Minutes	314 ± 18 (13)	290 ± 16 (10)	n.s.
0 Minutes	304 ± 14 (13)	283 ± 18 (10)	n.s.
90 Minutes	393 ± 8 (11)	383 ± 8 (9)	n.s.
210 Minutes	392 ± 9 (8)	331 ± 17 (8)	<0.05
Terminal	354 ± 14 (12)	328 ± 17 (10)	n.s.

Table X. Comparison of Hematocrit and Plasma Protein Concentration during a Period of Hemorrhagic Hypotension in Mature Leghorn Hens Anesthetized with Intramuscular Paraldehyde (1.25 ml/kg) or Phenobarbital Sodium (130 mg/kg).

	Paraldehyde	Phenobarbital	p
	Hematocrit (%)		
-30 Minutes	33.3 ± 1.8 (13)	28.0 ± 0.7 (10)	<0.01
0 Minutes	32.9 ± 1.1 (13)	27.6 ± 0.7 (10)	<0.01
90 Minutes	24.5 ± 1.1 (11)	19.5 ± 0.8 (9)	<0.01
210 Minutes	20.2 ± 1.2 (8)	16.9 ± 1.2 (8)	n.s.
Terminal	18.3 ± 1.2 (12)	15.9 ± 0.8 (10)	n.s.
	Plasma Protein Concentration (Gm%)		
-30 Minutes	4.97 ± 0.19 (13)	5.43 ± 0.29 (10)	n.s.
0 Minutes	4.64 ± 0.18 (13)	5.24 ± 0.30 (10)	n.s.
90 Minutes	3.41 ± 0.17 (11)	3.90 ± 0.34 (9)	n.s.
210 Minutes	2.83 ± 0.21 (8)	3.33 ± 0.25 (8)	n.s.
Terminal	2.93 ± 0.21 (12)	3.34 ± 0.24 (10)	n.s.

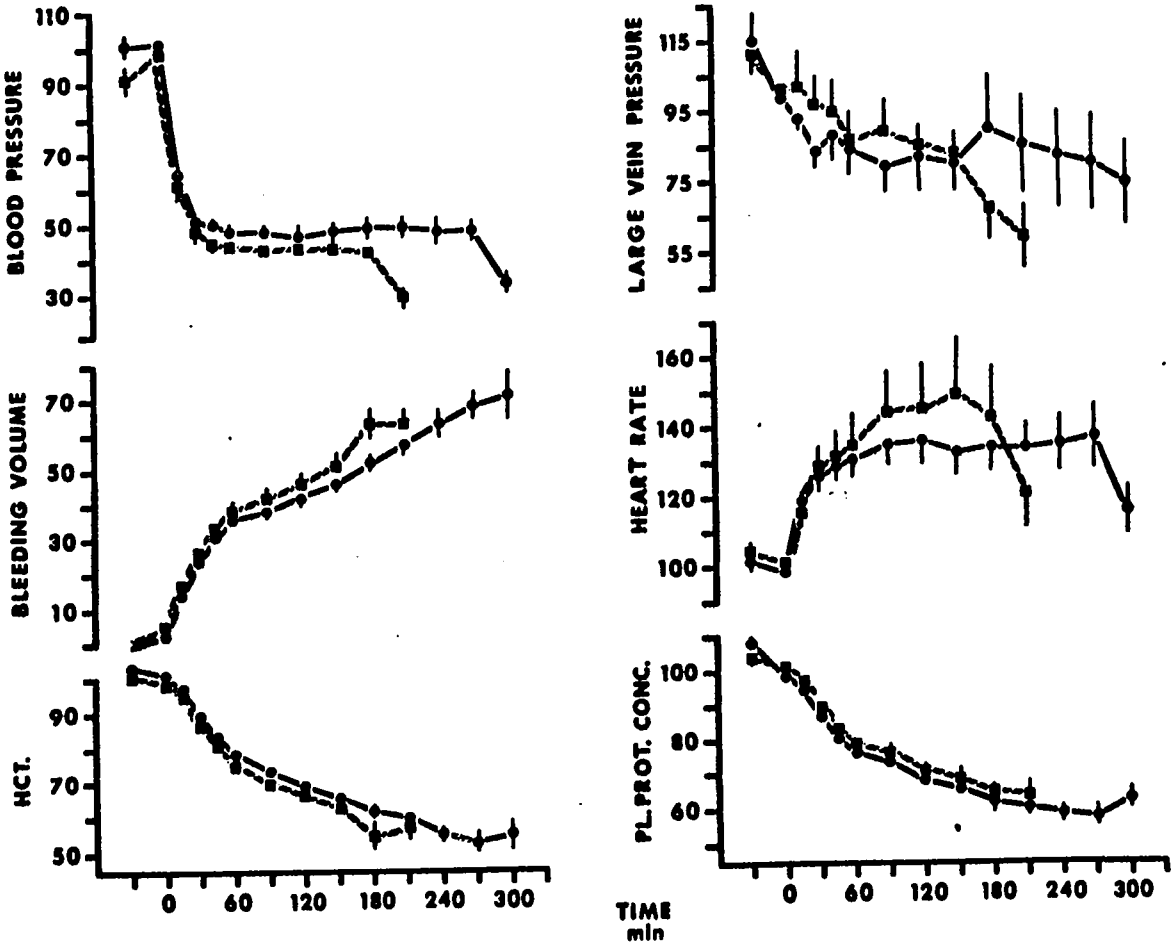

Figure 14. Comparison of the Course of Hemorrhagic Hypotension in Chickens Anesthetized with Barbiturate or Paraldehyde. Zero minutes is the start of systematic graded hemorrhage. All numbers on the ordinates are percentages. Zero minute measurements of blood pressure, large vein pressure, heart rate, hematocrit and plasma protein concentration are 100%. Bleeding volume is expressed as a percentage of initial measured blood volume. Closed squares and broken line are chickens anesthetized with phenobarbital sodium (130 mg/kg, im). Closed circles and solid lines are chickens anesthetized with paraldehyde (1.25 ml/kg, im). Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s of two points overlap, they are shown in only one direction.

The respiration rate (Fig. 15) was always lower in the phenobarbital group than it was in the paraldehyde group (significantly at 90 and 120 minutes) although in both series respiration rates rose with the initiation of hemorrhage but later fell again. The depressed respiration rate in the phenobarbital group may provide a clue to the reason for the shorter duration of the experiments in this group.

Fig. 16 is a reverse-time-plot (i.e., starting with death) of the hematocrit and plasma protein concentration. The final three measurements of each were plotted versus time to establish the presence or absence of hemoconcentration in the terminal stages of the experiments. There was no indication of significant hemoconcentration in either group.

Finally, Fig. 17 illustrates the massive hemodilution which has been a consistent finding in all our experiments on chickens. The shaded area between the two lines in each diagram represents the volume of extravascular fluid which was mobilized. Calculation of fluid mobilization after 3 hours at 50 mm Hg MAP in the hens anesthetized with phenobarbital showed that removal of 62% of the initial plasma volume resulted in a fall of measured plasma volume of 14%, i.e., an average fluid mobilization rate of 6.6 ml/kg/hr. This was not substantially different from the results with hens anesthetized with paraldehyde, where the corresponding values were 67% removal, 7% fall in measured plasma volume and 8.4 ml/kg/hr.

Comment. Comparison of the responses to a period of hemorrhagic hypotension in mature White Leghorn hens anesthetized with par-

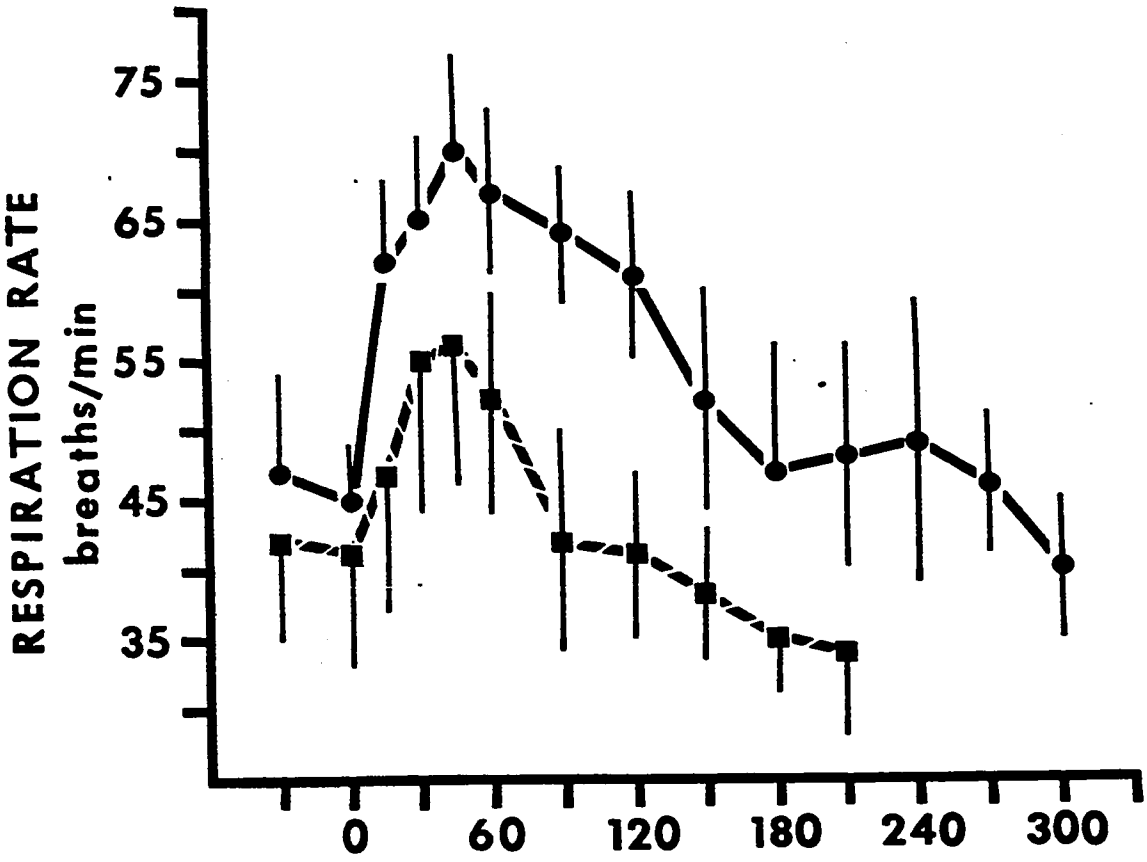

Figure 15. Comparison of Respiration Rates during Hemorrhagic Hypotension in Chickens Anesthetized with Barbiturate or Paraldehyde. Time in minutes is shown on the abscissa. Zero minutes is the start of systematic graded hemorrhage. Closed squares and broken line are chickens anesthetized with phenobarbital sodium (130 mg/kg, im). Closed circles and solid line are chickens anesthetized with paraldehyde (1.25 ml/kg, im). The vertical lines indicate the S.E. of each point. Where S.E.'s of two points overlap they are shown in only one direction.

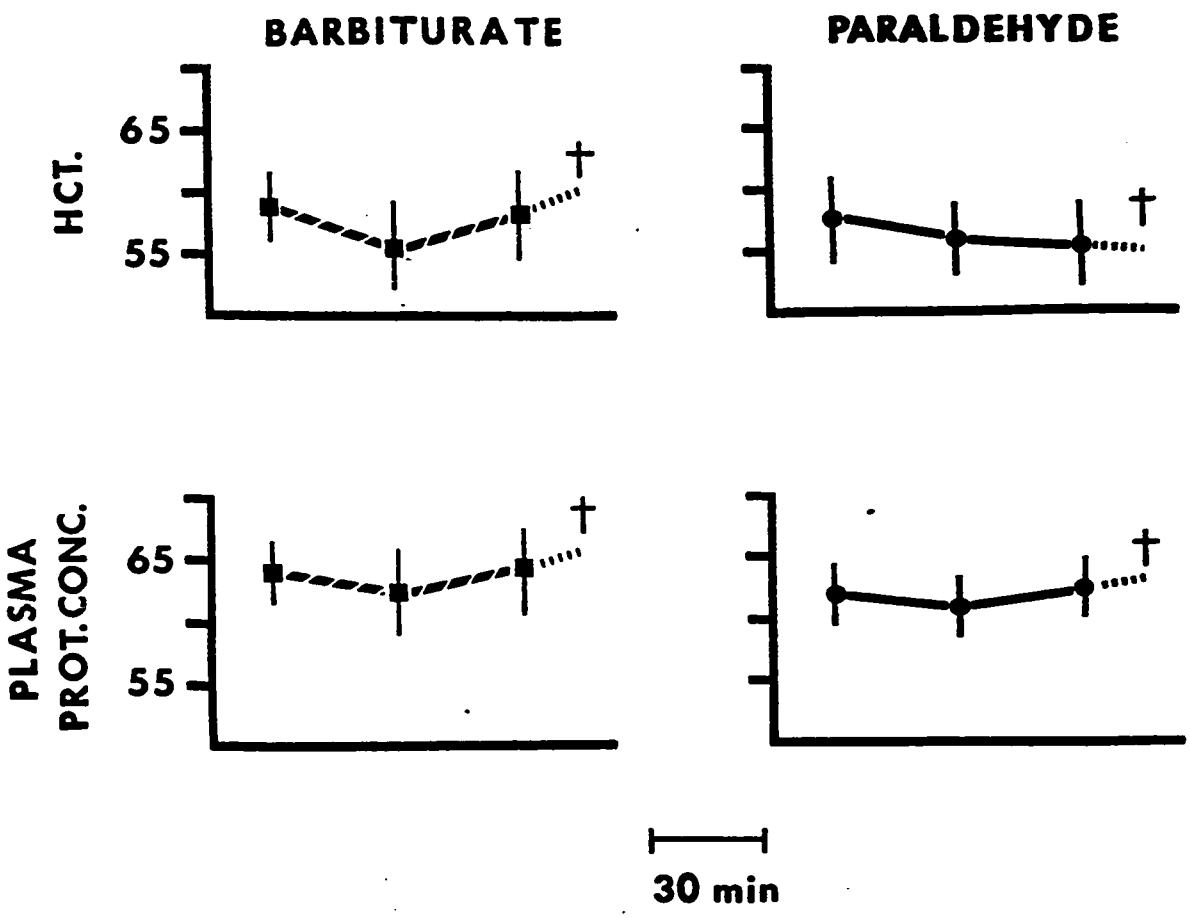

Figure 16. Terminal Effects of a Period of Hemorrhagic Hypotension on Hematocrit and Plasma Protein Concentration in Chickens Anesthetized with Barbiturate or Paraldehyde. All numbers on the ordinates are percentages of the value at zero minute. Corresponding times in individual experiments were related to time of death. The small cross indicates death of the animals and the last, next to last, and 3rd from last measurements are plotted for each group. The dotted lines are extrapolations from the final measurement to death. The vertical lines indicate the S.E. of each point.

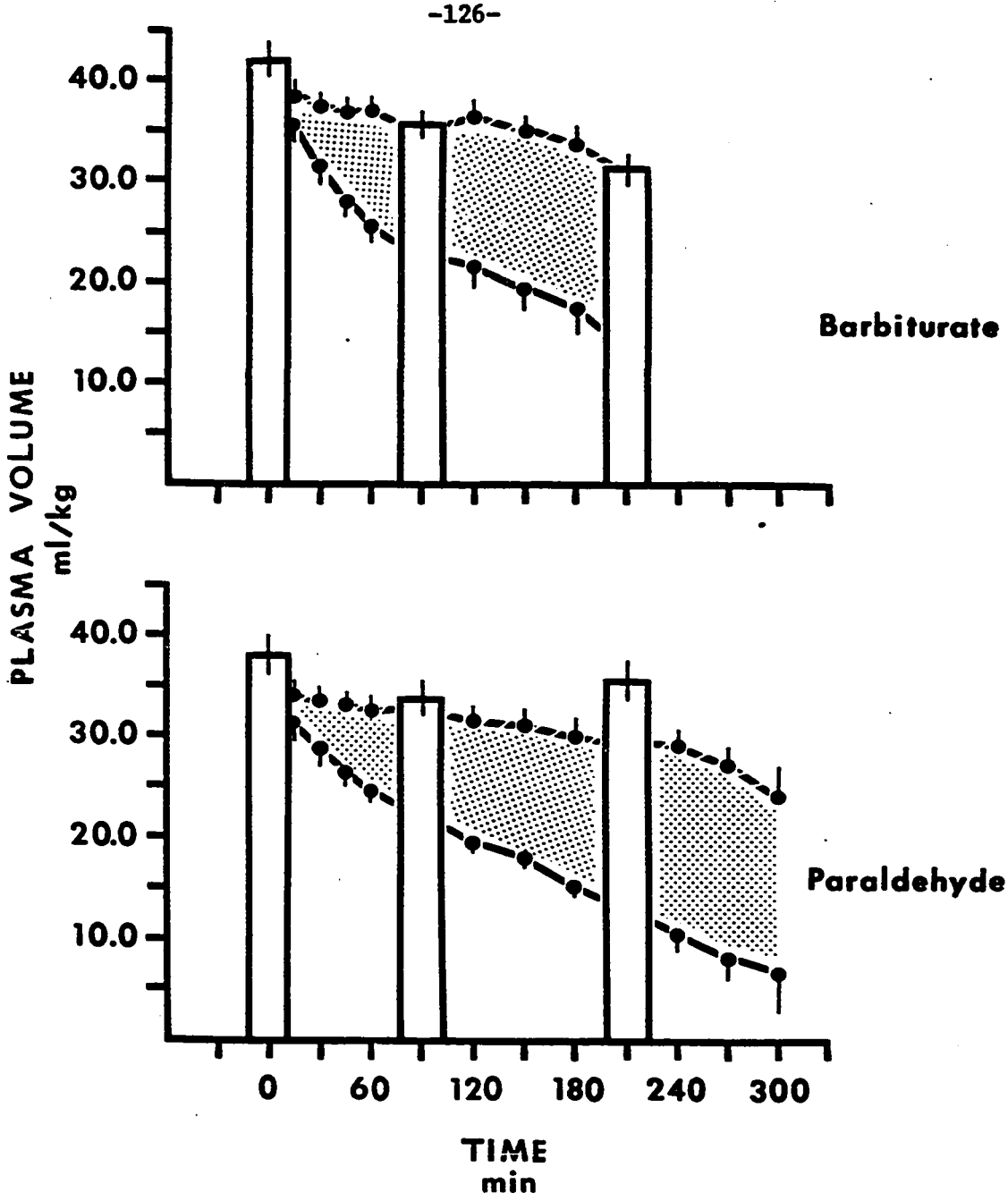

Figure 17. Comparison of Hemodilution in Chickens Anesthetized with Barbiturate or Paraldehyde. Bars indicate the measured plasma volume. The lower, solid line in each diagram is the initial plasma volume minus the volume of plasma removed. The upper, broken line in each diagram is the plasma volume estimated on the basis of protein dilution. Vertical lines indicate the S.E. of each point.

aldehyde or phenobarbital showed only one substantial quantitative difference. The "LT_{50}" was shorter in hens anesthetized with phenobarbital than in those anesthetized with paraldehyde. The only hint as to the reason for this difference appeared in a comparison of the respiration rates of the two groups. In the middle stages of the experiments, the respiration rate in the hens anesthetized with phenobarbital was significantly lower than that in those anesthetized with paraldehyde. Perhaps the shorter duration in the hens anesthetized with phenobarbital was the result of respiratory depression, a well-known consequence of barbiturate administration. Respiration rate alone is not a complete indication of the adequacy of respiration but a lowered respiration rate is at least one indication of respiratory depression. Other minor differences between the two groups which were statistically significant can be explained as results of seasonal variation or of the fact that the durations of the experiments were shorter in the phenobarbital groups, e.g., 210 minutes was near "terminal" in hens anesthetized with phenobarbital but not those with paraldehyde and therefore comparison of variables measured at 210 minutes in each group gave some significant differences. The striking similarity in responses was especially apparent in the "relative"changes (Fig. 14) and in the fluid mobilization (Fig. 17).

V. Comparison of the Effects of a Period of Hemorrhagic Hypotension in Chickens and Turkeys. Massive hemodilution following hemorrhage has also been reported to occur in other species of bird (Djojosugito et al., 1968). A few experiments were carried out on

turkeys to assess their response to a period of hemorrhagic hypotension because it was anticipated that a bird larger than a chicken, but with a similar response to hemorrhagic hypotension, might be useful in more detailed cardiovascular studies.

Method. Turkeys used in the present experiments were mature albino females which had been in the animal house for several months before use. Anesthetization and surgery in turkeys was identical to that described under general methods for chickens. Method of induction and maintenance of hemorrhagic hypotension (50 mm Hg) was also the same as used for chickens and is described under general methods. MAP was maintained at this pressure until death or for 4 hours. Plasma and blood volumes were measured before hemorrhage, at 90 and 210 minutes. Other cardiovascular parameters measured or calculated every 15 minutes during the first hour and every 30 minutes thereafter were: MAP, LVP, bleeding volume, heart rate, hematocrit, plasma protein concentration, plasma volume estimated on the basis of protein dilution, and initial plasma volume minus the volume of plasma removed. All techniques of measurement and methods of calculation are given under general methods.

Results. The present results obtained in turkeys are presented in comparison with data from mature White Leghorn hens from previous experiments. Cardiovascular variables measured during the pre-hemorrhage period (Table XI) showed a few minor differences between the two species. The MAP of chickens was significantly lower than that of

Table XI. Comparison of Selected Cardiovascular Variables in Chickens and Turkeys measured during the Pre-hemorrhage Period.

	Chickens n=13	Turkeys n=4	p
Weight (kg)	1.73 ± 0.08	8.18 ± 0.21	---
MAP (mm Hg)	107 ± 5	138 ± 17	<0.05
LVP (mm Hg)	15.3 ± 1.9	11.9 ± 0.3	n.s.
Heart Rate (beats/min)	314 ± 18	238 ± 16	<0.05
Hematocrit (%)	33.3 ± 1.8	34.2 ± 1.9	n.s.
Plasma Prot. Conc. (Gm%)	4.97 ± 0.19	5.06 ± 0.32	n.s.
Plasma Volume (ml/kg)	38.1 ± 2.0	28.0 ± 1.3	<0.01
Blood Volume (ml/kg)	57.2 ± 2.0	42.4 ± 0.9	<0.01

turkeys and the heart rate of the latter was also lower than that of the former. Plasma and blood volumes (ml/kg) of chickens were higher than those of turkeys. These slight differences indicated that caution was necessary in comparing these variables.

The duration of hypotension for the turkeys can only be stated as an approximate figure (Table XII) because three of the four experiments were terminated after 4 hours and the fourth turkey died after 3 hours of hypotension. The bleeding volumes (ml/kg) of turkeys (Table XII) tended to be lower than those of chickens but the differences were not statistically significant. On a percentage basis, i.e., percent of in vivo blood volume, the IBV's of chickens and turkeys were 22.0 and 23.4% respectively and MBV's were also similar (65.4 and 66.0%, respectively). The SBV/IBV ratios for chickens and turkeys were 1.97 and 1.83, respectively, whereas Hollenberg's data for dogs bled to 35 mm Hg MAP gave an SBV/IBV ratio of 0.16 (1965). These figures indicate the large amount of blood which can be removed from these two species of fowl after reaching a set hypotensive level. Bleeding volumes (percent, Table XIII) at selected times were similar in both species although turkeys had a tendency to have slightly higher bleeding volumes and after 90 minutes of hemorrhage, this difference was significant.

The "relative" (percentage, see legend Fig. 18) changes in cardiovascular variables measured following hemorrhage were generally similar in both species and differed only slightly in a quantitative manner (Fig. 18). Both species showed a marked inability to maintain MAP with hemorrhage and pressure was usually easily lowered to 50 mm

Table XII. Bleeding Volumes and Duration of Hemorrhagic Hypotension in Chickens and Turkeys.

	Chickens n=13	Turkeys n=4	p
IBV (ml/kg)	12.6 ± 1.7	9.9 ± 1.7	n.s.
MBV (ml/kg)	37.6 ± 3.1	28.0 ± 3.0	n.s.
SBV (ml/kg)	24.8 ± 3.2	18.1 ± 3.0	n.s.
Minutes at 50 mm Hg	302 ± 44	240	---

Table XIII. Bleeding Volume (percent of measured blood volume) during a Period of Hemorrhagic Hypotension in Chickens and Turkeys.

	Chickens	Turkeys	P
30 Minutes	24.5 ± 1.3 (9)	28.1 ± 2.6 (4)	n.s.
60 Minutes	34.1 ± 2.0 (9)	41.1 ± 3.0 (4)	n.s.
90 Minutes	37.8 ± 2.1 (8)	49.7 ± 3.4 (4)	<0.05
210 Minutes	56.9 ± 2.5 (6)	66.9 ± 8.8 (3)	n.s.

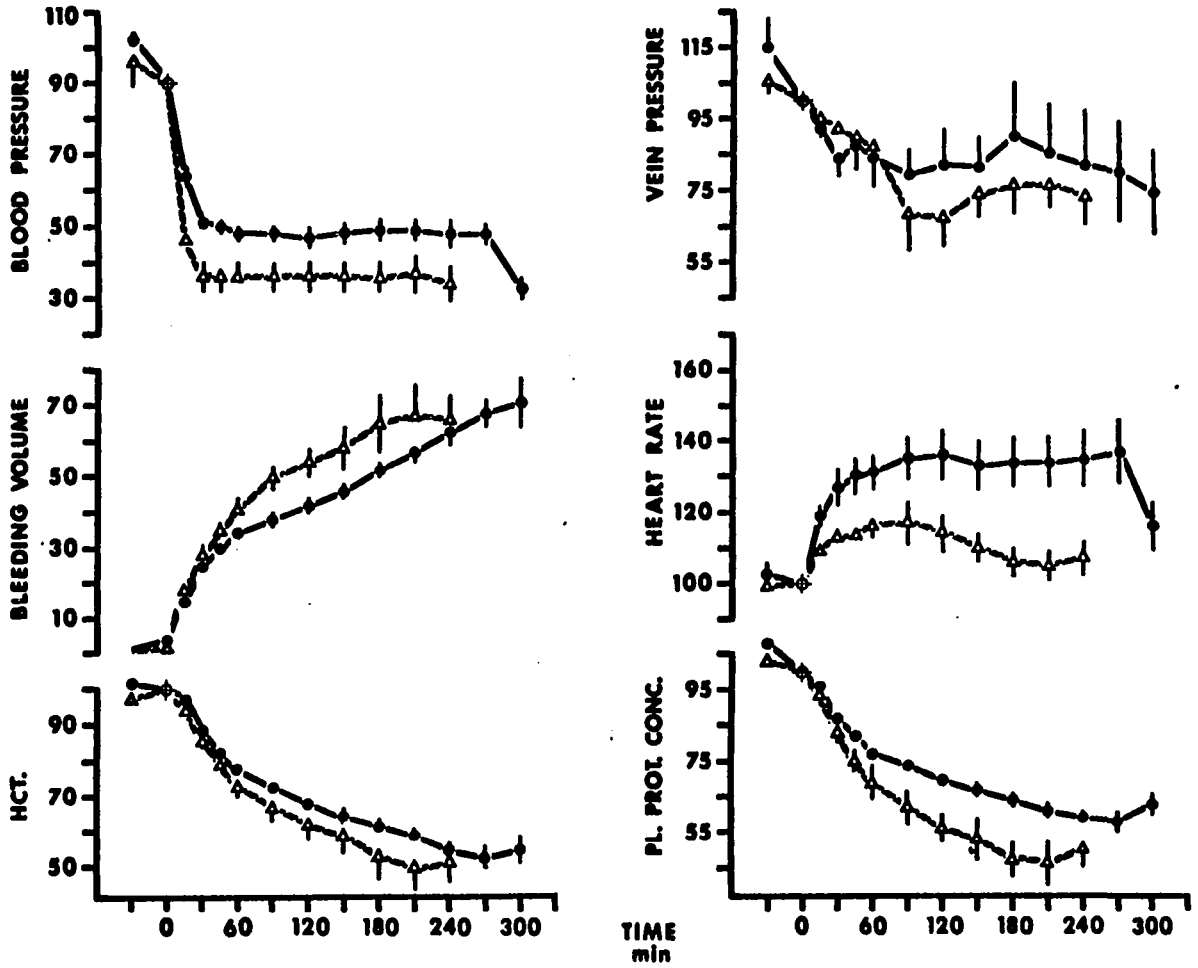

Figure 18. Comparison of the Course of Hemorrhagic Hypotension in Chickens and Turkeys. Zero minutes is the start of hemorrhage. All numbers on the ordinates are percentages. Zero minute measurements of blood pressure, large vein pressure, heart rate, hematocrit and plasma protein concentration are 100%. Bleeding volume is expressed as a percentage of initial measured blood volume. Closed circles and solid lines are chickens and open triangles and broken lines are turkeys. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s of two points overlap, they are shown in only one direction.

Hg in less than 30 minutes with the rate of bleeding used (0.5 ml/kg/min). Chickens were able to increase their heart rate to a somewhat greater extent than turkeys were. The difference in rates of bleeding during the middle portion of the experiment has already been mentioned (Table XIII). There was also a tendency for hematocrit and plasma protein concentration to fall more quickly in turkeys (Fig. 18, Table XIV) and perhaps a slightly faster rate of hemodilution was the reason for the difference in bleeding rates. Changes in hematocrit and plasma protein concentration in turkeys were generally much similar to those reported for chickens in preceding sections. However, turkeys displayed a significant increase in hematocrit between the first measurement and the start of hemorrhage which could possibly be attributed to mobilization of a small amount of erythrocyte-rich fluid from the spleen. This explanation was supported by the fact that during this same pre-hemorrhage period, the plasma protein concentration fell.

Fig. 19 is a graphical depiction of fluid mobilization in the two species of bird. The shaded area between the two lines in each diagram indicates influx to fluid into the vascular space. The amount of fluid mobilization did not appear to be markedly different in these birds. Based upon the <u>in vivo</u> measured plasma volume after 3.5 hours of hypotension, an average of 67% of the plasma volume of chickens had been removed, resulting in a net decrease in plasma volume of 7% and representing an average fluid mobilization rate of 8.4 ml/kg/hr. In turkeys, 74% of the plasma volume had been removed, measured plasma volume had decreased 12%, and the average fluid mobiliza-

Table XIV. Effects of a Period of Hemorrhagic Hypotension on Hematocrit and Plasma Protein Concentration in Chickens and Turkeys.

	Chickens	Turkeys	p
	Hematocrit (%)		
0 Minutes	32.9 ± 1.1 (13)	35.5 ± 2.1 (4)	n.s.
30 Minutes	29.0 ± 1.2 (12)	30.6 ± 2.1 (4)	n.s.
60 Minutes	25.6 ± 1.1 (13)	26.1 ± 2.0 (4)	n.s.
90 Minutes	24.5 ± 1.1 (11)	23.9 ± 2.3 (4)	n.s.
210 Minutes	20.2 ± 1.1 (8)	18.6 ± 3.0 (3)	n.s.
	Plasma Protein Concentration (Gm%)		
0 Minutes	4.64 ± 0.18 (13)	4.89 ± 0.29 (4)	n.s.
30 Minutes	4.12 ± 0.17 (12)	4.06 ± 0.32 (4)	n.s.
60 Minutes	3.60 ± 0.16 (13)	3.37 ± 0.33 (4)	n.s.
90 Minutes	3.41 ± 0.17 (11)	3.04 ± 0.28 (4)	n.s.
210 Minutes	2.83 ± 0.21 (8)	2.20 ± 0.40 (3)	n.s.

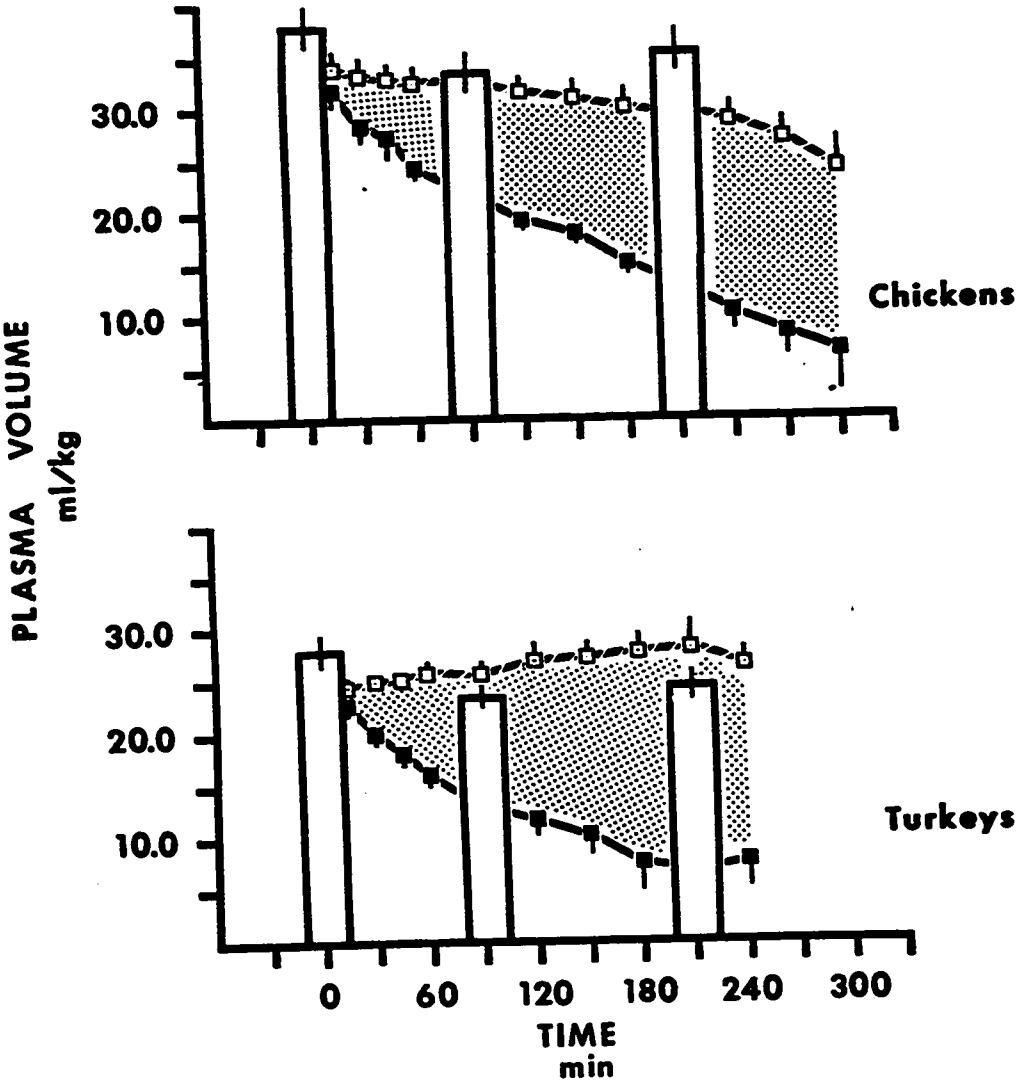

Figure 19. Comparison of Hemodilution in Chickens and Turkeys. Bars indicate the measured plasma volume. The closed squares and solid line in each diagram are the initial plasma volume minus the volume of plasma removed. The open squares and broken line in each diagram are the plasma volume estimated on the basis of protein dilution. Vertical lines indicate the S.E. of each point.

tion rate was 5.8 ml/kg/hr. The fluid mobilization rate expressed as ml/kg/hr was higher in chickens than in turkeys but as the percentage of the plasma volume mobilized per hour, the two were nearly identical, 22%/hr for chickens and 21%/hr for turkeys.

Comment. The two species of fowl, chickens and turkeys, reacted in essentially an identical manner when subjected to a period of hemorrhagic hypotension (Fig. 18). Both showed a marked inability to maintain their MAP with hemorrhage although it was possible to remove 65-70% of the initial blood volume during 4-5 hours of bleeding. The plasma removed was almost quantitatively (approximately 90%) replaced by the mobilization of extravascular fluid. Fluid mobilization was mirrored in marked falls in hematocrit and plasma protein concentration (45-55%). Although the falls in hematocrit were similar in both species of fowl after the start of hemorrhage, turkeys appeared to be able to mobilize some erythrocyte-rich pool early in the experiments as evidenced by an increase in hematocrit (plasma protein concentration was falling) during the prehemorrhage period.

The basic difference between dogs (and other mammals) and these two species of bird following hemorrhage appears to be two-fold. First, dogs are able to maintain their MAP while 10-20% of the blood volume is removed, whereas MAP of the fowl rapidly falls to the preset level with this amount of hemorrhage and can then be maintained at this level for a long time by further hemorrhage at a rate which matches the rate of fluid mobilization. Second, the fowl respond to hemorrhage by a mobilization of extravascular fluid which is both lar-

ger in volume and more rapid in rate than dogs are capable of (see detailed comparison in preceding sections).

VI. Effect of Reinfusion of Plasma or Erythrocytes on the Course of Hemorrhagic Hypotension in Chickens. It would be of interest to know what factor(s) eventually led to death in the previous experiments on chickens. It was earlier noted that artificial ventilation tended to extend the duration of experiments especially in younger chickens (see Section III). Also, respiratory depression was implicated as a cause of death in chickens anesthetized with barbiturate (see Section IV). The possibility that the ultimate death of chickens subjected to a period of hemorrhagic hypotension resulted from a lack of oxygen delivery to the tissues was thought to be worthy of further consideration. Also, it seemed possible that the reason the hemodilution slowed and eventually stopped could be the result of lowered plasma oncotic pressure, i.e., plasma oncotic and capillary hydrostatic pressures became equal. Whether this stage of hemodilution is approached in prolonged hemorrhagic hypotension in chickens is of some interest. The following experiments were an attempt to examine these points more closely.

Methods. Two groups of chickens were used in these experiments. The first half of the experiment was identical in each group. The surgical procedure was as described under general methods for cannulation of a single ischiatic artery and the ipsilateral external iliac vein. All chickens had a plastic catheter inserted into their trachea

to ensure a free airway. The cardiovascular parameters measured in these experiments were: MAP, LVP, bleeding volume, heart rate, hematocrit, and plasma protein concentration. The techniques of measurement are described under general methods. Measurements were recorded every 15 minutes for the first hour and every 30 minutes thereafter. The 0 minutes plasma volume minus the volume of plasma removed and the estimate of the plasma volume based on the protein dilution were also calculated as described in general methods at these same time intervals. In addition, the plasma and blood volumes were measured at 0 and 90 minutes, and one hour following reinfusion.

The animals were bled to 50 mm Hg MAP as described under general methods. After one hour of bleeding, all the blood obtained was removed from the reservoir and separated into plasma and cells by centrifugation. While this was being done, bleeding was continued for another hour. At the end of the second hour, the animals were divided into two groups. The first group (PLAS) received an intravenous infusion of all the plasma obtained from the blood collected in the first hour of bleeding. The second group (RBC) received a similar intravenous infusion of all cellular material (mostly erythrocytes) from the blood collected during the first hour. The erythrocytes were resuspended in an equal volume of 0.9% NaCl solution and reoxygenated before reinfusion.

The reinfusions were given during a 10-15 minute period and a further 10-15 minute period was allowed for equilibration following which the MAP was again lowered to 50 mm Hg by bleeding at the same rate as was initially used to lower MAP. The MAP was then maintain-

ed at 50 mm Hg until death. The moment of beginning this second bleedout phase is designated as 0' minutes and all other times from 0' minutes until death are distinguished from the corresponding times in the first bleeding phase by the (') symbol. From 0' minutes onward, the bleeding volume was recorded as the net volume removed, i.e., total bleedout minus the volume reinfused. The non-cellular volume reinfused was added to "residual" (0 minutes volume minus volume removed) and "estimated" (estimated on the basis of protein dilution) plasma volumes and the calculations were continued.

It was felt that these procedures would separate the chickens into groups with a high (RBC) and low (PLAS) oxygen-carrying capacity during the later stages of the experiments. It was also hoped that one group (PLAS) would have a relatively higher plasma protein concentration during the critical terminal stages of the experiments when the plasma protein osmotic pressure might be the factor limiting further mobilization of extravascular fluid.

Results. The volume of plasma reinfused was 10.1 $\pm$ 1.5 ml/kg with a total protein concentration of 3.56 $\pm$ 0.21 Gm%. The volume of cellular fluid was 7.4 $\pm$ 1.1 ml/kg with a hematocrit of 45.9 $\pm$ 1.7%. Plasma could not be completely excluded from the cellular pellet following centrifugation and the 4.0 $\pm$ 1.0 ml/kg of cell-free fluid contained in the cellular reinfusion had a protein concentration of 0.66 $\pm$ 0.07 Gm%. Following reinfusion the MAP was very easily lowered to 50 mm Hg by bleeding at the original rate, usually in less than 15 minutes, in both groups.

ed at 50 mm Hg until death. The moment of beginning this second bleedout phase is designated as 0' minutes and all other times from 0' minutes until death are distinguished from the corresponding times in the first bleeding phase by the (') symbol. From 0' minutes onward, the bleeding volume was recorded as the net volume removed, i.e., total bleedout minus the volume reinfused. The non-cellular volume reinfused was added to "residual" (0 minutes volume minus volume removed) and "estimated" (estimated on the basis of protein dilution) plasma volumes and the calculations were continued.

It was felt that these procedures would separate the chickens into groups with a high (RBC) and low (PLAS) oxygen-carrying capacity during the later stages of the experiments. It was also hoped that one group (PLAS) would have a relatively higher plasma protein concentration during the critical terminal stages of the experiments when the plasma protein osmotic pressure might be the factor limiting further mobilization of extravascular fluid.

Results. The volume of plasma reinfused was 10.1 $\pm$ 1.5 ml/kg with a total protein concentration of 3.56 $\pm$ 0.21 Gm%. The volume of cellular fluid was 7.4 $\pm$ 1.1 ml/kg with a hematocrit of 45.9 $\pm$ 1.7%. Plasma could not be completely excluded from the cellular pellet following centrifugation and the 4.0 $\pm$ 1.0 ml/kg of cell-free fluid contained in the cellular reinfusion had a protein concentration of 0.66 $\pm$ 0.07 Gm%. Following reinfusion the MAP was very easily lowered to 50 mm Hg by bleeding at the original rate, usually in less than 15 minutes, in both groups.

The weights, bleeding volumes and total minutes at 50 mm Hg for the two groups (Table XV) were similar to those reported in the previous experiments. Neither procedure prolonged the experiments or altered the MBV. Tables XVI, XVII and Fig. 20 summarize the results of these experiments. The heart rates (Table XVI) showed an interesting difference between the two groups. The initial heart rate was slightly higher in the PLAS group than in the RBC group, but this difference was barely significant at the $p < 0.05$ level using the *t*-test for unpaired data. The apparent difference in "relative" heart rate (Fig. 20) is a result of the unexplained higher initial "absolute" heart rate in the PLAS group. The higher heart rate in the RBC group terminally was very significant in both absolute units (see Table XVI) and as a percent of the 0 minutes heart rate (see Fig. 20). Perhaps the terminal fall in heart rate seen in the PLAS group (low oxygen-carrying capacity) and in previous experiments was due to a lack of oxygen supply to the myocardium or pacemaker tissue resulting from very low hematocrits.

The hematocrits and plasma protein concentrations in Table XVII, with one exception, reflect the expected changes resulting from these procedures. The unexpected result was that the difference in hematocrits at 0 minutes was significant (probably a random difference due to low sample numbers). During the first two hours, changes in hematocrit and plasma protein concentration were identical in both groups (Fig. 20). Following reinfusion, the hematocrit was markedly higher in the RBC group than in the PLAS group (highly significant, Table XVII) because the reinfusion fluids affected the hematocrit in opposite

Table XV. Weights and Bleeding Volumes of Chickens whose Plasma or Erythrocyte Volume was Supplemented during the Period of Hemorrhagic Hypotension.

	Plasma Reinfusion n=5	Erythrocyte Reinfusion n=6	p
Weight (kg)	1.57 ± 0.10	1.53 ± 0.08	n.s.
IBV (ml/kg)	11.7 ± 2.7	10.8 ± 1.9	n.s.
MBV (ml/kg)	32.2 ± 3.2	30.7 ± 3.3	n.s.
SBV (ml/kg)	19.7 ± 5.6	19.9 ± 3.1	n.s.
Total minutes at 50 mm Hg	238 ± 42	252 ± 25	n.s.

Table XVI. Heart Rate (beats/min) in Chickens whose Plasma or Erythrocyte Volume was Supplemented during the Period of Hemorrhagic Hypotension.

	Plasma Reinfusion	Erythrocyte Reinfusion	P
0 Minutes	321 ± 8	271 ± 17	<0.05
120 Minutes	370 ± 10	365 ± 13	n.s.
0' Minutes	350 ± 16	348 ± 18	n.s.
120' Minutes	291 ± 14	347 ± 10	<0.01

Table XVII. Hematocrit and Plasma Protein Concentration in Chickens whose Plasma or Erythrocyte Volume was Supplemented during the Period of Hemorrhagic Hypotension.

	Plasma Reinfusion	Erythrocyte Reinfusion	p
	Hematocrit (%)		
0 Minutes	25.9 ± 0.9	30.4 ± 1.5	<0.05
120 Minutes	18.6 ± 0.6	20.7 ± 1.9	n.s.
0' Minutes	15.8 ± 0.6	26.8 ± 1.3	<0.001
120' Minutes	13.1 ± 0.7	23.1 ± 1.0	<0.001
	Plasma Protein Concentration (Gm%)		
0 Minutes	4.08 ± 0.27	4.41 ± 0.26	n.s.
120 Minutes	3.01 ± 0.12	3.23 ± 0.31	n.s.
0' Minutes	3.34 ± 0.13	3.13 ± 0.33	n.s.
120' Minutes	2.94 ± 0.05	2.87 ± 0.40	n.s.

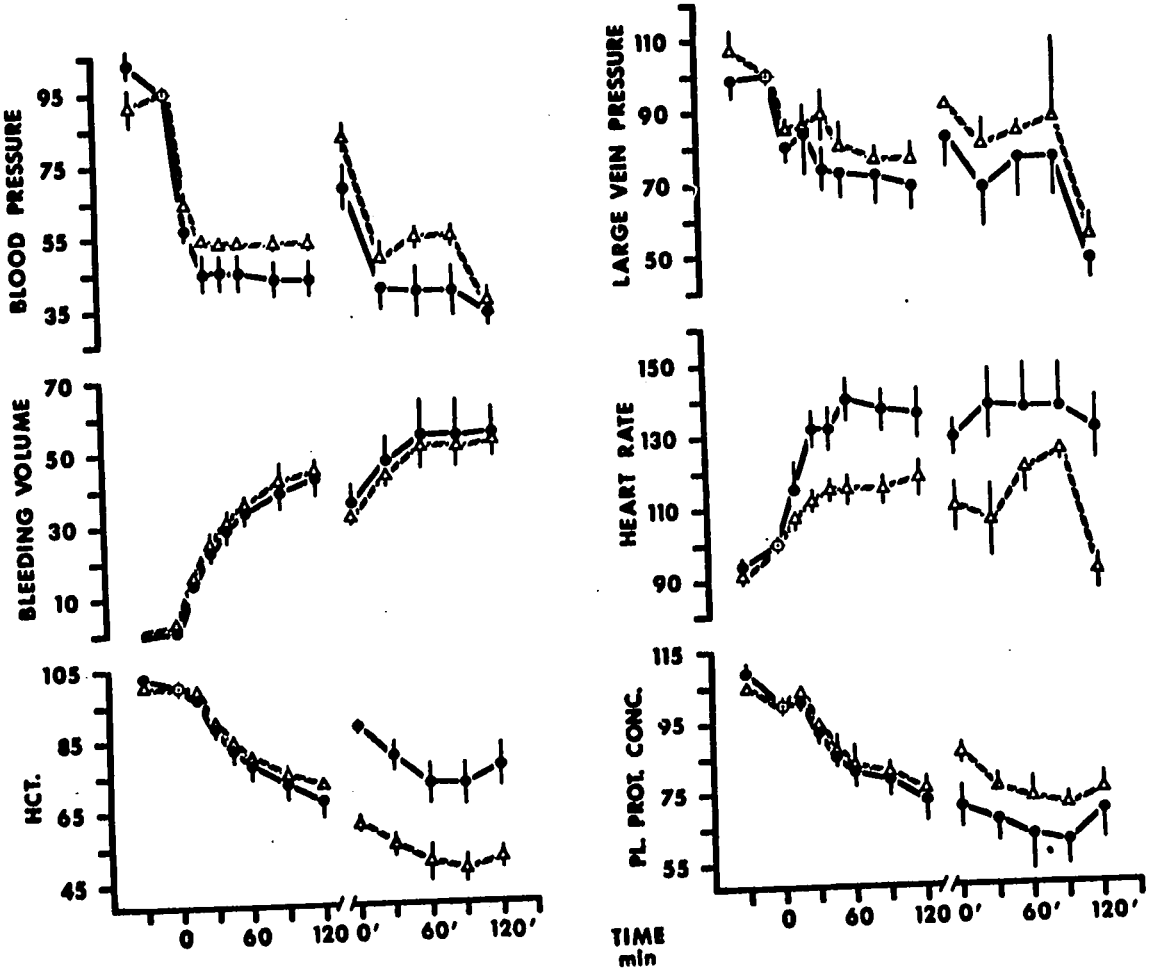

Figure 20. Comparison of the Course of Hemorrhagic Hypotension in Chickens Reinfused with Plasma or Erythrocytes after Two Hours of Hypotension. Zero minutes is the start of Hemorrhage. All numbers on the ordinates are percentages. Zero minute measurements of blood pressure, large vein pressure, heart rate, hemocrit and plasma protein concentration are 100%. Bleeding volume is expressed as a percentage of measured blood volume. The break in the abscissa indicates reinfusion and the symbol (') differentiates corresponding times in the first and second periods of hemorrhage. Closed circles and solid lines represent erythrocyte reinfusion group and open triangles and broken line represent plasma reinfusion group. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s overlap, they are shown in only one direction.

directions in each group. The hematocrit in the PLAS group fell to levels significantly lower than those seen in previous experiments (cf. 120' minutes in Table XVIII and terminal values in Table V, Section III). In the PLAS group, although the plasma protein concentration was higher, the difference was not statistically significant. However, if it is considered that the plasma protein concentration in the PLAS group increased 0.33 Gm% while during the same period it decreased 0.10 Gm% in the RBC group, the actual difference between the two concentrations is 0.43 Gm% rather than the 0.21 Gm% seen in Table XVII. The increase in plasma protein concentration is a real change because it occurred in every case. The overall falls in protein concentration in both groups was similar to those found in previous experiments. It appears that the objective of an increased terminal plasma protein concentration in one of the groups (PLAS) was not achieved. All other parameters (Fig. 20) are very similar in both groups and all the "relative"changes are similar to those seen in previous experiments.

Fig. 21 shows the massive hemodilution which was very similar to that recorded for other groups of chickens. Also evident in these diagrams, especially in the PLAS group (upper) is the shift of the dilution curves resulting from reinfusion. It appeared that the curves were merely shifted out of phase and no other differences were apparent.

Comment. It does not appear from these experiments that a relatively greater oxygen-carrying capacity of blood increases the duration of hypotension tolerated, the amount of blood which can be removed

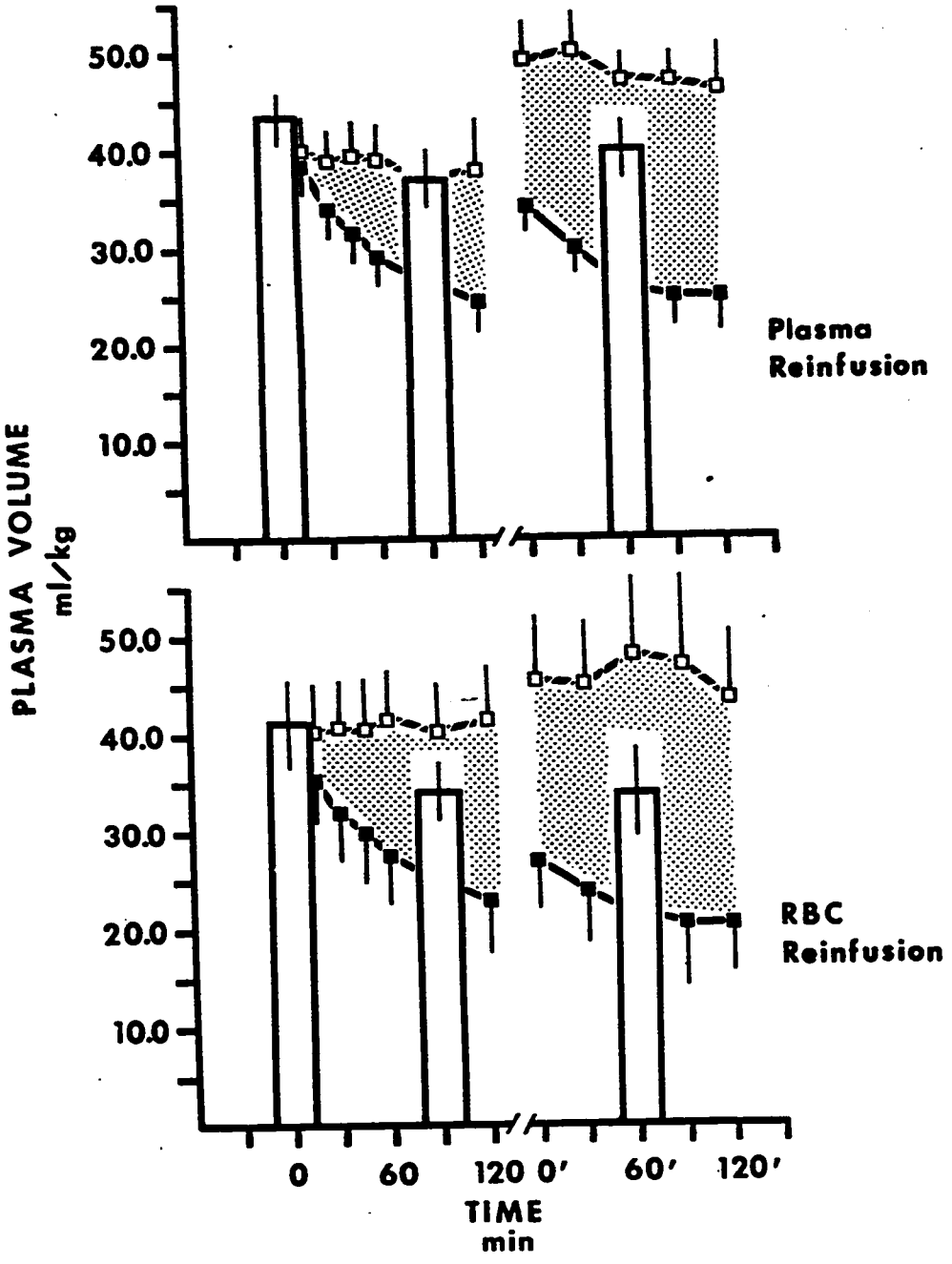

Figure 21. Comparison of Hemodilution in Chickens Reinfused with Plasma or Erythrocytes after Two Hours of Hypotension. The break in the abscissa indicates reinfusion. Bars are measured plasma volume. The closed squares and lower, solid line in each diagram is the initial plasma volume minus the volume of plasma removed. The open squares and upper, broken line in each diagram is the plasma volume estimated on the basis of protein dilution. Vertical lines indicate the S.E. of each point.

or the fluid mobilization during a period of hemorrhagic hypotension in chickens. The one beneficial effect of an increased oxygen-carrying capacity of the blood is that it prevents the terminal decline in heart rate which is usually seen following such prolonged hemorrhagic hypotension. It is suggested that the terminal decline in heart rate is related to an inadequate oxygen supply to the myocardium or pacemaker tissue late in the hypotension period, which is accentuated by the low hematocrit at this stage.

The attempt to increase fluid mobilization by increasing the terminal plasma protein concentration was not successful. The procedure followed for the reinfusion of plasma in these experiments did not raise the plasma protein concentration to an amount which was sufficient to alter the course of the experiment.

In conclusion, it is not known exactly what ultimately causes death after a prolonged period of hemorrhagic hypotension in chickens. However, except for the very terminal stages, the oxygen-carrying capacity of the blood appears to be compatible with continued compensation.

B. Changes in Peripheral Resistance and in Circulating Catecholamines during Hemorrhage in Chickens.

VII. Cardiac Output and Total Peripheral Resistance in Chickens During Hemorrhagic Hypotension and the Effect of Noradrenaline Infusion.

The following experiments were performed to assess changes in CO and TPR following hemorrhage in chickens. Because of the earlier observation of a rapid fall in MAP with hemorrhage, no marked increase in

TPR was expected and therefore an attempt was made to artificially induce generalized vasoconstriction during hemorrhage by the continuous infusion of noradrenaline. The overall effect of noradrenaline infusion on the course of hemorrhagic hypotension in chickens was also of some interest in view of the observation that catecholamine infusion alone can induce a state of shock in dogs (Erlanger & Gasser, 1919 and Yard & Nickerson, 1956).

Method. Animals used in these experiments were from a single flock of mature White Leghorn hens and the groups of experiments were done in sequence (i.e., individual experiments were not alternated between groups). One group served as a control (hemorrhage only) and the other was continuously infused with noradrenaline during hemorrhage (NA group). Anesthetization and surgical preparation were as described under general methods and monitoring MAP, bleeding, and withdrawing blood through the cuvette for measurement of CO were all accomplished through a single arterial (ischiatic) cannula. CVP was measured in the thoracic venous bed by means of a catheter in an external jugular vein and a catheter in an external iliac vein was used for infusion of noradrenaline.

Noradrenaline infusion and hemorrhage were started at the same time (0 minutes) and continued until cardiovascular collapse appeared imminent, when 20% of the MBV was reinfused. Following partial reinfusion, net bleeding volume was held constant but noradrenaline infusion was continued and the birds were followed until death or for 90 minutes (see below). The purpose of the partial reinfusion was

to prolong the final stages of hypotension because experiments in the preceding sections (no reinfusion) showed that death usually occurred too quickly for close examination of the terminal events. In four preliminary experiments, all the blood remaining in the reservoir was returned to the animal as the MAP began its terminal decline but, three hours after this reinfusion (total duration of experiments > 7 hours), all the animals were still alive and 3 of 4 were awake, alert and appeared none the worse for fear. This observation, coupled with the excellent 48-hour survival following complete reinfusion (Section II) led to adoption of partial reinfusion. Noradrenaline was freshly prepared each day by dilution of a stock solution of l-noradrenaline bitartrate with 0.9% NaCl and infused by means of a Harvard Infusion-Withdrawal Pump (Model 600-900). The speed of the pump was set to deliver 0.0194 ml/min (5 µg/kg/min, calculated as free base).

Measurements made at 15 minute intervals for the duration of the experiment were: MAP, CVP, bleeding volume and hematocrit. Plasma protein concentration, and plasma and blood volumes were not measured in these experiments. CO was measured (single determination) and TPR calculated at 30 minute intervals with one additional measurement at 15 minutes. This schedule of CO measurement was established to minimize addition of exogenous fluid (1.1 ml per measurement). All methods of measurement and calculation are given under general methods.

Hens were bled to 50 mm Hg MAP as described under general methods and maintained at this pressure by further bleeding until partial

reinfusion. Blood was reinfused in 10-15 minutes and a similar period was allowed for mixing and equilibration before measurements were resumed. The moment of resumption of cardiovascular measurements was designated as 0' minutes and all other times from 0' minutes onward were distinguished from the corresponding times in the bleeding phase by the (') symbol. After reinfusion, any blood removed for samples was replaced by an equal volume from the reservoir (i.e., no change in net bleeding volume). During the initial stages of the bleeding, when the animals were rapidly compensating, it was often difficult to maintain MAP constant at 50 mm Hg (partly because the measurement of CO interfered with bleeding) and MAP sometimes rose as high as 60 mm Hg, however, great care was taken to record the exact MAP at the time of measurement of CO to ensure accurate calculation of TPR.

Results. With the exception of hematocrit, there were no significant differences between groups for the initial measurements of cardiovascular variables (Table XVIII). The difference in hematocrits is not likely due to seasonal variation, as all experiments were completed over a short period (one month). The calculated t-value for this difference barely exceeded the tabled t-value at $p < 0.05$ and perhaps a few more experiments in each group would have closed the gap between means sufficiently to render this difference not statistically significant. Because of this difference, however, comparison of changes in hematocrit were made on the basis of "relative" values.

In the NA group, IBV was significantly higher than in the control

Table XVIII. Comparison of Initial Measurements of Cardiovascular Variables in Chickens used in Study of Cardiac Output and TPR.

	Control n=8	Noradrenaline Infusion n=7	p
MAP (mm Hg)	135 ± 9	134 ± 12	n.s.
CVP (mm Hg)	2.3 ± 0.3	3.1 ± 0.5	n.s.
Cardiac Output (ml/kg/min)	296 ± 43	351 ± 37	n.s.
TPR (PRU)	0.525 ± 0.069	0.382 ± 0.035	n.s.
Hematocrit (%)	34.5 ± 1.3	30.9 ± 0.7	<0.05

group indicating the larger amount of blood which must be removed to lower MAP to the present hypotensive level during noradrenaline infusion (Table XIX). Noradrenaline infusion, however, did not have any significant effect on MBV and therefore, the SBV's of the two groups were also markedly different. The observation that noradrenaline infusion altered the relationship of IBV and SBV in a manner which made hemorrhage in chickens appear more similar to that in dogs (see Section II), is extremely interesting. Further, in view of the marked alterations in IBV and SBV, it is interesting that noradrenaline infusion had minimal effects on changes in hematocrit (Table XX). After 60 minutes of bleeding, fluid mobilization (indicated by decreased hematocrit) was slightly greater in the NA group than that in the control group and the bleeding volume at this time was also significantly higher in the NA group (see also Fig. 22). These differences cannot be attributed to fluid added by noradrenaline infusion, for after one hour, a total of only 1.2 ml of saline would have been added and, because this fluid contains no protein, it would probably not be completely retained within the vascular space. The overall hemodilution seen in these experiments is similar to that found in previous studies although perhaps slightly augmented, probably because of fluid being added during measurements of CO. The effect of adding fluid is particularly apparent as "relative" hematocrit fell quite markedly in both groups (Table XX, Fig. 22) during the post-reinfusion period, a time during which a significant fall in hematocrit was never seen in previous experiments. More detailed discussion of the effect of noradrenaline infusion on hemodilution is included in association

Table XIX. Weights, Bleeding and Reinfusion Volumes, and Durations of Hypotension in Chickens Subjected to Hemorrhage and Partial Reinfusion and in Chickens Subjected to Similar Treatment plus Continuous Infusion of Noradrenaline (5 µg/kg/min, calculated as free base).

	Control n=8	Noradrenaline Infusion n=7	p
Weight (kg)	1.86 ± 0.08	1.94 ± 0.05	n.s.
IBV (ml/kg)	14.5 ± 2.4	24.0 ± 2.4	<0.05
MBV (ml/kg)	37.4 ± 5.0	32.5 ± 2.3	n.s.
SBV (ml/kg)	22.9 ± 4.5	8.5 ± 1.2	<0.05
Minutes from 0 to Reinfusion	171 ± 18	136 ± 15	n.s.
Reinfusion Volume (ml/kg)	7.5 ± 1.0	6.5 ± 0.5	n.s.
Minutes from 0' to Death	65 ± 16	35 ± 14	n.s.

Table XX. Effect of Hemorrhage and Partial Reinfusion on "Relative" Hematocrit in Normal Chickens and Chickens Continuously Infused with Noradrenaline (5 μg/kg/min, calculated as free base).

	Control n=8	Noradrenaline Infusion n=7	p
	Relative Hematocrit (%)		
-30 Minutes	101.0 ± 0.9	100.9 ± 0.8	n.s.
15 Minutes	92.6 ± 1.1	94.7 ± 2.6	n.s.
60 Minutes	71.9 ± 2.4	64.3 ± 2.3	<0.05
Pre-reinfusion	50.2 ± 3.4	51.6 ± 4.2	n.s.
Post-reinfusion	66.7 ± 4.5	73.4 ± 4.4	n.s.
Terminal	56.7 ± 2.5	56.5 ± 1.5	n.s.

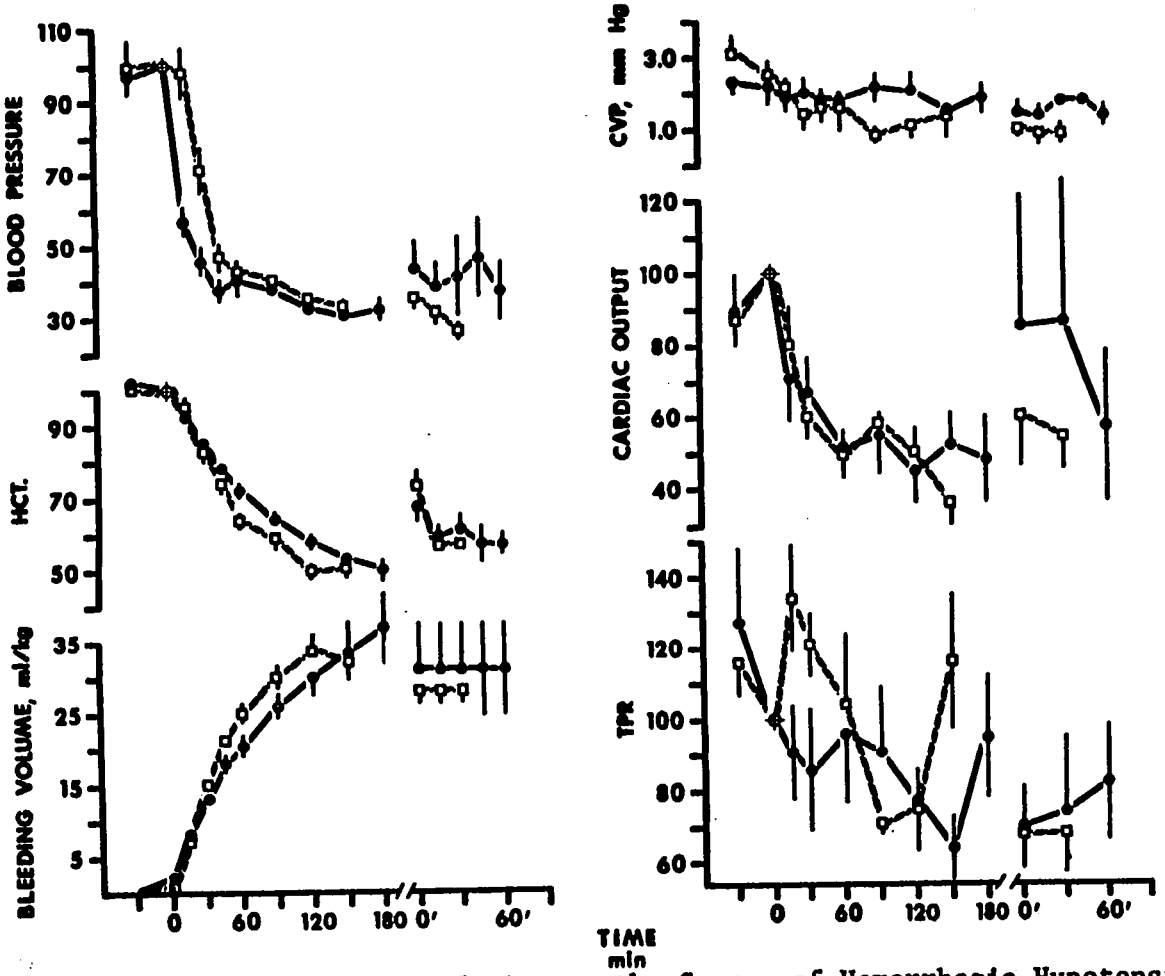

Figure 22. The Effects of Noradrenaline infusion on the Course of Hemorrhagic Hypotension (including Cardiac Output and Total Peripheral Resistance) in Chickens Subjected to Hemorrhage and Partial Reinfusion. Zero minutes is the start of hemorrhage. Except for bleeding volume and CVP, all numbers on the ordinates are percentages. Zero minute measurements are 100%. The break in the abscissa indicates partial reinfusion and the symbol (') differentiates the corresponding times before, and after partial reinfusion. The time scale is slightly expanded following partial reinfusion to accommodate plotting more points. The closed circles and solid lines represent the control, and open squares and broken lines the noradrenaline infusion (5 µg/kg/min, calculated as free base) groups. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s overlap, they are shown in only one direction.

with subsequent experiments (Section IX) where plasma volumes were measured. The larger IBV in the NA group meant that the time taken to reach the preset hypotensive level was greater in this group. However, the average durations from the beginning of hemorrhage until partial reinfusion and from partial reinfusion until death were shorter (not significant) in the NA group (Table XIX).

Noradrenaline infusion prevented the early, rapid fall in MAP which is usually seen during bleeding in chickens. Average MAP (Table XXI, Fig. 22) in the NA group had not decreased significantly from 0 minutes after 15 minutes of bleeding while, in the control group, MAP had decreased 43% during this same time.

The changes in CO were almost identical in both groups (Table XXII, Fig. 22). The final CO before partial reinfusion was 48 $\pm$ 12% of the 0 minutes value in the control group, and 36 $\pm$ 6% in the NA group. Partial reinfusion usually increased CO (in all hens except two in the NA group). The average TPR's also exhibited no significant differences between the two groups. It had been expected that TPR after 15 minutes of bleeding would be higher in the NA group because of the significantly higher MAP (Table XXI) while the CO's were essentially the same at this time. Closer examination of Table XXII shows that the average TPR <u>decreased</u> in the control group from 0 to 15 minutes while it <u>increased</u> during this same time period in the NA group. Considering the large standard errors (10-15%) and the fact that the 0 minutes value was higher in the control group than in the NA group, it was thought the "relative" TPR's (Fig. 22) might show this difference to be significant. The average "relative" TPR's

Table XXI. Effect of Hemorrhage and Partial Reinfusion on MAP in Normal Chickens and Chickens Continuously Infused with Noradrenaline (5 µg/kg/min, calculated as free base).

	Control n=8	Noradrenaline Infusion n=7	p
	MAP (mm Hg)		
0 Minutes	134 ± 11	134 ± 5	n.s.
15 Minutes	81 ± 8	132 ± 8	<0.001
60 Minutes	60 ± 4	58 ± 4	n.s.
Pre-reinfusion	45 ± 2	44 ± 3	n.s.
Post-reinfusion	57 ± 7	49 ± 3	n.s.
Terminal	52 ± 8	36 ± 4	n.s.

Table XXII. Effect of Hemorrhage and Partial Reinfusion on Cardiac Output and TPR in Normal Chickens and Chickens Continuously Infused with Noradrenaline (5 μg/kg/min, calculated as free base).

	Control n=8	Noradrenaline Infusion n=7	p
	Cardiac Output (ml/kg/min)		
0 Minutes	382 ± 85	413 ± 36	n.s.
15 Minutes	236 ± 41	321 ± 47	n.s.
60 Minutes	167 ± 21	204 ± 36	n.s.
Pre-reinfusion	148 ± 28	140 ± 18	n.s.
Post-reinfusion	278 ± 55	238 ± 54	n.s.
Terminal	222 ± 57	216 ± 43	n.s.
	TPR (PRU)		
0 Minutes	0.456 ± 0.061	0.341 ± 0.042	n.s.
15 Minutes	0.366 ± 0.026	0.438 ± 0.044	n.s.
60 Minutes	0.419 ± 0.108	0.337 ± 0.054	n.s.
Pre-reinfusion	0.409 ± 0.084	0.367 ± 0.044	n.s.
Post-reinfusion	0.219 ± 0.015	0.248 ± 0.060	n.s.
Terminal	0.316 ± 0.123	0.245 ± 0.061	n.s.

after 15 minutes of bleeding were 91 ± 13% in the control group and 134 ± 15% in the NA group and were not quite significantly different. Nevertheless, consideration of the unaveraged data does indicate that this was a real difference. In the control group, after 15 minutes of bleeding, 2 of 8 animals had an increased TPR at 15 minutes although some of these increases probably were within the range of error of the method. The fact remains, however, that TPR did not decrease in the NA group between 0 and 15 minutes, while during this same period, half of the animals in the control group had a decrease in TPR of 20% or more.

Tachyphylaxis to noradrenaline developed fairly rapidly and TPR was consistently decreased or unchanged late in the experiments in most animals in both groups. Arbitrarily selecting a 20% change as significant, the control group at 120 minutes had 2 decreased TPR's and 3 unchanged, whereas the NA group had 1 increased and 4 decreased. This (and the averaged TPR's) indicated a general tendency for TPR to fall slightly later in the experiments. Just prior to partial reinfusion, the TPR tended to increase again, and the average pre-reinfusion values were 95 ± 17% of zero minute values in the control group, and 116 ± 19% in the NA group. Following partial reinfusion, TPR tended to decrease, and did not seem to increase again in either group.

Comments. It appears from these experiments that hemorrhage in chickens does not usually result in a generalized vasoconstriction of sufficient proportion to be detected in measurements in TPR. The

CO falls, as expected, but at roughly the same rate as the MAP, and there is little change in TPR with perhaps even a tendency for it to decrease slightly during prolonged periods of hemorrhagic hypotension. These results are similar to those obtained by Abel et al. (1967) in dogs but unlike those of Reynell et al. (1955) who showed, initially at least, an increase in TPR. The present experiments indicate a lack of active vasoconstriction in chickens during hemorrhage, an hypothesis which is readily supported by the observation of a rapid fall in MAP. A certain proportion of chickens (in these experiments, 2 of 8) respond to hemorrhage with a slight increase of TPR, at least during the early stages of hemorrhage but this increased TPR may be partly passive, resulting from elastic recoil of resistance vessels (i.e., inherent elasticity of vascular tissue) perfused at a decreased transmural pressure (i.e., stretch reduced).

Continuous infusion of a rather large dose of noradrenaline during the period of hemorrhage modified the response of the chickens only slightly. In contrast, smaller doses of noradrenaline infused into dogs initially increase blood pressure which then becomes stabilized in the normal range although cardiac output is greatly reduced, i.e., TPR is greatly increased (Yard & Nickerson, 1956). Similarly in chickens, the start of noradrenaline infusion was usually marked by a pressor response and during the early stages of hemorrhage there was a small, and probably real increase in TPR although it could not be shown to be statistically significant. This effect of noradrenaline did not last and had completely disappeared 90 minutes after the start of bleeding. The net effect was an altered relationship between IBV and SBV and slightly greater fluid mobilization in the earlier

part of the experiments suggesting that noradrenaline enhanced the rate of compensation during the early stages. This could be due to relatively greater vasoconstriction in the precapillary segment of resistance, accentuating the fall in capillary hydrostatic pressure and aiding the mobilization of extravascular fluid. This mechanism operates in cats (Öberg, 1964) and dogs (Haddy et al., 1965) during hemorrhage.

Although it did appear that the group infused with noradrenaline did not survive as long, there were no statistically significant terminal differences between the two groups. The maximal amounts of blood removed and the total fluid mobilization were similar in both groups and hemoconcentration and decompensation were not seen in either one. In fact, following partial reinfusion, hemodilution, which was at least partly due to exogenous fluid introduced in the measurement of CO, was seen in both groups. Thus, although noradrenaline infusion may not contribute directly to death in chickens through a mechanism such as that proposed by Hollenberg (1965) for dogs, it may indirectly shorten the period of compensation by simply speeding it up.

The overall most striking observation is a lack of any substantial effect of prolonged administration of such a high dose of noradrenaline (5 μg/kg/min, calculated as free base). This dose of noradrenaline is more than adequate to produce shock in dogs even when there is no bleeding (Yard & Nickerson, 1956). In this laboratory, doses of noradrenaline up to 20 μg/kg/min, calculated at the free base, have been continuously infused into chickens for more than 5 hours without causing shock.

VIII. Regional Blood Flow and Vascular Resistance in the Ischiatic and Mesenteric Beds during Hemorrhagic Hypotension. Generally, changes

in TPR are not good estimations of vasoconstriction throughout the body because of the parallel arrangement of regional vascular beds which allows marked changes to occur in a particular regional bed without any significant alteration in TPR. Because of ease of access, the limbs of animals are often studied for regional changes in blood flow and vascular resistance. Gesell and Moyle (1922) used a simple drop counter to measure the venous drainage from the hindlimb of dogs subjected to graded hemorrhage and found that the decrease in blood flow was greater than could be accounted for by the fall in blood pressure. Others have also observed a decreased blood flow and vasoconstriction in hindlimbs of dogs in hemorrhagic shock (see Freeman et al., 1938; Eckstein et al., 1946). Although measurements in limbs reflect changes in both skin and skeletal muscle flow and resistance, Selkurt and Rothe (1961) showed that there was a marked increase in vascular resistance in gracilis muscle, with its nervous connections, during the early stages of hemorrhagic hypotension which was only decreased slightly by reinfusion of the withdrawn blood. Moreover, resistance remained considerably above the prehemorrhage value throughout the decompensatory phase of normovolemic shock.

Virtually all investigators agree that the intestine is one of the organs most likely to be critical in the development of irreversible shock. Mesenteric vascular resistance in dogs was found to increase markedly soon after the beginning of hemorrhage (Selkurt et al., 1947); it declined later in the oligemic period, although it remained above control levels. In the late stages of hypovolemia, it again rose sharply, and reinfusion resulted in only a temporary decline to control levels or

below. The critical nature of decreased flow to the intestine has been most extensively investigated by Lillehei and his associates (see Lillehei et al., 1962; Lillehei et al., 1964). They noted that if the intestinal circulation was maintained during shock almost all dogs survived. These dogs did not show hemoconcentration and plasma volume loss characteristic of irreversible shock, and at sacrifice their intestinal mucosa was normal. These same results were obtained in chronic Eck fistula dogs, i.e., all portal blood diverted into the inferior vena cava, thus, indirect liver perfusion as a result of superior mesenteric arterial perfusion was not a factor in promoting survival in these experiments.

The following experiments were carried out to assess changes in regional blood flow and vascular resistance in the leg and mesenteric vascular beds during hemorrhagic hypotension in the chicken. The ischiatic arterial flow to the leg supplies blood mostly to skin and skeletal muscle, and the cranial mesenteric artery is the major blood supply to the intestine of the chicken.

Methods. All animals used in this study were mature White Leghorn hens anesthetized and surgically prepared as described under general methods. Measurements made initially, and at 15 minute intervals for the duration of the experiments were: MAP, CVP, regional flow, bleeding volume, hematocrit and plasma protein concentration. In addition, plasma and blood volumes were measured initially, at 120 minutes after the start of bleeding, and immediately after partial reinfusion. The techniques for measuring these cardiovascular variables are given under

general methods. Values for regional vascular resistance, initial plasma volume minus the volume of plasma removed, and plasma volume estimated on the basis of protein dilution were calculated from the above measurements. These calculations are described under general methods.

After waiting one hour to allow recovery from surgical preparation, the animals were bled to 50 mm Hg MAP as described under general methods. MAP was maintained at this level by further bleeding as necessary until its terminal decline was apparent when 20% of the MBV was reinfused over a 10-15 minute period. After waiting another 10-15 minutes, measurements and calculations were continued until death, or, for 90 minutes. The resumption of measurements is designated as 0' minutes and the symbol (') is used to differentiate corresponding times before and after partial reinfusion. The calculation of initial plasma volume minus the volume of plasma removed and plasma volume based on protein dilution were not continued because the exact nature of the blood reinfused (plasma volume and protein content) was not determined. Following reinfusion, any blood removed for use as samples was replaced by an equal volume from the reservoir, i.e., bleeding volume was held constant.

Results. Comparison of the initial measurements of cardiovascular variables (Table XXIII) showed only minor differences. Although the MAP's of each group were significantly different at this point (-30 minutes), at the start of the bleeding (0 minutes), MAP had fallen slightly in the ischiatic flow group and remained unchanged in the mesenteric flow group and the difference at this time was not statistically significant. The

Table XXIII. Comparison of Initial Measurement of Cardiovascular Variables in Chickens used to Study Flow and Resistance in Ischiatic and Mesenteric Vascular Beds during Hemorrhagic Hypotension.

	Ischiatic n=15	Mesenteric n=9	p
MAP (mm Hg)	127 ± 5	107 ± 10	<0.05
CVP (mm Hg)	2.6 ± 0.3	2.9 ± 0.3	n.s.
Hematocrit (%)	31.2 ± 0.7	33.4 ± 0.7	<0.05
Plasma Protein Conc. (Gm%)	4.80 ± 0.19	4.78 ± 0.20	n.s.
Flow (ml/min)	10.7 ± 1.0	36.6 ± 3.2	---
Resistance (PRU)	12.1 ± 0.8	2.96 ± 0.40	---
Plasma Volume (ml/kg)	41.4 ± 2.0	40.6 ± 2.0	n.s.
Blood Volume (ml/kg)	60.2 ± 3.0	60.7 ± 2.5	n.s.

only difference in treatment between the two groups was the more extensive surgery in the mesenteric flow group which was accompanied by some manipulation of the intestine to expose the cranial mesenteric artery. This was an additional stress and may explain the slightly lower initial MAP in this group. There was an unexplained small, but statistically significant, difference in hematocrits in the two groups, therefore, any further comparison of hematocrits will be done using the "relative" measurements. Flow and resistance in the two vascular beds will also be compared on the basis of "relative" measurements because of the obvious difference in total flow to the two regions.

The MBV in the mesenteric flow group was significantly less than that in the ischiatic flow group, and, because the IBV's were almost identical, the SBV in the former group was also significantly lower than that in the latter (Table XXIV). As a constant percentage of the MBV was reinfused in each case, the reinfusion volume of the mesenteric flow group was also lower than that in the ischiatic flow group. These differences may reflect the relative lengths of the bleeding periods in the two groups as the average duration from 0 minutes to partial reinfusion tended to be shorter in the mesenteric flow group (not significant). However, both these groups had bleeding periods shorter than those of previous experiments (Section III) which was probably due to the additional surgery necessary to implant probes. The average duration from partial reinfusion to death was shorter (not significantly) in the mesenteric flow group than that in the ischiatic flow group (animals which survived longer that 90 minutes were taken to be 90 minute survivors), thus, the former group tended to compensate least, judged by

Table XXIV. Weights, Bleeding and Reinfusion Volumes, and Durations of Hypotension in Chickens used to Study Flow and Resistance in Ischiatic and Mesenteric Vascular Beds during Hemorrhagic Hypotension.

	Ischiatic n=15	Mesenteric n=9	p
Weight (kg)	1.77 ± 0.07	1.85 ± 0.09	n.s.
IBV (ml/kg)	14.0 ± 1.1	14.7 ± 1.7	n.s.
MBV (ml/kg)	33.3 ± 1.6	26.8 ± 2.3	<0.05
SBV (ml/kg)	19.4 ± 1.6	12.1 ± 2.0	<0.05
Minutes from 0 to Reinfusion	176 ± 16	154 ± 13	n.s.
Reinfusion Volume (ml/kg)	6.6 ± 0.3	5.4 ± 0.5	<0.05
Minutes from 0' to Death	73 ± 7	53 ± 10	n.s.

MBV's and duration of hypotension, probably because of more extensive surgical preparation in this group.

The course of hemorrhagic hypotension in these groups (Tables XXV and XXVI, Fig. 23), although shorter in duration, was very similar to that already described in preceding sections. During the later part of the bleeding period, hematocrit and plasma protein concentration (Table XXV) remained higher in the mesenteric flow group than in the ischiatic flow group but this was only significant in the case of hematocrit. This difference was probably the result of a diminished ability to compensate in the former group which in turn resulted from the additional surgical trauma. The maximum bleeding volume (Table XXVI, Fig. 23) was less in the mesenteric flow group (44%) than it was in the ischiatic flow group (57%) and both these bleeding volumes were lower than those previously reported as the maximum (65-70%, Section III), probably for the same reason (surgical trauma).

Fig. 24 is a diagram of fluid mobilization and, although there seems to be slightly greater fluid mobilization in the ischiatic flow group than in the mesenteric flow group, the differences were not statistically significant. After two hours of bleeding the average volume of plasma removed was 48% of the initial volume in the former group and 37% in the latter group. During this same period, the total amounts of fluid mobilized in the two groups were 37 and 27% of the initial measured plasma volume respectively and the average rates of fluid mobilization during this two hour period were 7.7 ml/kg/hr in the ischiatic flow group and 5.6 ml/kg/hr in the mesenteric flow group, indicating once again the great capability of the chicken to mobilize extravascular fluid.

Table XXV. Effect of Hemorrhage and Partial Reinfusion on "Relative" Hematocrit and Plasma Protein Concentration in Chickens used to Study Flow and Resistance in Ischiatic and Mesenteric Vascular Beds.

	Ischiatic n=15	Mesenteric n=9	p
	Relative Hematocrit (%)		
-30 Minutes	107.0 ± 0.6	106.9 ± 1.8	n.s.
15 Minutes	96.3 ± 0.9	98.1 ± 1.0	n.s.
90 Minutes	67.6 ± 2.4	75.6 ± 2.8	<0.05
Pre-reinfusion	57.2 ± 2.9	67.6 ± 3.5	<0.05
Post-reinfusion	68.2 ± 3.0	76.2 ± 3.1	n.s.
Terminal	69.9 ± 2.5	73.7 ± 4.3	n.s.
	Relative Plasma Protein Concentration (%)		
-30 Minutes	110.3 ± 1.5	110.2 ± 2.6	n.s.
15 Minutes	98.3 ± 1.2	100.3 ± 1.2	n.s.
90 Minutes	70.5 ± 1.9	76.7 ± 2.4	n.s.
Pre-reinfusion	66.0 ± 2.1	69.3 ± 3.1	n.s.
Post-reinfusion	73.5 ± 2.6	75.2 ± 3.0	n.s.
Terminal	71.0 ± 2.9	73.4 ± 5.4	n.s.

Table XXVI. Bleeding Volume during Hemorrhage and Partial Reinfusion in Chickens used to Study Flow and Resistance in Ischiatic and Mesenteric Vascular Beds.

	Ischiatic n=15	Mesenteric n=9	p
	Bleeding Volume (ml/kg)		
15 Minutes	9.1 ± 0.2	9.1 ± 0.1	n.s.
90 Minutes	24.3 ± 1.0	21.2 ± 2.1	n.s.
Pre-reinfusion	33.2 ± 1.6	26.3 ± 2.4	<0.05
Post-reinfusion	26.7 ± 1.4	23.6 ± 2.1	n.s.

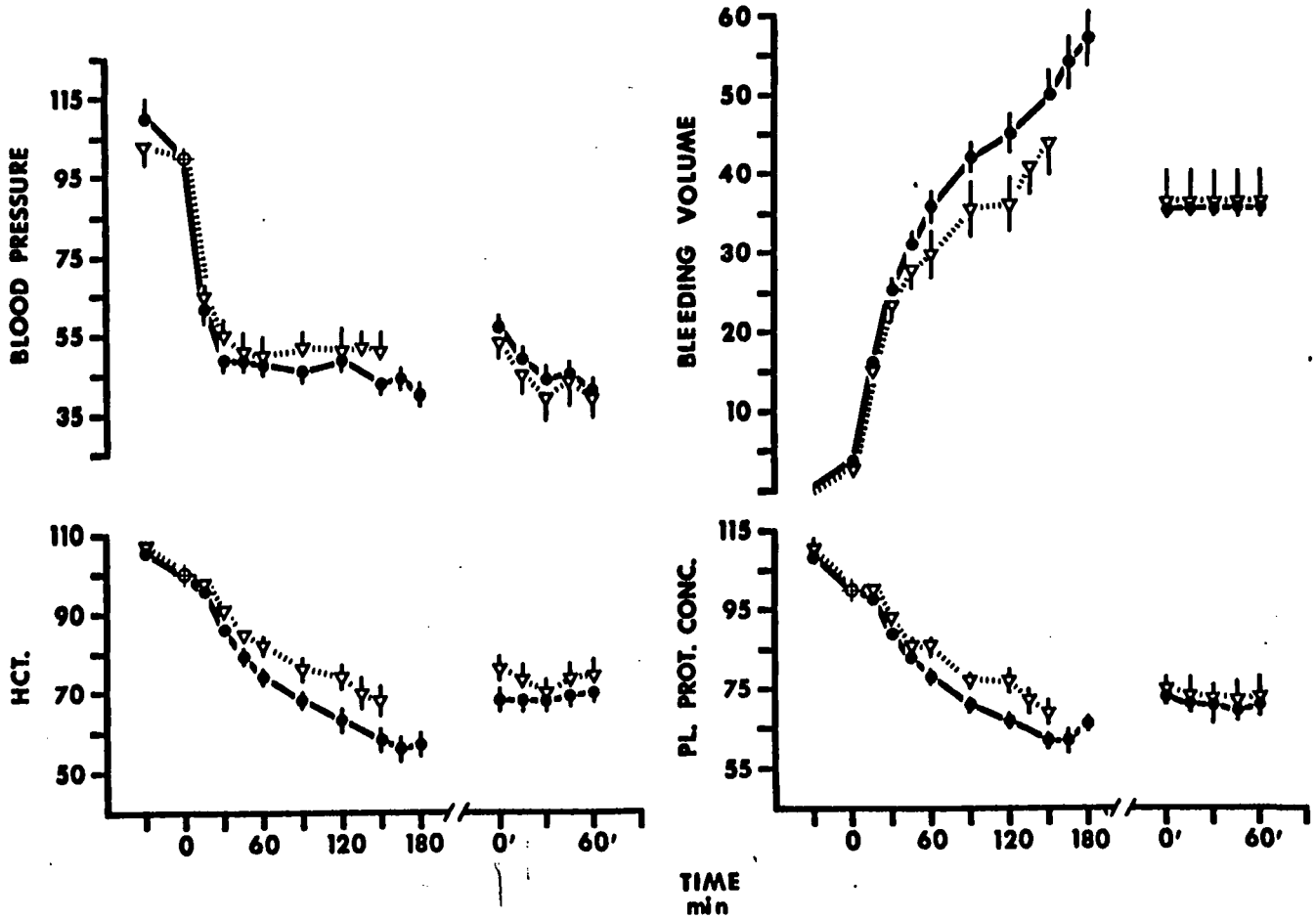

Figure 23. Comparison of the Effect of Hemorrhage and Partial Reinfusion in Chickens used to Study Flow and Resistance in the Ischiatic and Mesenteric Vascular Beds. Zero minutes is the start of Hemorrhage. All numbers on the ordinates are percentages. Zero minute measurements of blood pressure, hematocrit and plasma protein concentration are 100%. Bleeding volume is expressed as a percent of the initial measured blood volume. The break in the abscissa indicates partial reinfusion and the symbol (') differentiates the corresponding times before, and after partial reinfusion. The time scale is slightly expanded following partial reinfusion to accommodate plotting more points. The closed circles and solid lines represent the ischiatic flow group and the open triangles and broken lines represent the mesenteric flow group. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s overlap, they are shown in only one direction.

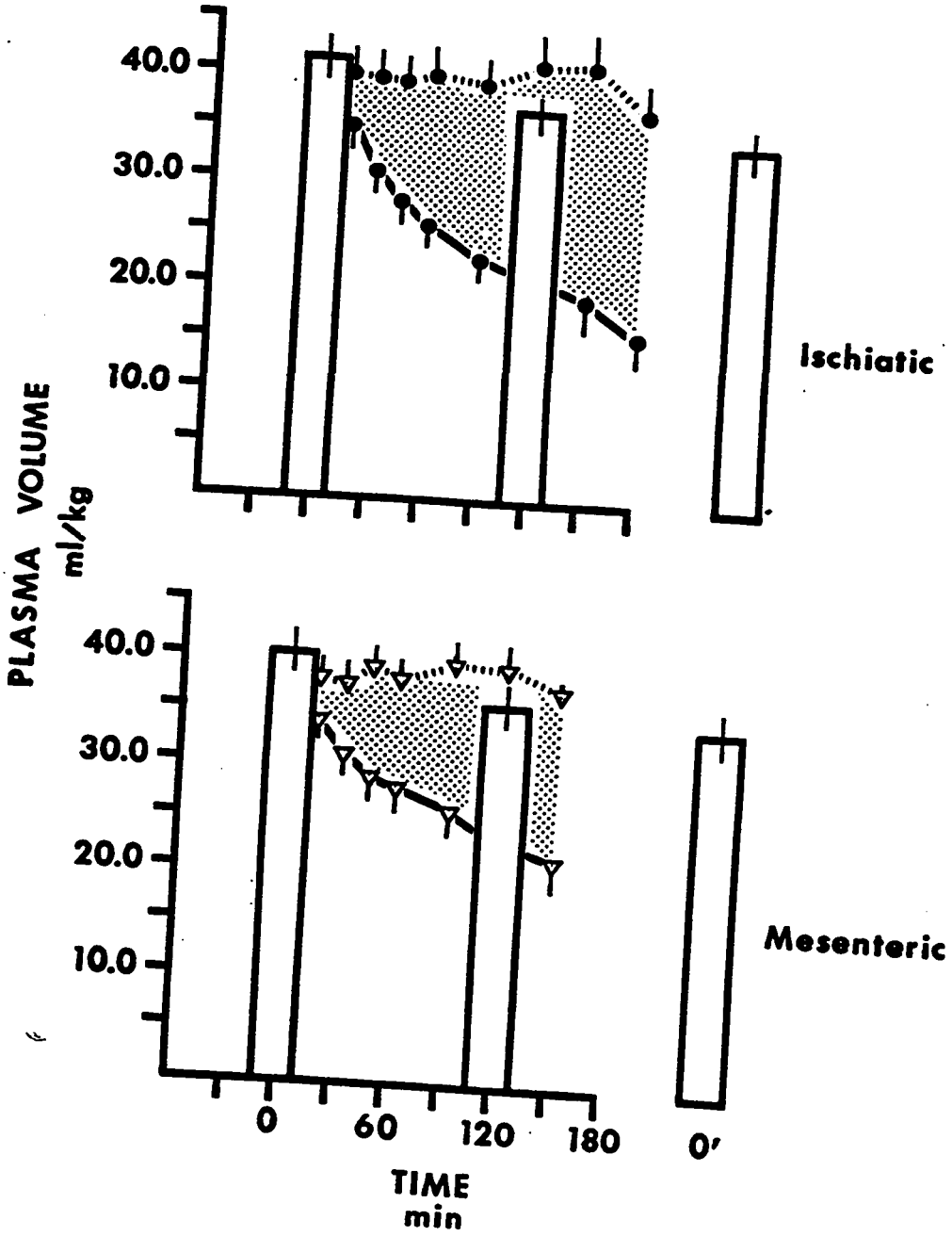

Figure 24. Comparison of Hemodilution following Hemorrhage and Partial Reinfusion in Chickens used to Study Ischiatic and Mesenteric Flow and Vascular Resistance. Bars indicate the measured plasma volume. The lower solid line in each diagram is the initial plasma volume minus the volume of plasma removed. The upper, broken line in each diagram is the plasma volume estimated on the basis of protein dilution. Vertical lines indicate the S.E. of each point. Zero prime indicates the plasma volume measurement made following partial reinfusion.

The effects of this schedule of hemorrhage and reinfusion on blood flow and total regional resistance are shown in Table XXVII and Fig. 25. The flow in both vascular beds decreased with hemorrhage but, except for immediately before partial reinfusion and during the post-reinfusion period, this decrease was not proportionately greater than the fall in MAP. Just before partial reinfusion, flow started to fall sharply in both groups. Following partial reinfusion, it rose again and, finally, in the terminal stages of the experiment, flow again fell sharply. Generally, the magnitude of decreased average flow was similar in both vascular beds (Table XXVII, Fig. 25).

There was a slight tendency for average resistance in the mesenteric bed to increase at the beginning of hemorrhage, however, this increase was not statistically significant when compared to the ischiatic bed where average resistance remained unchanged (see 15 minutes, Table XXVII). The increased mesenteric resistance was not sustained, because at 90 minutes, the average resistance in both beds was slightly decreased. The average total vascular resistance increased sharply just before partial reinfusion in both beds, fell again following partial reinfusion but remained slightly elevated over control, and then increased markedly again near the end of the experiment. In both cases, when the resistance began to increase sharply, the MAP was usually 40-45 mm Hg and falling.

For a more detailed analysis of the individual results, a change from control of greater than 10% was arbitrarily considered to be significant. The 15 minute "relative" resistance values in the mesenteric group, average change in total vascular resistance was a 15% increase, showed 4 increased, 3 decreased and 2 remained unchanged. Thus,

Table XXVII. Effect of Hemorrhage and Partial Reinfusion on "Relative" Flow and Resistance in Ischiatic and Mesenteric Vascular Beds.

	Ischiatic n=15	Mesenteric n=9	p
	Relative Flow (%)		
-30 Minutes	107 ± 5	105 ± 6	n.s.
15 Minutes	57 ± 7	63 ± 7	n.s.
90 Minutes	52 ± 4	61 ± 8	n.s.
Pre-reinfusion	29 ± 6	35 ± 4	n.s.
Post-reinfusion	48 ± 8	39 ± 5	n.s.
Terminal	27 ± 8	23 ± 8	n.s.
	Relative Resistance (%)		
-30 Minutes	102 ± 2	98 ± 10	n.s.
15 Minutes	99 ± 10	115 ± 12	n.s.
90 Minutes	91 ± 12	88 ± 10	n.s.
Pre-reinfusion	232 ± 79	146 ± 22	n.s.
Post-reinfusion	126 ± 19	131 ± 21	n.s.
Terminal	262 ± 99	177 ± 38	n.s.

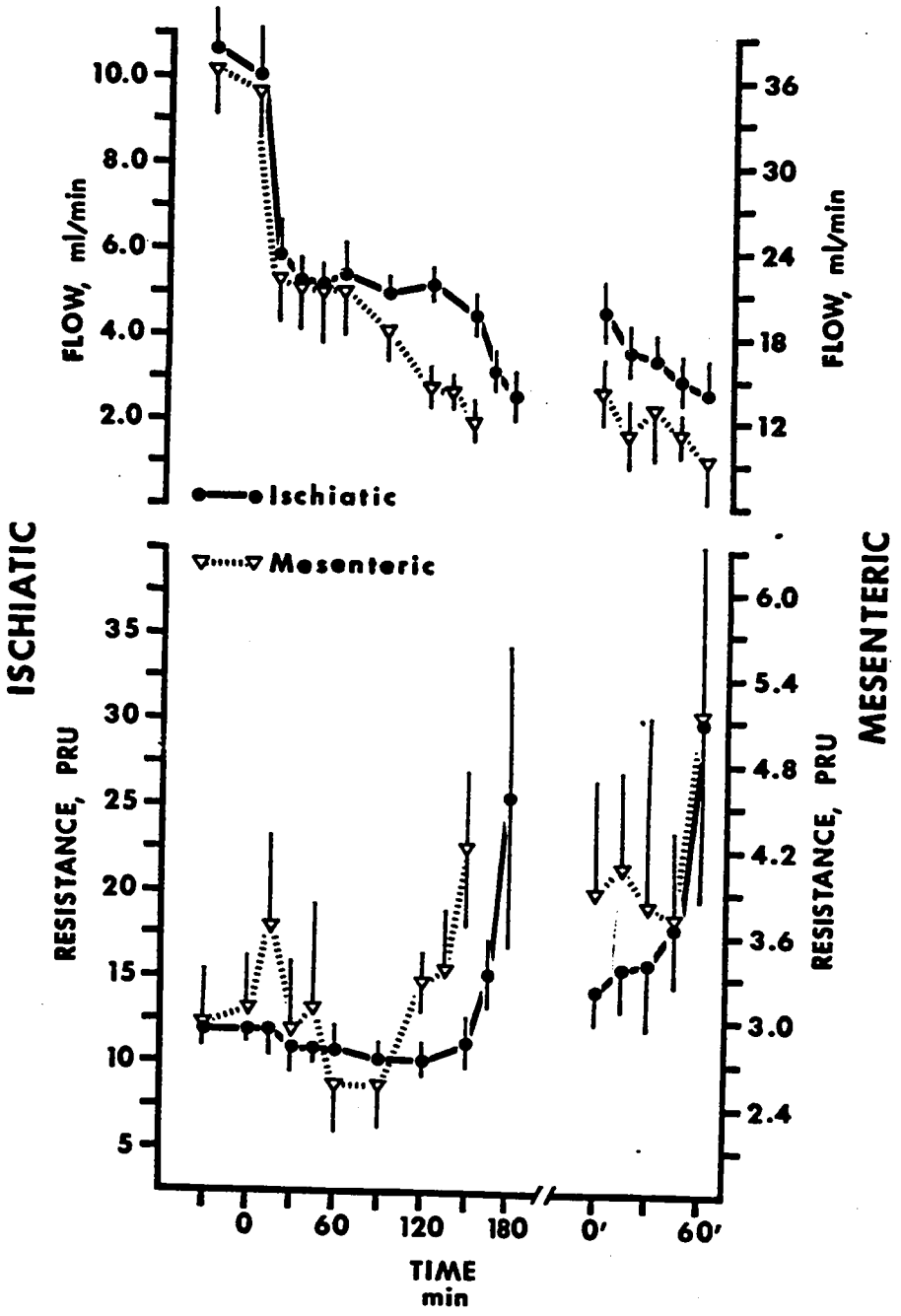

Figure 25. Comparison of the Effect of Hemorrhage and Partial Reinfusion on Flow and Vascular Resistance in the Ischiatic and Mesenteric Vascular Beds. The scales for flow and resistance are arranged along the ordinates so that the two beds are comparable in this diagram. Zero minutes is the start of hemorrhage. The break in the abscissa indicates partial reinfusion and the symbol (') differentiates the corresponding times before, and after partial reinfusion. The time scale is slightly expanded following partial reinfusion to accommodate plotting more points. Vertical lines indicate the S.E. of each point. If S.E.'s overlap, they are shown in only one direction.

there was no general trend. At 90 minutes, where the average total vascular resistance in the mesenteric bed was decreased 12%, 2 were increased and the remainder decreased or unchanged, perhaps indicating a trend toward decreased vascular resistance in the mesenteric vascular bed later in the bleeding phase. Just before partial reinfusion all but 2 were increased by variable amounts (average increase 46%). In the survivors following partial reinfusion, the post-reinfusion and terminal measurements showed all hens but 2 had increased mesenteric vascular resistance and the average increases were 31 and 77% at these two times respectively. These observations indicate a general tendency for resistance to rise during the late bleeding phase, fall slightly, but remain above control after partial reinfusion and rise sharply terminally. There was no apparent difference between the one animal in this group (i.e., mesenteric flow group) which lasted longer than 90 minutes after partial reinfusion and the remainder of the animals which survived partial reinfusion. Resistances in this animal showed a slight decrease (11%) at 15 minutes, a slight increase at 90 minutes (15%), an increase at pre-reinfusion (26%), little change from control after partial reinfusion (increased 8%) and a marked increase 90 minutes after partial reinfusion (117%).

A similar consideration of ischiatic vascular resistance gave an approximately equal number of increases and decreases with a few unchanged at 15 and 90 minutes, when the average changes were a 1% and 9% decrease, respectively. Just before partial reinfusion, half the animals showed very marked increases (>150%) while the remainder were unchanged or slightly decreased. MAP in those chickens which showed a

marked increase in resistance was low (40-47 mm Hg) and falling but a few hens with even lower MAP's (35 mm Hg) did not show the sharp increase just before partial reinfusion. In the survivors following partial reinfusion, those animals that had high pre-reinfusion resistance had this value reduced, although the values remained above control, and the others showed no change (i.e., greater than 10%) following partial reinfusion. Terminally, increases and decreases were again equally divided, but increases were quantitatively greater and the net result was an average increase. Thus, a qualitative consideration of the ischiatic vascular resistance also showed no definite pattern of change between those hens that survived 90 minutes or longer and those that did not. In general, ischiatic vascular resistance remained unchanged during the bleeding period, rose in half the animals just before partial reinfusion, decreased somewhat following partial reinfusion, but remained above control, and rose again terminally. The other half of the hens had quantitatively smaller <u>decreased</u> ischiatic vascular resistance during the last part of the experiment.

<u>Comment</u>. There was no definite pattern of vascular resistance change in either the leg or gut of the chicken during hemorrhagic hypotension. Blood flow falls in both these regions during hemorrhage, but the average fall is not significantly different from the average fall in MAP and small increases and decreases in vascular resistance were found in approximately equal numbers of animals. These results are in marked contrast to the large and sustained increases in vascular resistance seen in these two beds following hemorrhage in dogs (<u>see</u> Review of

Literature for references).

Towards the end of the bleeding phase in the present experiments, there was a more definite tendency for vascular resistance to increase in both beds, but this was not universally found in all animals. This increased resistance declined somewhat after partial reinfusion and rose again terminally. In the ischiatic flow group, those animals which did not show an increased resistance in the terminal stages of the bleeding period often did not show any further change following partial reinfusion and sometimes the resistance even increased. Likewise, in the mesenteric flow group, partial reinfusion did not always result in a fall in resistance and, in a few animals, there was an increase in vascular resistance.

The sudden and marked increases in vascular resistance which occurred in the terminal stages of the experiments or prior to partial reinfusion are an interesting phenomenon. The exact origin of the increased resistance at these times is unknown but may be due to "critical closure". "Critical closure" results when the transmural pressure becomes too low to oppose the elastic recoil of small resistance vessels which then close. This phenomenon is more often seen when "tonus" of these vessels is high (as would be expected during a prolonged period of hemorrhagic hypotension). Because MAP was always falling (MAP at the time in question was of the order of 40 mm Hg) in those animals which displayed the sudden rise in vascular resistance, we favor this explanation. Increases in vascular resistance in the mesenteric bed during the earlier hemorrhage period tended to be greater than those in the ischiatic bed. However, generally the increases in

both beds were small enough to be partially due to a passive increase in resistance which results from the elastic recoil of small resistance vessels when transmural pressure is reduced. In this case, the transmural pressure is not reduced sufficiently to lead to "critical closure".

Because the dog has marked pathological changes in the gut following hemorrhage (see Lillehei et al., 1964), several postmortem examinations of the gut of chickens were made during these and preceding experiments. Gross pathological changes were never seen even in animals subjected to several hours of hemorrhagic hypotension. In a few cases, histological sections of small intestine were examined and these also were essentially normal. The absence of pathological changes in the gut probably reflects the absence of substantial vasoconstriction in the mesenteric vascular bed following hemorrhage in the chicken.

The results varied from animal to animal, but in general, changes in resistance in either direction were small. This bears out our observations on the lack of change in TPR and the rapid fall in MAP during hemorrhage in chickens. In conclusion, it can be said that substantial active vasoconstriction does not occur in either mesenteric or ischiatic vascular beds during hemorrhagic hypotension in the chicken.

IX. Regional Blood Flow and Segmental Vascular Resistance in the Ischiatic Bed during Hemorrhagic Hypotension in Chickens and the Effect of Noradrenaline Infusion. The experiments reported thus far demonstrated no organized or consistent changes in vascular resistance in the chicken following hemorrhage which could be interpreted as either

a compensatory or decompensatory response. Constant total regional resistance, however, does not preclude changes in segmental resistance. The latter could play a role in the mobilization of extravascular fluid which is the main response following hemorrhage in the chicken. The work of others in cats (see Mellander & Lewis, 1963; Öberg, 1964) has shown that sympathetic activity can increase the pre- to postcapillary resistance ratio following hemorrhage and lead to extravascular fluid mobilization by lowering capillary hydrostatic pressure. This fluid absorption occurs even when the inflow and outflow pressures are constant and therefore cannot be attributed to a change in MAP. Consequently, such a mechanism, if activated during hemorrhage, would tend to augment the fall in capillary hydrostatic pressure due to the fall in MAP. This mechanism can also operate to mobilize extravascular fluid during the initial stage of bleeding in species such as the dog where MAP does not fall substantially until 15-20% of the blood volume has been removed. These authors have found this mechanism in the cat to exist predominately in skeletal muscle vascular beds and, in view of the relative amount of skeletal muscle in the overall body composition, have assumed this tissue to be the major site of extravascular fluid absorption.

Hollenberg (1965) demonstrated the importance of segmental resistance in the development of irreversible hemorrhagic shock in dogs. He found a progressive rise in small vein resistance during the late stages of oligemia which resulted in a rise in capillary hydrostatic pressure and loss of vascular fluid by ultrafiltration. This change is manifest in hemoconcentration, one of the signs of impending irreversibility. The terminal increase in small vein resistance occurred in both hindquarters and mesenteric vascular beds, although it seemed to begin earlier and

increase more rapidly in the mesenteric bed.

Most of the techniques in common use today for the study of venous tone monitor the weight or volume of a tissue segment, and thus responses of the larger capacitance veins dominate the results. The technique that most closely approximates a measurement of postcapillary resistance is that developed by Haddy et al. (1954). "Small vein" pressure is measured through a small polyethylene catheter introduced into a vein in a retrograde direction and "wedged" as far peripherally as possible, presumably at the level of veins too small to allow the catheter to enter. This provides a measurement of the end pressure in the vessels just proximal to the catheter (VWP).

The following experiments were carried out to assess changes in segmental resistance which may occur during hemorrhagic hypotension in the chicken. The effects of continuous noradrenaline infusion during hemorrhagic hypotension on these segmental resistances were also determined.

Method. A modification of the Haddy technique was used. Pressures were monitored in a large artery, a small vein (wedged catheter) and the central venous bed. Special care was taken with the wedged catheter and any experiments wherein the three criteria for "wedge pressure" (see General Methods) were not met, were rejected. This effectively divided the total regional resistance into two segments, one which is mainly arterial (MAP to VWP), and the other which is venous (VWP to CVP). The capillary hydrostatic pressure may also be calculated knowing these measurements by the formula of Pappenheimer and Soto-Rivera (1948). Using the VWP as outflow pressure (pV), calculated values for capillary

hydrostatic pressure appear to be too high, but this relationship (see formula in general methods) has been consistently shown and changes in calculated capillary hydrostatic pressure must reflect capillary events. We intended to study these segments of vascular resistance in both the ischiatic and mesenteric vascular beds, however, even using soft polyethylene catheters and being as gentle as possible, attempts at retrograde cannulation of a small vein in the mesentery always resulted in rupture of afragile small vein and no wedge pressures could be obtained. The control experiments described in this section are those for which total ischiatic resistance has already been reported (Section VIII).

All animals used in this study were mature White Leghorn hens anesthetized and surgically prepared as described under general methods. The following measurements were made initially and at 15 minute intervals for the duration of the experiments: MAP, VWP, CVP, regional flow, bleeding volume, heart rate, hematocrit and plasma protein concentration. In addition, the plasma and blood volumes were measured initially, after 120 minutes of bleeding, and following reinfusion of 20% of the MBV. The techniques for all these measurements are given under general methods. From these measurements, capillary hydrostatic pressure, arterial and venous resistance, plasma volume based on the dilution of protein, and initial plasma volume minus the volume of plasma removed were calculated as described in general methods.

The animals in these experiments were divided into two groups. One group served as a control and the other group (NA group) received a continuous infusion of noradrenaline beginning at the start of hemorrhage

(0 minutes). The dosage of noradrenaline used was 5 µg/kg/min, calculated as the free base. The solution used for infusion was freshly prepared daily by dilution of a stock solution of l-noradrenaline bitartrate with 0.9% NaCl and was administered through a catheter in an external iliac vein using a Harvard Infusion-Withdrawal Pump (Model 600-900) set at a speed to deliver 0.0194 ml/min of fluid. This amount of fluid would not substantially effect any of the measurements. Both groups of animals were bled to 50 mm Hg MAP as described under general methods and maintained at this pressure by further bleeding as necessary until the terminal decline in MAP, when 20% of the MBV was reinfused over a 10-15 minute period. After waiting 10-15 minutes, the final measurement of plasma and blood volumes was made and all other measurements and calculations were resumed at 15 minute intervals until death, or, for 90 minutes. The resumption of measurements is designated as 0' minutes and the symbol (') is used to differentiate the corresponding times before, and after partial reinfusion. Because the exact composition of the blood reinfused from the reservoir (i.e., volume of plasma and protein concentration) was not determined, calculation of initial plasma volume minus the volume of plasma removed and plasma volume estimated on the basis of protein dilution were not continued past partial reinfusion. After partial reinfusion, any blood removed as samples was replaced by an equal volume from the reservoir, i.e., bleeding volume was held constant.

Results. A comparison of the initial measurements and calculations of cardiovascular parameters in the two groups of chickens (Table XXVIII) showed that, except for CVP, the values were virtually the same in the

Table XXVIII. Initial Measurements of Cardiovascular Variables in Chickens used to Study Flow and Segmental Resistance in the Ischiatic Vascular Bed during Hemorrhage and Partial Reinfusion and Similar Treatment plus Continuous Noradrenaline Infusion (5 µg/kg/min, calculated as free base).

	Control n=15	Noradrenaline Infusion n=11	p
MAP (mm Hg)	127 ± 5	115 ± 8	n.s.
CVP (mm Hg)	2.6 ± 0.3	3.9 ± 0.2	<0.01
VWP (mm Hg)	21.3 ± 1.6	18.2 ± 1.1	n.s.
CP (mm Hg)	36.9 ± 3.9	30.6 ± 2.3	n.s.
Flow (ml/min)	10.7 ± 1.1	9.2 ± 1.3	n.s.
Arterial Resistance (PRU)	10.3 ± 0.8	12.1 ± 1.3	n.s.
Venous Resistance (PRU)	1.83 ± 0.28	1.73 ± 0.22	n.s.
Heart Rate (beats/min)	297 ± 12	314 ± 21	n.s.
Hematocrit (%)	31.2 ± 0.7	31.9 ± 0.8	n.s.
Plasma Protein Conc. (Gm%)	4.80 ± 0.19	4.61 ± 0.25	n.s.
Plasma Volume (ml/kg)	41.4 ± 2.0	38.0 ± 3.1	n.s.
Blood Volume (ml/kg)	60.2 ± 3.0	55.7 ± 4.8	n.s.

two groups. There was a small (1 mm Hg), but statistically significant difference in the CVP's in the two groups. There is no apparent reason for the higher CVP in the NA group prior to the beginning of the infusion. It could be due to a chance difference in the height of the transducer or in placement of the tip of the CVP catheter.

As was found in previous experiments (Section VII), the infusion of noradrenaline resulted in a large IBV (Table XXIX). The MBV's were the same in both groups, and the SBV of the NA group was significantly lower than that of the control group. Both groups of hens had shorter bleeding periods than those found earlier (Section III) which is attributed to the extra surgical preparation, however, the average duration of the bleeding period was significantly shorter in the NA than in the control group. Thus, although total compensatory ability, judged by MBV's, was not altered by noradrenaline infusion, the average time of arrival of MBV was significantly hastened by the drug infusion. On the other hand, noradrenaline infusion had no significant effect on the average duration from partial reinfusion until death (animals that survived more than 90 minutes were considered to be 90 minute survivors). Thirteen of 15 in the control group survived partial reinfusion, and of these, 7 lived 90 minutes or longer. In the NA group, 8 of 11 survived partial reinfusion, and four of these lived 90 minutes or longer. Thus, both the average durations of survival and the number of animals surviving the complete experiment showed no detrimental effect of the noradrenaline infusion.

Table XXX and Fig. 26 show the changes in hematocrits and plasma protein concentrations in the two groups. These changes were generally

Table XXIX. Weights, Bleeding and Reinfusion Volumes, and Durations of Hypotension in Chickens used to Study Flow and Segmental Resistance in the Ischiatic Vascular Bed during Hemorrhage and Partial Reinfusion and Similar Treatment plus Continuous Noradrenaline Infusion (5 μg/kg/min, calculated as free base).

	Control n=15	Noradrenaline Infusion n=11	p
Weight (kg)	1.77 ± 0.07	1.67 ± 0.09	n.s.
IBV (ml/kg)	14.0 ± 1.1	23.4 ± 2.4	<0.001
MBV (ml/kg)	33.3 ± 1.6	33.9 ± 3.6	n.s.
SBV (ml/kg)	19.4 ± 1.6	10.6 ± 1.9	<0.01
Minutes from 0 to Reinfusion	176 ± 16	125 ± 14	<0.05
Reinfusion Volume (ml/kg)	6.6 ± 0.3	6.8 ± 0.7	n.s.
Minutes from 0' to Death	73 ± 7	62 ± 11	n.s.

Table XXX. Effect of Hemorrhage and Partial Reinfusion on Hematocrit and Plasma Protein Concentration in Normal Chickens and Chickens Continuously Infused with Noradrenaline (5 µg/kg/min, calculated as free base).

	Control n=15	Noradrenaline Infusion n=11	p
	Hematocrit (%)		
15 Minutes	28.1 ± 0.6	28.6 ± 1.6	n.s.
90 Minutes	19.8 ± 0.9	17.8 ± 1.2	n.s.
Pre-reinfusion	16.8 ± 1.1	16.6 ± 1.1	n.s.
Post-reinfusion	19.8 ± 1.1	19.4 ± 1.5	n.s.
Terminal	20.3 ± 1.1	19.3 ± 1.3	n.s.
	Plasma Protein Concentration (Gm%)		
15 Minutes	4.33 ± 0.21	3.99 ± 0.31	n.s.
90 Minutes	3.04 ± 0.18	2.49 ± 0.24	n.s.
Pre-reinfusion	2.82 ± 0.15	2.52 ± 0.25	n.s.
Post-reinfusion	3.20 ± 0.22	2.82 ± 0.33	n.s.
Terminal	3.08 ± 0.19	2.78 ± 0.32	n.s.

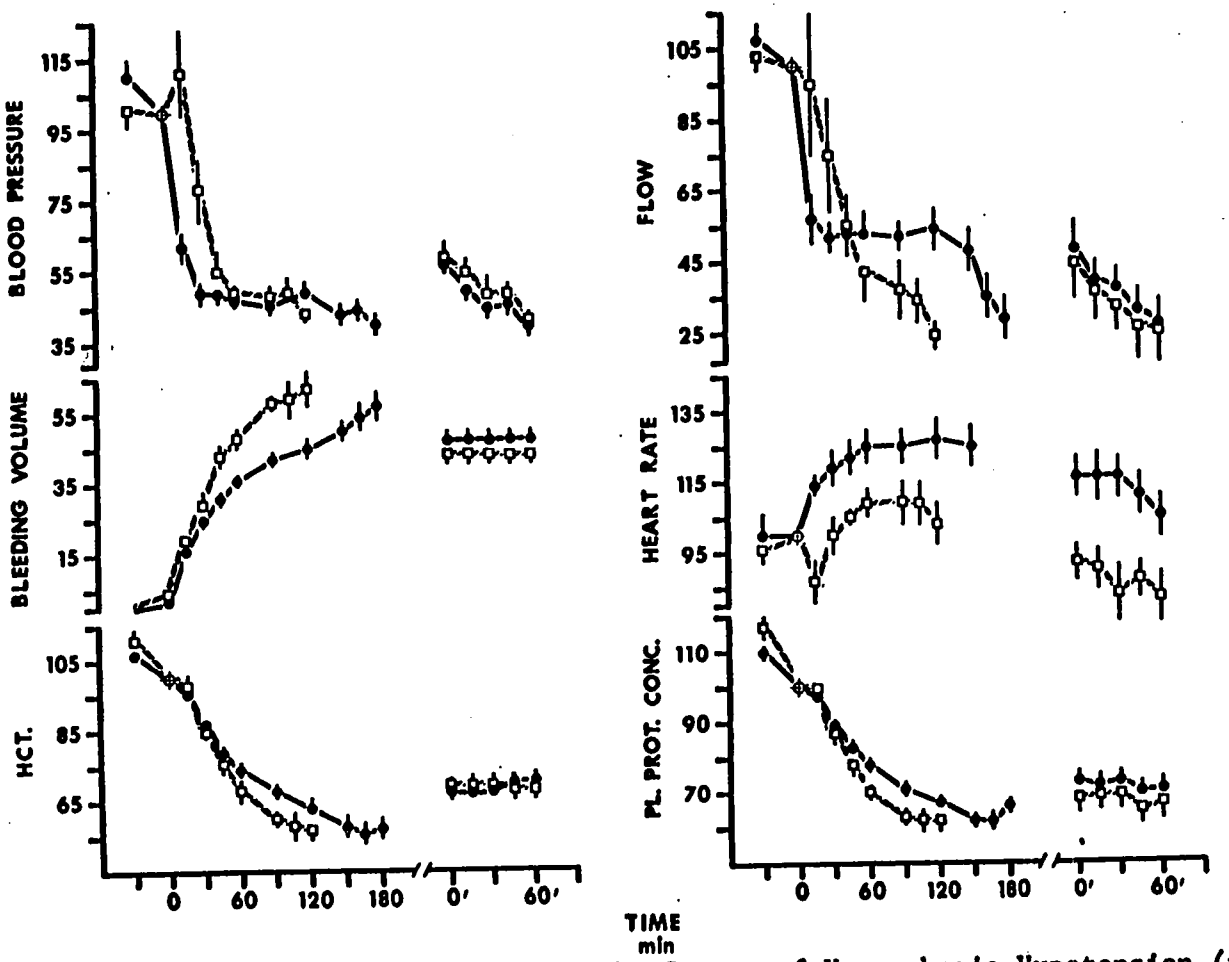

Figure 26. The Effects of Noradrenaline Infusion on the Course of Hemorrhagic Hypotension (including Ischiatic Regional Flow) in Chickens used to Study Ischiatic Segmental Resistances following Hemorrhage and Partial Reinfusion. Zero minutes is the start of hemorrhage. All numbers on the ordinates are percentages. Except for Bleeding Volume, zero minute measurements are 100%. Bleeding Volume is expressed as a percentage of initial measured blood volume. The break in the abscissa indicates partial reinfusion and the symbol (') differentiates the corresponding times before, and after partial reinfusion. The time scale is slightly expanded following partial reinfusion to accommodate plotting more points. The closed circles and solid lines represent the control, and open squares and broken lines the noradrenaline infusion (5 µg/kg/min, calculated as free base) groups. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s overlap, they are shown in only one direction.

similar to those found in preceding experiments except for two differences. First, in the initial few minutes (not apparent in Table XXX) of noradrenaline infusion the hematocrit and plasma protein concentration fell more slowly in this group (in cases where MAP increased, hematocrit and plasma protein concentration also increased) and second, both hematocrit and plasma protein concentration were lower at 90 minutes in the NA group than in the control group (not significant compared in absolute units). The average "relative" decrease in hematocrit at 90 minutes (see Fig. 26) in the NA group (40%) was significantly greater ($p<0.05$) than that in the control group (32%). Likewise, the fall in plasma protein concentration at 90 minutes in the NA group (37%) was significantly greater ($p<0.01$) than that of the control group (29%). This is the period when the arterial resistance and the CP were different in the two groups (see below). The "relative" bleeding volume also became significantly greater in the NA than in the control group during the 60 to 90 minute period (Fig. 26). These observations indicate some effect of noradrenaline infusion on fluid mobilization at this time. As an interesting sidelight to Fig. 26, the NA group showed a marked bradycardia at the start of the infusion. This was probably reflex in origin and the result of increased MAP, and indicates that the chicken is not completely devoid of baroreceptors (see General Discussion).

The extent of hemodilution in the two groups is shown in Fig. 27. The total fluid mobilized was very similar in both groups, 20.6 ml/kg in the control and 21.3 ml/kg in the NA group. However, the average rate of fluid mobilization during the initial two hour portion of the bleeding period was greater in the NA group (10.7 ml/kg/hr) than it was in the

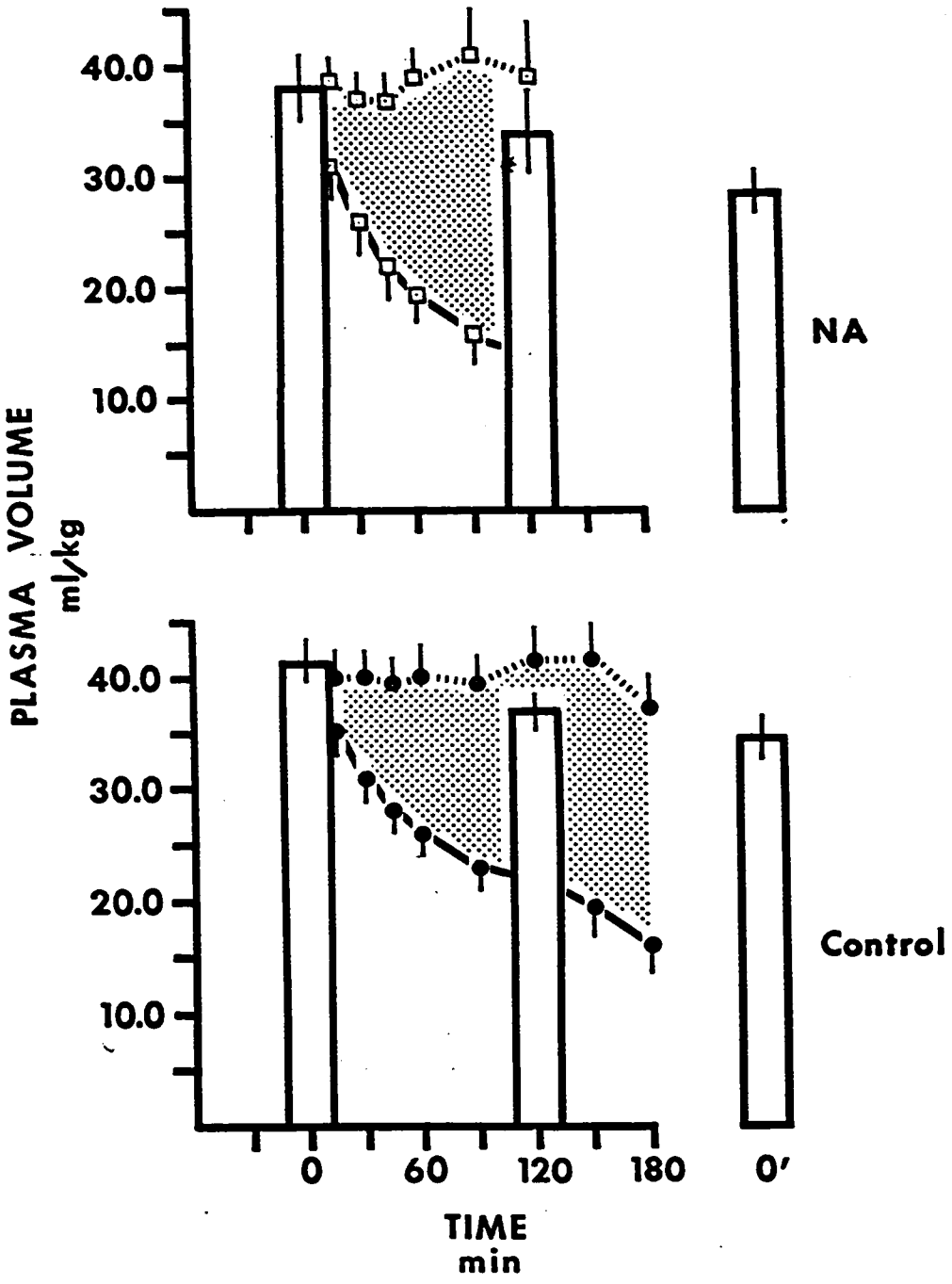

Figure 27. Effect of Noradrenaline Infusion on Hemodilution in Chickens used to Study Ischiatic Segmental Resistances following Hemorrhage and Partial Reinfusion. Bars indicate the measured plasma volume. The lower solid line in each diagram is the initial plasma volume minus the volume of plasma removed. The upper, broken line in each diagram is the plasma volume estimated on the basis of protein dilution. Vertical lines indicate the S.E. of each point. Zero prime indicates the plasma volume measurement made following partial reinfusion.

control group (7.7 ml/kg/hr).

The MAP, capillary hydrostatic pressure (CP) and VWP at selected intervals in the two groups are shown in Table XXXI. The only statistically significant difference was the higher MAP in the NA group after 15 minutes of hemorrhage. These pressures, and the CVP, are also shown in Fig. 28. The shift of the MAP curve to the right by the NA infusion is apparent from this figure. Also in Fig. 28, it can be seen that CP and VWP changes follow those in MAP. CVP remains relatively unchanged in both groups although the difference between groups which existed from the beginning is apparent in this figure. During the period from 60 to 90 minutes, MAP was constant in both groups, but the CP, and to a lesser extent the VWP, were falling in the NA group. This suggests that some precapillary change might be causing these pressures to fall. Interestingly, these changes occurred during a period when there was an increased rate of fluid mobilization (see above). During the terminal stages of bleeding and in the post-reinfusion period, the VWP fell in parallel with all the other pressures in both groups. This is contrary to the terminal <u>increase</u> in VWP found by Hollenberg (1965) in dogs.

Blood flow (Table XXXII, Fig. 26) initially tended to decrease more slowly in the NA than in the control group, but this difference was not statistically significant at the 15 minute measurement. After 90 minutes, however, flow had fallen significantly <u>more</u> in the NA than in the control group. During the terminal stages of the bleeding and post-reinfusion periods, regional flows behaved similarly in both groups. They fell in the later stages of the bleeding period, rose slightly after reinfusion and declined terminally. The changes in flow and MAP (Fig. 26) are

Table XXXI. Effect of Hemorrhage and Partial Reinfusion on MAP, CP, and VWP in Normal Chickens and Chickens Continuously Infused with Noradrenaline (5 µg/kg/min, calculated as free base).

	MAP (mm Hg)			CP (mm Hg)			VWP (mm Hg)		
	Control n=15	Noradrenaline Infusion n=11	p	Control n=15	Noradrenaline Infusion n=11	p	Control n=15	Noradrenaline Infusion n=11	p
15 Minutes	72 ± 5	125 ± 13	<0.001	27.1 ± 3.6	32.4 ± 2.4	n.s.	17.0 ± 1.5	19.0 ± 1.2	n.s.
90 Minutes	51 ± 2	56 ± 3	n.s.	21.9 ± 1.8	18.4 ± 2.6	n.s.	14.0 ± 1.2	11.1 ± 1.5	n.s.
Pre-reinfusion	45 ± 1	48 ± 1	n.s.	16.1 ± 2.4	14.2 ± 2.2	n.s.	10.8 ± 1.3	10.5 ± 1.6	n.s.
Post-reinfusion	65 ± 5	65 ± 6	n.s.	22.9 ± 2.5	22.0 ± 2.9	n.s.	16.4 ± 1.5	14.6 ± 1.6	n.s.
Terminal	46 ± 5	45 ± 4	n.s.	12.4 ± 2.7	18.7 ± 3.2	n.s.	10.6 ± 1.6	12.5 ± 1.7	n.s.

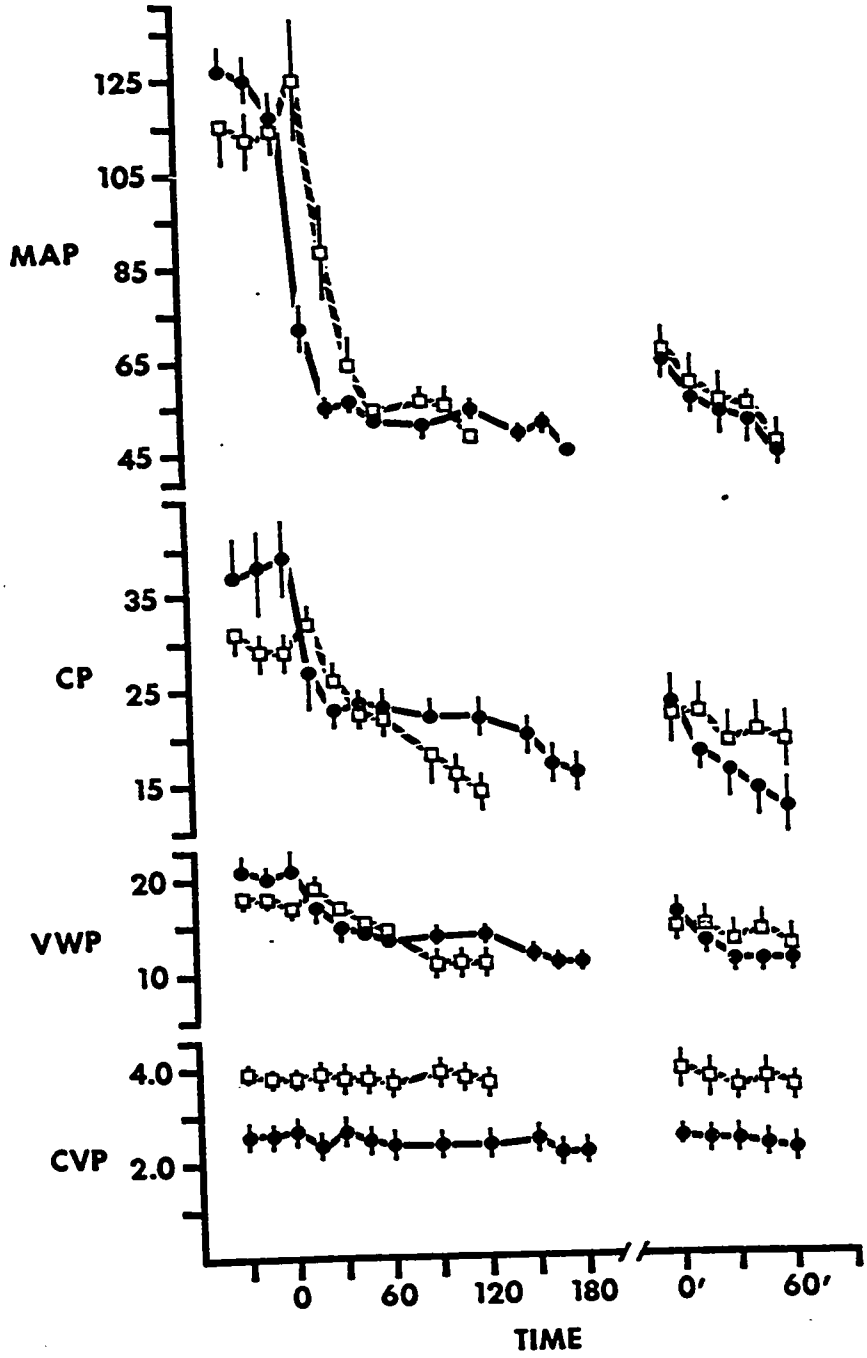

Figure 28. Effect of Noradrenaline Infusion on Various Pressures in the Ischiatic Vascular Bed of Chickens following Hemorrhage and Partial Reinfusion. All numbers on the ordinates are mm Hg. The break in the abscissa indicates partial reinfusion and the symbol (') differentiates the corresponding times before, and after partial reinfusion. The time scale is slightly expanded following partial reinfusion to accommodate plotting more points. The closed circles and solid lines represent the control, and open squares and broken lines the noradrenaline infusion (5 µg/kg/min, calculated as free base) groups. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s overlap, they are shown in only one direction.

Table XXXII. Effect of Hemorrhage and Partial Reinfusion on Flow and Total Regional Resistance in the Ischiatic Vascular Bed in Normal Chickens and Chickens Continuously Infused with Noradrenaline (5 μg/kg/min, calculated as free base).

	Control n=15	Noradrenaline Infusion n=11	p
	Flow (ml/min)		
15 Minutes	5.9 ± 0.8	7.6 ± 1.1	n.s.
90 Minutes	5.0 ± 0.4	3.0 ± 0.3	<0.01
Pre-reinfusion	2.6 ± 0.6	2.0 ± 0.3	n.s.
Post-reinfusion	4.6 ± 0.7	3.6 ± 0.5	n.s.
Terminal	2.7 ± 0.8	2.1 ± 0.7	n.s.
	Total Regional Resistance (PRU)		
15 Minutes	12.0 ± 1.6	18.8 ± 2.1	<0.05
90 Minutes	10.3 ± 1.1	18.8 ± 2.1	<0.01
Pre-reinfusion	25.6 ± 8.7	23.5 ± 2.8	n.s.
Post-reinfusion	14.3 ± 1.9	18.6 ± 1.5	n.s.
Terminal	29.9 ± 11.0	27.4 ± 6.0	n.s.

reflected in the total regional resistance values, which are also shown in Table XXXII. The resistance was significantly higher in the NA group at both 15 and 90 minutes and also higher following partial reinfusion, but this was not significant. The pre-reinfusion and terminal values were similar in both groups.

The observation of increased average vascular resistance in the NA group at 15 and 90 minutes of the bleeding period was supported by a qualitative consideration of the data. For this purpose, a 10% change in resistance was arbitrarily considered to be indicative of a real change. In the control group, after 15 minutes, equal numbers of animals had increased and decreased resistance and a few were unchanged, whereas in the NA group, all but one animal had an increased resistance at this time. The tabulation of numbers with increased, decreased, or unchanged resistance at 90 minutes was much the same as at 15 minutes. On a percentage basis, the average change in resistance in the NA group was a 31% increase at 15 minutes and a 38% increase at 90 minutes. In the control group, the corresponding figures were a 1% and 9% decrease. Following partial reinfusion, the average resistance fell somewhat from the pre-reinfusion measurement but remained increased over control in both groups (26% in the control and 39% in the NA groups).

In Table XXXIII (also Fig. 29), the total regional resistance in the two groups is subdivided into its arterial and venous segments. As can be seen, the venous resistance changes roughly follow those on the arterial side. Notably, however, the arterial (and total) resistance during the bleeding period was higher in the NA group than it was in the control

Table XXXIII. Effect of Hemorrhage and Partial Reinfusion on Arterial and Venous Segments of Resistance in the Ischiatic Vascular Bed in Normal Chickens and Chickens Continuously Infused with Noradrenaline (5 µg/kg/min, calculated as free base).

	Control n=15	Noradrenaline Infusion n=11	p
	Arterial Resistance (PRU)		
15 Minutes	9.6 ± 1.5	15.2 ± 1.8	<0.05
90 Minutes	8.1 ± 1.1	16.0 ± 2.0	<0.01
Pre-reinfusion	17.8 ± 4.5	23.7 ± 4.5	n.s.
Post-reinfusion	11.5 ± 1.7	15.8 ± 1.5	n.s.
Terminal	26.0 ± 8.9	23.2 ± 5.2	n.s.
	Venous Resistance (PRU)		
15 Minutes	2.45 ± 0.36	2.40 ± 0.45	n.s.
90 Minutes	2.24 ± 0.25	2.80 ± 0.53	n.s.
Pre-reinfusion	4.39 ± 1.69	3.20 ± 0.73	n.s.
Post-reinfusion	2.77 ± 0.36	2.94 ± 0.47	n.s.
Terminal	3.02 ± 1.29	5.20 ± 1.35	n.s.

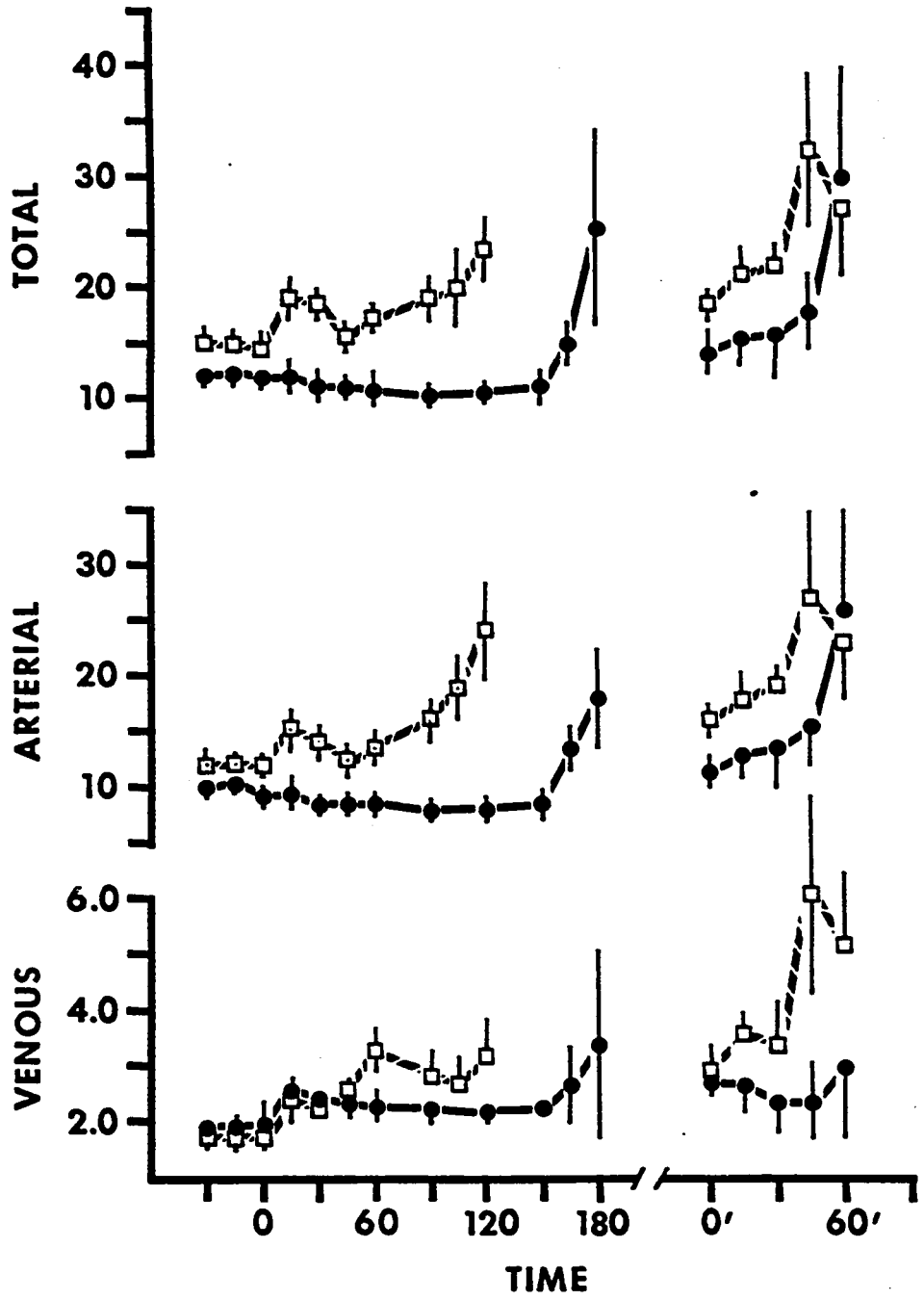

Figure 29. Effect of Noradrenaline Infusion on Ischiatic Segmental Resistances in Chickens following Hemorrhage and Partial Reinfusion. All resistances on the ordinates are in arbitrary PRU. The break in the abscissa indicates partial reinfusion and the symbol (') differentiates the corresponding times before, and after partial reinfusion. The time scale is slightly expanded following partial reinfusion to accommodate plotting more points. The closed circles and solid lines represent the control, and open squares and broken lines the noradrenaline - infusion (5 µg/kg/min, calculated as free base) groups. Vertical lines indicate the S.E. of each point. Where no S.E.'s are shown, they are included in the limits of the symbols. If S.E.'s overlap, they are shown in only one direction.

group although venous resistances were essentially the same. This indicates that differences between the two groups in total regional resistance were almost entirely due to differences on the arterial side. The initial resistance increase in the NA group declined somewhat in the middle portion of the bleeding period although it remained above that of the control group. This response (tachyphylaxis) to NA infusion is similar to that observed by TPR measurements in previous experiments (Section VII). The pre-reinfusion measurements were similar in both groups.

After partial reinfusion, the differences between the two groups were not significant. The resistance changes between 60 and 90 minutes are of special interest in view of the observation of an increased rate of fluid mobilization at this time. MAP was constant in both groups during this period, while the CP fell slightly (not significant) in the NA group (Table XXXI, Fig. 28). During this same period, the venous resistance was essentially identical in the two groups, but the arterial resistance was significantly higher in the NA group. An increased arterial and unchanged venous resistance would explain the lower CP during this period in the NA group and this relationship would also promote greater fluid absorption.

As was noted in previous experiments, there was no hemoconcentration in the post-reinfusion period and this was consistent with the finding that VWP fell, and venous and arterial resistances increased at about the same rate (Figs. 28 and 29). Thus, the mechanisms which Hollenberg (1965) proposed to explain decompensation and hemoconcentration in dogs does not seem to exist in chickens.

Comment. Except for the very terminal stages, there was little change in segmental or total resistance in the ischiatic vascular bed when chickens were subjected to hemorrhage alone. The suggestion is made that active vasoconstriction makes no substantial contribution to the massive fluid mobilization seen in this species. In the absence of active vasoconstriction, a given fall in MAP will be transferred almost directly and quantitatively to the capillary portion of vascular beds where fluid movements occur. It was a regular and consistent finding in chickens that the MAP falls very markedly in response to even a small hemorrhage. Thus, in these experiments, a fall in MAP from 117 to 55 mm Hg over a 30 minute period resulted in a 40% decrease in calculated CP. A decrease of CP of this magnitude (perhaps 8-10 mm Hg) generalized over the skeletal muscle mass of the body is more than adequate to explain the volume of fluid mobilization seen following hemorrhage in the chicken. The CFC (see Review of Literature for definition of CFC, capillary filtration coefficient) of skeletal muscle of ducks is reported to be 0.5 ml/min x kg x mm Hg (Djojosugito et al., 1968). Assuming a similar value for chickens and a skeletal muscle mass approximately 50% of body weight, a 10 mm Hg decrease in CP would give a maximum rate of fluid mobilization (skeletal muscle only) of 2.5 ml/kg/min. Even if this rate diminished rapidly with hemodilution, it is still several-fold higher than the average fluid mobilization rates observed in these experiments (approximately 0.1 ml/kg/min).

The effects of the noradrenaline infusion show that the vessels of the chicken can respond to large amounts of exogenous catecholamines. However, as was found with the previous study of the effect of noradrenaline

infusion and hemorrhage on TPR (Section VII), the response was very small compared to that of mammals. Noradrenaline infusion had little or no effect on the venous segment of resistance, and only increased the arterial resistance slightly.

During the early stages of these experiments, when noradrenaline elevated the MAP, there was a slight retardation of fluid mobilization. A pressor response would tend to elevate CP and offset the small increase in the arterial resistance tending to lower CP. However, in the period during which MAP was held constant, fluid tended to be mobilized at a slightly accelerated rate. Increased mobilization rate was evidenced by an accelerated rate of bleeding necessary to maintain the MAP at the preset hypotensive level and by more rapid hemodilution. The interpretation that this is the result of an increased pre- to postcapillary resistance ratio is supported by the measurements of segmental resistances. It can be concluded that the mechanism of fluid mobilization proposed by Mellander & Lewis (1963) and Öberg (1964) exists in the chicken, but is poorly developed and is not substantially activated during hemorrhage alone.

The noradrenaline infusion did not seem to have any marked effect on the total amount of compensation, as judged by the maximum amount of blood removed. However, because of the increased rate of compensation, the limit of blood removal was reached sooner when noradrenaline was infused. The absence of a direct contribution of noradrenaline to peripheral vascular failure in chickens is contrary to the response in mammals. Because of the report of Hollenberg (1965) of the terminal increase in VWP in dogs, it is interesting to examine this measurement in the chicken, a species where terminal hemoconcentration is not seen during prolonged

hemorrhagic hypotension. The decompensatory increase in VWP which he reported to occur in dogs was never seen in chickens, either during pure hemorrhagic hypotension or hemorrhagic hypotension coupled with noradrenaline infusion. Thus, the chicken seems to possess only a rudimentary ability to develop arterial resistance and almost no ability to develop venous resistance. The lack of a vasoconstrictive compensatory response to hemorrhage, far from being a disadvantage, appears to provide greater tolerance to hemorrhage in this species.

The changes in segmental resistances and the effect of noradrenaline infusion on them were measured only in the ischiatic bed. However, the preceding observations on the effect of hemorrhage alone on total resistance in the mesenteric bed showed that it responds in a similar manner, both qualitatively and quantitatively. There is no reason to believe that the segmental vascular arrangement is different in the mesenteric bed, and the total resistance in this bed probably also reflects changes in the arterial segment. In a few experiments in which NA was infused and the total vascular resistance in the mesenteric bed was examined, a similar, small response to noradrenaline was seen.

X. Plasma Catecholamines during Hemorrhagic Hypotension in Chickens. Reports in the literature reveal two interesting observations concerning catecholamines in chicken plasma. First, total plasma catecholamine concentration is much higher (approximately 7.5 µg/l, vs. 1 µg/l in mammals) than that of mammals and second, the amount of adrenaline in chickens plasma is 3 to 4 times the amount of noradrenaline (Lin & Sturkie, 1968), whereas in mammals, the two are approximately equal

or there is slightly more noradrenaline (see Chien, 1967 for references on mammals). Histochemical evidence from blood vessels of chickens indicates that a substantial proportion of sympathetic nerves are truly adrenergic rather than noradrenergic (see Akester & Mann, 1969), which may partly explain the high proportion of circulating adrenaline.

The chicken's inability to develop vascular resistance during hemorrhage indicates that adjustment of the circulation by the sympathetic nervous system is poorly developed in this species. It has been reported in the literature that chickens have a lack of baroreceptors in the head and neck region (McGinnis & Ringer, 1966, 1967) and preceding experiments in this study showed that they have a marked inability to maintain their MAP after even a small hemorrhage. Also, the vascular response to exogenously administered catecholamines and adrenergic blocking agents is poor in chickens (see Harvey & Nickerson, 1951). Therefore, the possibility exists that lack of sympathetic circulatory adjustment in hemorrhage could result from : a) a relative lack of sensory input (i.e., baroreceptors), b) insensitivity to released sympathetic neurohumoral mediator, or, c) both of the above. It was felt some useful information with regard to these possibilities would be obtained by estimating levels of catecholamines in the plasma of chickens during hemorrhagic hypotension. A large increase of catecholamines in plasma would be a general indication that the "sympatho-adrenal" system was activated during hemorrhage. In dogs, lowering MAP to 40 mm Hg causes approximately a 50-fold increase in adrenaline and a 10-fold increase in noradrenaline (Millar & Benfey, 1958; Greever & Watts, 1959; Walton et al., 1959; Rosenberg et al., 1961). However, the adrenal medulla in mammals is a major source of catecholamines

following hemorrhage (see Chien, 1967 for references) and since the cardiovascular system is primarily under the influence of catecholamines released locally rather than those liberated from the adrenal medulla (Celander, 1954), the circulating levels do not necessarily give quantitative information of the sympathetic influence on the cardiovascular system in these species.

Method. Anesthetization, surgical preparation, technique of hemorrhage, and method of measurement of catecholamines are given under general methods. Four groups of mature White Leghorn hens, two control and two experimental, were used. One control group was made up of chickens subjected to the usual surgical preparation used in preceding experiments. Chickens in the second control group were anesthetized for two hours with paraldehyde and without bleeding. Chickens in the two experimental groups were subjected to one and two hours of hemorrhagic hypotension. Because of the volume of blood necessary to assay catecholamines, only one sample was taken from each chicken and the collection of the sample terminated the experiment.

Results. The levels (Table XXXIV) of adrenaline and noradrenaline in the plasma of the two control groups were higher than those previously reported for chickens (Lin & Sturkie, 1968). Higher levels of plasma catecholamines in the present experiments could be explained by a difference in experimental conditions. In these experiments samples were collected over a relatively longer period (105 seconds) from anesthetized birds subjected to a certain amount of surgical trauma, whereas, in the

Table XXXIV. Effects of Hemorrhagic Hypotension on Plasma Levels of Adrenaline and Noradrenaline in the Chicken.

	n	Adrenaline (µg/l)	Noradrenaline (µg/l)
Control, 0 Minutes	6	14.4 ± 2.9	5.3 ± 1.4
Control, 2hr. Anesthesia	4	14.7 ± 3.1	3.5 ± 1.5
One Hour at 50 mm Hg	7	23.3 ± 3.5	11.7 ± 1.6*
Two Hours at 50 mm Hg	9	93.5 ± 14.9**	22.4 ± 3.2**

* Significantly different from both control groups $p<0.05$

** Significantly different from both control groups $p<0.01$

experiments of Lin & Sturkie, birds were unanesthetized and samples were of pooled blood obtained by heart puncture. The possibility of a sex difference cannot be dismissed as their birds were males whereas, in the present study, all birds were hens. Total catecholamines in chicken plasma were high in comparison to values reported for dogs. In fact, the control levels in chicken plasma approached the maximum plasma levels reported in some studies of dogs (Millar & Benfey, 1958). Another interesting observation concerning the control measurements of catecholamines was the relative amounts of adrenaline and noradrenaline and existence of substantially more adrenaline than noradrenaline in chicken plasma was confirmed by the present experiments.

Assuming that control levels of catecholamines in the chicken, although much higher than those of dogs in absolute units, are an indication of the resting "tone" of the "sympatho-adrenal" system (Table XXXIV, Fig. 30) the chicken's "relative" ability to increase plasma catecholamine levels during hemorrhage was less than that of the dog. During the first hour of hemorrhage, the relative increases in adrenaline and noradrenaline were of the same magnitude (approximately 2-fold), however, after two hours, the amount of adrenaline had increased (7-fold) more than noradrenaline (4-fold). Compared to the 50-fold adrenaline and 10-fold noradrenaline increases in dog plasma after a similar level of hemorrhagic hypotension, the increases in circulating catecholamines in chicken plasma were relatively smaller.

Comment. These experiments show the chicken's plasma catecholamine levels during hemorrhagic hypotension. Compared to the relative increase

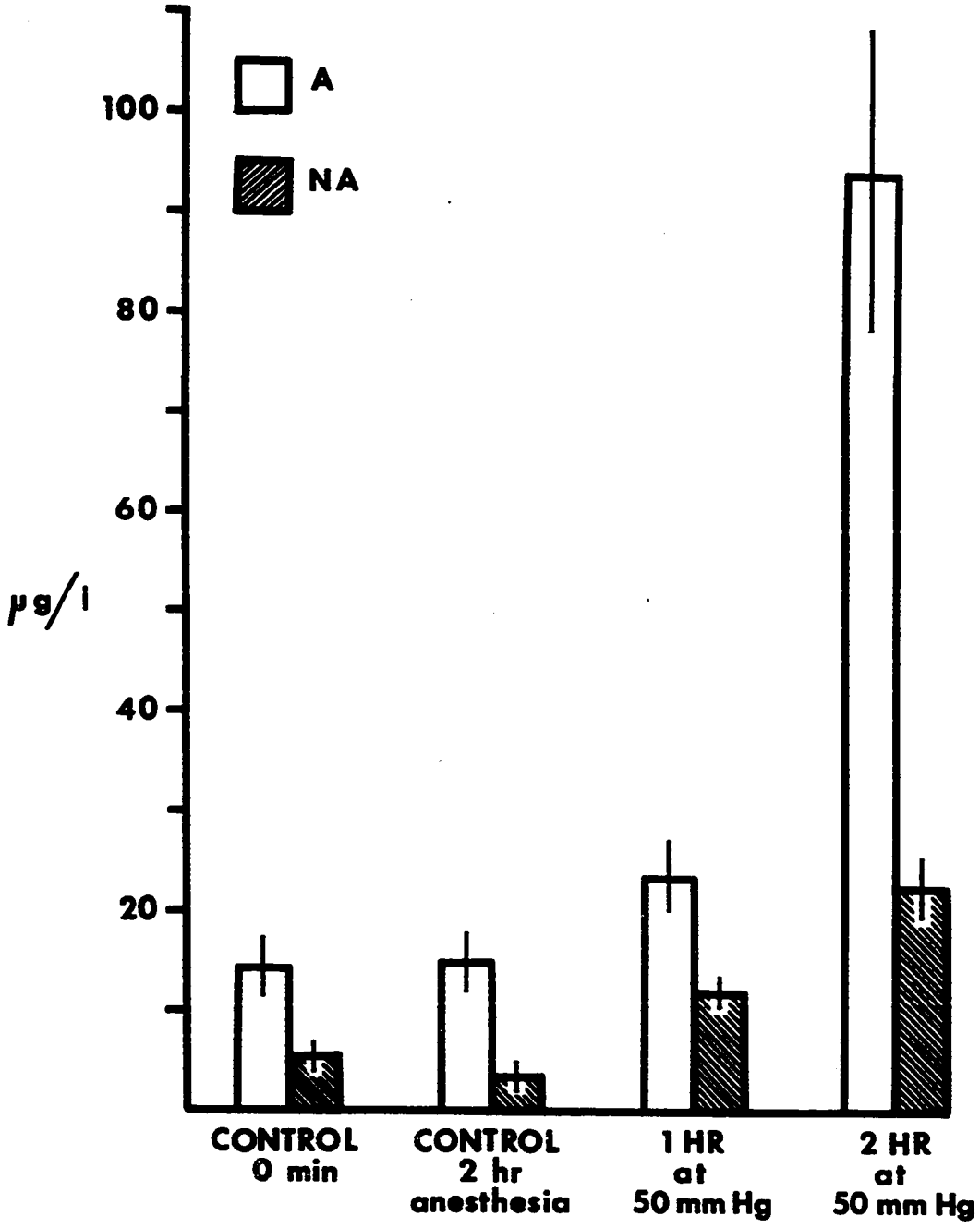

Figure 30. Effect of Hemorrhagic Hypotension on Circulating Levels of Plasma Adrenaline and Noradrenaline in Chickens. The vertical lines indicate the S.E.'s of each point.

of these substances in dog plasma following a similar amount and duration of hemorrhage, the increases in chicken plasma were not particularly outstanding. The major feature of the response to hemorrhage in chickens is a massive hemodilution and therefore concentrations may not accurately reflect the total increase in plasma catecholamines. However, plasma dilution does not entirely reconcile the differences between dogs and chickens because dilution of protein and erythrocytes (and presumably catecholamines) was never substantially greater than half.

The relative abundance of adrenaline in chicken plasma under control circumstances is unexplained and awaits further study. In the dog, plasma noradrenaline usually exceeds adrenaline (Rosenberg et al., 1961) or the two are approximately equal (Millar & Benfey, 1958). The physiological significance of the large amounts of adrenaline in chicken plasma is not clear. This adrenaline could simply indicate a relatively active adrenal medulla. However, there have been reports which suggest a substantial proportion of sympathetic nerves in the chicken contained adrenaline rather than noradrenaline (Akester & Mann, 1969). The finding of greater outpouring of adrenaline than noradrenaline during hemorrhagic hypotension in chickens was a similar phenomenon to that seen in dogs (Millar & Benfey, 1958). However, whether the source of this adrenaline during hemorrhage in chickens is mostly the adrenal medulla or true adrenergic sympathetic nerves is not certain.

In summary, it appears that the ability to respond to hemorrhagic hypotension by outpouring of catecholamines is present in the chicken although relatively somewhat less than that in the dog. This indicates the lack of a vascular response to hemorrhage in chickens may be largely

due to an insensitivity to locally released or circulating catecholamines rather than decreased ability to sense a fall in MAP. The existence of high catecholamine levels in plasma under control conditions is compatible with this interpretation and suggestive of a permanent tachyphylaxis to catecholamines in chickens.

8. GENERAL DISCUSSION

In the present experiments, it was found that chickens (and turkeys) present a rather characteristic pattern of response following hemorrhage which is markedly different from that of mammals (and diving birds, see below). In general, there are three points of difference between the reaction of these birds and that of mammals during experimental hemorrhagic hypotension. First, the MAP of both species of fowl falls very rapidly after even a small hemorrhage; second, hemodilution is both more rapid and larger in birds; and finally, chickens do not appear to develop the "irreversibility" and hemoconcentration which is characteristic of hemorrhagic shock in mammals. Results of the hemodynamic studies on chickens reported here led to the proposal that all these phenomena are related to a lack of active reflex vasoconstriction in this species.

The observed phenomena following hemorrhage in the chicken cannot be attributed to an effect of the anesthetic agent (paraldehyde) because similar results were obtained using a more common agent (barbiturate). In fact, the only difference between experiments with paraldehyde and those with barbiturate was that the latter caused respiratory depression and experiments were generally shorter when it was used (Section IV). Younger chickens anesthetized with paraldehyde also seemed to die because of respiratory difficulties which could be prevented by artificial ventilation, but artificial ventilation had no significant effect in experiments on mature hens anesthetized with this agent (Section III).

Mobilization of fluid into the vascular space in chickens appears to result solely from a fall in MAP transferred directly to capillaries

with little or no assistance from precapillary vasoconstriction (see below). The lack of active vasoconstriction is inferred from the characteristic rapid fall in MAP after even a small volume of hemorrhage. In several series of experiments in this study, removal of blood samples to measure plasma volume (less than 3% of *in vivo* blood volume) resulted in a significant decrease in MAP. In dogs, it is reported that *more* than 10% of the *in vivo* blood volume must be removed before there is *any* fall in MAP (Chien, 1958). The rate of bleeding used in the present experiments (0.5 ml/kg/min) rapidly lowered the MAP of chickens to 50 mm Hg, usually in less than 20 minutes. This is a slow rate of bleeding compared to mammals. Hollenberg (1965) reported hemorrhage at a rate of 2 ml/kg/min was necessary to lower MAP of dogs to 50 mm Hg within 15 minutes. Thus, the initial response to hemorrhage is clearly different in these two species.

Reflex vasoconstriction mediated through the sympathetic nervous system undoubtedly plays a role in the ability of dogs (and other mammals) to maintain MAP following hemorrhage. Probably one of the most important parts of this homeostatic mechanism is the sensory "baroreceptors" located in the aortic arch and carotid sinus. The area in the chicken homologous to the carotid sinus in mammals is at the entrance to the thoracic cavity along the common carotid artery. It is distal to the subclavian and proximal to the vertebral artery. It has been reported that occlusion of both carotid arteries of Leghorn hens results in no change in MAP and leads to the conclusion that there are no functional baroreceptors in the head and neck of these hens. A pressor response to occlusion of the *entire* (carotid and

vertebral arteries) blood supply to the head and neck region is attributed to cerebral ischemia (McGinnis & Ringer, 1966, 1967). Thus, it appears that the inability of chickens to maintain MAP following hemorrhage may be at least partly related to a deficiency in the sensing elements which respond to changes in blood pressure in mammals.

The inability to maintain MAP following hemorrhage implies a failure of the chicken to develop active vasoconstriction, but the correctness of this inference has been confirmed in several ways in the present study. Measurement of $\overline{TPR}$ (Section VII) and regional resistances in the intestine and leg (Section VIII) revealed no significant evidence of active vasoconstriction during hemorrhagic hypotension in the chicken. Individual experiments gave somewhat variable results but, in general, any changes were small and the average cardiac output and regional flow fell in proportion to the fall in MAP. This is in definite contrast to mammals, which show marked and sustained vasoconstriction following hemorrhage in both regional vascular beds mentioned above (see Review of the Literature for references) and, at least in the bleeding phase, increased TPR (see Section VII for references). Just prior to reinfusion or terminally, an increase in resistance was seen in both the mesenteric and ischiatic vascular beds but, because MAP was very low and falling at this time, this was considered to be a passive increase due to collapse of small vessels resulting from a decreased transmural pressure, i.e., "critical closure" (see Section VII). Subdivision of the ischiatic regional vascular resistance into an arterial and venous segment (Section IX) revealed that the total regional resistance was almost entirely arterial and that

there was never any indication of an independent change in either segment.

During hemorrhagic hypotension accompanied by continuous noradrenaline infusion, there was an increase in both total peripheral (Section VII) and ischiatic regional (Section IX) resistance. Increased TPR was not sustained, however, and disappeared after a short time in spite of continued noradrenaline infusion. On the other hand, elevation of ischiatic regional resistance during hemorrhage and noradrenaline infusion was maintained throughout the hypotensive period although there was some indication of tachyphylaxis. The average increases in both TPR and ischiatic regional resistance were small (20-35%) and might partially be accounted for on the basis of a passive resistance increase as transmural pressure was lowered by hemorrhage (i.e., elastic recoil of vessels). The increased ischiatic vascular resistance during hemorrhage and noradrenaline infusion was entirely in the arterial segment (MAP to VWP segment) and there was no significant effect on resistance in the venous segment (VWP to CVP segment). Normally, an increased precapillary resistance would favor hemodilution but, when the MAP was also elevated, as it often was with the start of noradrenaline infusion, the small increase in arterial resistance was not able to offset the effect of increased MAP on the capillary hydrostatic pressure, and ultrafiltration of vascular fluid occurred (see below). After reaching the preset hypotensive level (MAP held constant), however, the elevated ischiatic arterial resistance slightly increased the rate of fluid mobilization (see Section IX). The magnitude of the response to noradrenaline infusion was rather meager in view of the dosage given.

This dose (5 µg/kg/min, calculated as free base) given to dogs, is more than sufficient to produce shock in the absence of bleeding (Yard & Nickerson, 1956). The small response to noradrenaline infusion could be due to insufficiency of α-adrenergic receptors in the vasculature of chickens, or to a poorly developed contractile mechanism, i.e., shortage of smooth muscle in the vessels, or both. Others have also reported limited responses to noradrenaline administration in chickens (Harvey & Nickerson, 1951).

Once the MAP of chickens is lowered to the preset hypotensive level, however, it begins to rise again quite rapidly and bleeding must be continued in order to keep the pressure down. Experiments carried out to assess the maximum volume which could be removed (Section III), revealed that, before death occurred (about 5 hours of hemorrhage), a volume equivalent to 70% of the measured in vivo blood volume had been taken out. This volume of fluid is greater than can be removed from most mammals during acute hemorrhage and the MBV (percent) of the chickens in these experiments is greater than that reported by Hollenberg (1965) for dogs (about 55%). A further important difference between hemorrhage in dogs and that in chickens is that more blood is removed from the latter after reaching the preset hypotensive level (in these experiments, twice as much as was necessary to lower MAP to 50 mm Hg), whereas in dogs, more blood is removed to attain the desired pressure. To illustrate this difference it was noted earlier (Section II) that the ratio of SBV to IBV was 0.16 in dogs while a similar ratio for chickens was 1.9. This difference between the two species results

first, from the much smaller volume required to initially lower MAP in chickens and second, from a greater MBV in the latter species.

The large volume of hemorrhage in chickens is accompanied by a massive mobilization of extravascular fluid which is mirrored in dramatic decreases in hematocrit and plasma protein concentration. Measured plasma volume actually decreases very little during the hemorrhage, which indicates the degree of replacement of plasma by non-vascular fluid. Removal of more than 80% of the measured *in vivo* plasma volume was achieved although measured plasma volume diminished by only about 10% (Section III). Hollenberg (1965) has reported maximum plasma volume replacement in dogs to be 25%, but only slightly more than half of this is protein-poor fluid (protein dilution approximately 16%). In the present experiments in chickens, hematocrit and plasma protein concentration were diluted to half their starting levels. During prolonged hemorrhage (Section III), a small amount of protein was added to the vascular space and plasma protein concentration did not fall as rapidly as hematocrit did at this time. The massive fluid mobilization in chickens occurs completely unaided by constriction of precapillary resistance vessels in skeletal muscle, as occurs in dogs and cats (*see* Haddy *et al.*, 1965; Öberg, 1964). During hemorrhage in chickens, there was no change in either arterial or venous resistance segments in the leg, except for a passive terminal increase (Section IX). Although we were unable to confirm this observation in another vascular bed because of technical difficulties, the skeletal muscle circulation is considered to be the most important in hemodilution (Mellander & Lewis, 1960; Öberg, 1964). Therefore, the movement of fluid into the

vascular space following hemorrhage in chickens results from a fall in capillary hydrostatic pressure which is solely the result of a fall in MAP. It was calculated from these experiments that the fall in MAP is almost quantitatively transferred to the capillaries, and a decrease in capillary hydrostatic pressure of the calculated magnitude (40%), generalized over the skeletal muscle mass of the body, is more than sufficient to account for the observed fluid movement. With continuous infusion of noradrenaline, however, the precapillary resistance was slightly but significantly increased and during the period in which MAP was held constant, fluid mobilization occurred at a faster rate. Thus, a mechanism for aiding fluid mobilization by constriction of precapillary resistance vessels is present in the chicken, but it appears not to be activated during uncomplicated hemorrhage when the major factor which governs capillary hydrostatic pressure, and thus the rate and direction of fluid movement, is the MAP. Noradrenaline infusion did not increase the total amount of fluid mobilized but reduced the time required to reach MBV due to the increased rate of fluid mobilization.

On the other hand, under conditions in which the MAP was elevated, ultrafiltration of vascular fluid occurred. When noradrenaline was infused (Sections VII and IX), there was no hemodilution during the first minutes of hemorrhage unless MAP fell and, if MAP during this time was above the zero minutes measurement, hemoconcentration occurred. In an experiment which was not reported above, a large elevation of MAP (25 mm Hg) by infusion of noradrenaline resulted in an increase in both hematocrit (35.0 to 38.4%) and plasma protein concentration (4.98 to 5.21 Gm%).

Ultrafiltration due to increasing MAP is also seen in J4 of Fig. 1 (Methods Section) which is a regression plot of RISA activity versus time. In this experiment, MAP slowly rose (about 10 mm Hg) during the measurement of the plasma volume and, although there was no significant change in hematocrit or plasma protein concentration, the slope of the regression line was definitely positive. This phenomenon was rarely seen in hundreds of plasma volume measurements and can only be interpreted as a loss of protein-poor fluid from the vascular space.

In the later stages of hypotension, there was never a significant phase of hemoconcentration and our experiments revealed that, except for perhaps a very short preterminal period, hemorrhagic hypotension in chickens never became irreversible to retransfusion. In one series of animals (Section II), after 3-4 hours of hypotension, reinfusion of the shed blood gave an excellent 48-hour survival rate (14/17). In a small number of animals (not reported in detail, but referred to in Section VII), reinfusion of all the blood near the point of terminal cardiovascular collapse returned normal cardiovascular function with no tendency toward deterioration after 3 hours.

The coincident lack of active vasoconstriction and a significant phase of hemoconcentration correspond with the suggestion of Hollenberg (1965) that hemoconcentration results from a progressive rise in small vein wedge pressure and loss of a plasma ultrafiltrate (and later, sequestration of whole blood) and that the further decrease in circulating volume leads directly to irreversibility. A rise in venous "wedge"

pressure independent of flow and MAP was never seen in skeletal muscle of the leg of chickens (Section IX). The "wedge" pressure always passively followed MAP. Although noradrenaline infusion shortened the period of compensation, this was due to acceleration of the rate of compensation and not to vasoconstriction in small veins leading to decompensation. The total amount of fluid mobilized during hemorrhage seems to be rather constant in any one species or preparation and cannot be altered substantially by reinforcing or blocking sympathetic activity (Hollenberg, 1965). However, the rate of fluid movement, and thus the time taken to reach the point of reversal, are affected by sympathetic activity. Fluid mobilization is slower in dogs which are totally sympathectomized (Chien, 1958) or have received phenoxybenzamine, an α-adrenergic blocking agent (Hollenberg, 1965), whereas it is speeded by sympathetic stimulation (Mellander & Lewis, 1963; Öberg, 1964), or administration of noradrenaline (Hollenberg, 1965) and is delayed following α-adrenergic blockade (H.C. Wiggers et al., 1948; Hollenberg, 1965). Aside from fluid mobilization, however, vasoconstriction in the later stages of severe and prolonged hemorrhagic shock is definitely deleterious and, as Hollenberg's work has suggested, leads to irreversibility in itself.

Earlier (see Review of Literature), the various hypotheses proposed to explain the development of irreversibility to retransfusion following a period of hemorrhagic hypotension were extensively reviewed. The present experiments are relevant to that discussion because the

chicken does not develop "irreversibility". In conjunction with the absence of irreversibility, there is no independent increase in small vein wedge pressure in skeletal muscle of chickens. Hollenberg (1965) had reported that this is one of the tissues in which an increase of this pressure is observed during the later stages of hemorrhagic shock in dogs. The increase in small vein wedge pressure is highly correlated with the appearance of hemoconcentration, the step which probably leads to irreversibility in dogs (see discussion in Review of Literature). The concomittant lack of irreversibility and active vasoconstriction in the chicken provide evidence based on a clear species difference to support the concept that vasoconstriction late in shock is deleterious and, specifically, that the absence of constriction in small veins prevents the progressive hemoconcentration, to which Hollenberg (1965) attributed the major role in the development of irreversibility.

The eventual cause of death in chickens subjected to prolonged hemorrhagic hypotension is unknown. Except in the case of pullets (Section III), where inadequate respiration appeared to contribute, we were unable to find any event consistently leading to death. The cause of death may lie outside the cardiovascular system (e.g., respiratory system). Even with the very low hematocrits found late in the hypotensive period, the oxygen-carrying capacity of the blood appeared to be adequate for continued compensation because reinfusion of erythrocytes did not prolong nor increase compensation (Section VI).

In general, a lack of active vasoconstriction throughout the vascular tree appears to account for the observed differences between

chickens and mammals following hemorrhage. The reason for the absence of active vasoconstriction is not clear. There may be a relative absence of homeostatic mechanisms for control of the circulation in chickens but there are at least some reflexes. There is a cardio-accelerator nerve in chickens (Tummons & Sturkie, 1968) and tachycardia, which could be blocked by the β-adrenergic blocking agent propranolol, was always seen during hypotension (propranolol experiments not reported above). Also, increased MAP resulting from noradrenaline infusion caused bradycardia (Section IX). There is histochemical evidence (Akester & Mann, 1969) for the presence of adrenergic nerves, and pharmacological evidence (Harvey & Nickerson, 1951) for the existence of α-adrenergic receptors in the vasculature of chickens, although sensitivity to injected catecholamines is low. We have confirmed that reactivity to injected catecholamines is modest, for even large doses of noradrenaline infused over a long period, had little effect on the cardiovascular "non-response" to hemorrhagic hypotension in chickens.

The present measurement of circulating catecholamines confirms those reports in the literature (Lin & Sturkie, 1968) that show resting levels of catecholamines are several-fold higher in chickens than in mammals (Section X). The high concentrations of circulating catecholamines could contribute to the insensitivity of the vasculature to injected agents by production of permanent tachyphylaxis. During hemorrhagic hypotension, circulating catecholamines in chickens rise to levels which are not usually approached in mammals, however, in view of the resting levels, the increases are relatively less than those reported in mammals.

The evidence suggests that the reason for the lack of active vasoconstriction during hemorrhage is twofold; first, there is a relative lack of homeostatic control of the circulation of chickens, and second, the vasculature is relatively insensitive to the neurohumoral substances which are considered to mediate sympathetic vasoconstriction. Lack of response to catecholamines may be partially a receptor event (e.g., permanent tachyphylaxis due to high levels of circulating catecholamines).

While this work was in progress, Djojosugito et al. (1968) reported a small number of experiments which suggested that the rapid volume replacement in ducks was the result of a marked ability of this species to increase precapillary resistance. However, their experiments differed in some respects to those reported here. They removed a small volume of blood (10-15% of in vivo blood volume) over a short period (10 minutes) and followed the animal for another 20 to 30 minutes after which all the shed blood was reinfused. This procedure cannot be construed as a hemorrhagic shock model. In a few anesthetized ducks, they found a large increase in skeletal muscle precapillary vascular resistance but, in unanesthetized ducks, in spite of the fact that fluid mobilization was even more marked, TPR increased by only 15%. There is some difficulty in reconciling the magnitude of the reported increase in skeletal muscle regional resistance (300%) with the small change in TPR. One significant difference between ducks and chickens, however, was that in the former, up to 25% of the blood volume could be removed without any appreciable fall in MAP. In the present study on chickens,

20-25% of the *in vivo* blood volume was sufficient to lower MAP to 50 mm Hg. The differences in the responses of ducks and chickens indicate a marked difference in their ability to vasoconstrict. The observed increase in resistance in ducks may be related to the "diving" reflex of waterfowl. It is well known that ducks have highly developed and complex sympathetic reflexes which are activated during "diving". Upon diving, waterfowl develop a very marked bradycardia accompanied by intense vasoconstriction which serves to preserve the oxygen supply to "vital" areas. This response is reversed within 1-2 seconds of emersion to a marked tachycardia and profound vasodilatation (Folkow *et al.*, 1967). The intense vasoconstriction seen in the ducks following hemorrhage may be due to partial activation of this "diving" reflex. The present study of chickens shows no such intense reflex vasoconstriction is activated in this species following hemorrhage. In their paper on ducks (Djojosugito *et al.*, 1968), these authors also refer to two other papers "in press" which supposedly report hemodilution and resistance to irreversibility in some other species of bird, but these papers have not yet appeared.

9. SUMMARY AND CONCLUSIONS

In the present experiments, it was found that chickens (and turkeys) present a rather characteristic pattern of response following hemorrhage which is markedly different from that of mammals (and diving birds). In general, there are three points of difference between the reaction of these birds and that of mammals during experimental hemorrhagic hypotension. First, the mean arterial pressure of both species of fowl falls very rapidly after even a small hemorrhage; second, hemodilution is both more rapid and larger in the birds; and finally, chickens do not appear to develop the "irreversibility" and hemoconcentration which is characteristic of hemorrhagic shock in mammals. Results of the hemodynamic studies on chickens reported here led to the proposal that all these phenomena are related to a lack of active reflex vasoconstriction in this species.

Lack of active vasoconstriction means removal of blood results in an immediate fall in mean arterial pressure and since this fall in pressure is unopposed by changes in vessel "tone" it is transferred directly and almost quantitatively to the capillaries, leading to absorption of extravascular fluid according to the principles of the Starling Hypothesis. A lack of vasoconstriction in small veins during prolonged hemorrhagic hypotension ensures that capillary hydrostatic pressure does not increase terminally and explains the absence of hemoconcentration, fluid loss, and irreversibility. The evidence suggests that absence of active vasoconstriction may be partly due to chronic tachyphylaxis resulting from high levels of circulating catecholamines.

10. CLAIMS FOR ORIGINAL WORK

No systematic experimental study covering the purpose of this investigation has been previously reported. Some of the techniques described were originally adapted for this study in chickens.

The important new findings of these studies are:

1. Cardiac output (indicator-dilution techniques) of chickens may be calculated by means of the Dow formula after a slight modification.

2. The following phenomena are observed during hemorrhage in chickens:

a) The mean arterial blood pressure falls very rapidly following even small volumes of hemorrhage.

b) The fall in mean arterial pressure is accompanied by a rapid and large mobilization of extravascular fluid evidenced by a marked hemodilution.

c) Even during prolonged hemorrhagic hypotension, there is no evidence of a significant phase of hemoconcentration and chickens are very resistant to the development of "irreversibility".

3. The observed phenomena during hemorrhage in chickens are explained by an almost complete absence of active vasoconstriction and lend support to the concept that vasoconstriction late in shock plays a major role in the development of "irreversibility".

4. The lack of active vasoconstriction in the chicken may be partly due to chronic tachyphylaxis to sympathetic neurohumoral substances.

11. REFERENCES

Abel, F.L., J.A. Waldhausen, W.J. Daly and W.L. Pearce. Pulmonary blood volume in hemorrhagic shock in the dog and primate. Amer. J. Physiol. 213: 1072-1078 (1967).

Adolph, E.F., M.J. Gerbasi and M.J. Lepore. The rate of entrance of fluid into the blood in hemorrhage. Amer. J. Physiol. 104: 502-517 (1933).

Akester, A.R. Renal portal shunts in the kidney of the domestic fowl. J. Anat. 101: 569-594 (1967).

Akester, A.R. and S.P. Mann. Adrenergic and cholinergic innervation of the renal portal valve in the domestic fowl. J. Anat. 104: 241-252 (1969).

Allen, T.H., R.A. Walzer, K. Gregersen and M.I. Gregersen. Blood volume, bleeding volume and tolerance to hemorrhage in the splenectomized dog. Amer. J. Physiol. 196: 176-178 (1959).

Annino, J.S. Clinical Chemistry. 2nd Ed., Little, Brown & Co., Toronto, 1960.

Anton, A.H. and D.F. Sayre. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exp. Ther. 138: 360-375 (1962).

Artz, C.P., J.M. Howard, Y. Sako, A.W. Bronwell and T. Prentice. Clinical experiences in the early management of the most severely injured battle casualties. Ann. Surg. 141: 285-296 (1955).

Attar, S., W.H. Kirby Jr., C. Masitis, A.R. Mansberger Jr. and R.A. Crowley. Coagulation changes in clinical shock: I. Effect of hemorrhagic shock on clotting time in humans. Ann. Surg. 164: 34-40 (1966).

Attar, S.M., J. McLaughlin, A.R. Mansberger Jr. and R.A. Crowley. Prognostic significance of coagulation studies in clinical shock. Surg. Forum 17: 8-11 (1966).

Aub, J.C. A toxic factor in experimental traumatic shock. New Eng. J. Med. 231: 71-75 (1944).

Baker, C.H. Fibrinogen-I^{131}, T-1824, and red cell-Cr^{51} spaces following hemorrhage. Amer. J. Physiol. 205: 527-532 (1963).

Baker, C.H. and J.W. Remington. Fluid shifts after hemorrhage. Amer. J. Physiol. 201: 910-914 (1961).

Beecher, H.K., F.A. Simeone, C.H. Burnett, S.L. Shapiro, E.R. Sullivan and T.B. Mallory. The internal state of the severely wounded man on entry to the most forward hospital. Surgery 22: 672-711 (1947).

Blalock, A. Shock: Further studies with particular reference to the effects of hemorrhage. Arch. Surg. 29: 837-857 (1934).

Bloch, J.H., R.H. Dietzman, C.H. Pierce and R.C. Lillehei. Theories of the production of shock; a review of their relevance to clinical practice. Brit. J. Anaesth. 38: 234-250 (1966).

Bohr, D.F. and P.L. Goulet. Humoral and myogenic factors in shock: Evaluated by means of isolated arteriolar smooth muscle response. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, pp. 314-318.

Bradley, O.C. The Structure of the Fowl. 4th Ed., revised by T. Grahame, Oliver and Boyd, London, 1960.

Brand, E.D. and A.M. Lefer. Myocardial depressant factor in plasma from cats in irreversible post-oligemic shock. Proc. Soc. Exp. Biol. Med. 122: 200-203 (1966).

Brand, E.D. and A.M. Lefer. Influence of positive inotropic agents on the action of a myocardial depressant factor in the plasma of cats in postoligemic shock. Proc. Soc. Exp. Biol. Med. 126: 335-339 (1967).

Burton, R.R. and A.H. Smith. Blood and air volumes in the avian lung. Poult. Sci. 47: 85-91 (1968).

Cannon, W.B. Traumatic Shock. Appleton, New York, 1923.

Celander, O. The range of control exercised by the sympathico-adrenal system; a quantitative study on blood vessels and other smooth muscle effectors in the cat. Acta Physiol. Scand. 32: (suppl. 116) 1-132 (1954).

Chien, S. Quantitative evaluation of the circulatory adjustment of splenectomized dogs to hemorrhage. Amer. J. Physiol. 193: 605-614 (1958).

Chien, S. Role of the sympathetic nervous system in hemorrhage. Physiol. Rev. 47: 214-288 (1967).

Cobbold, A., B. Folkow, I. Kjellmer and S. Mellander. Nervous and local chemical control of pre-capillary sphincters in skeletal muscle as measured by changes in filtration coefficient. Acta Physiol. Scand. 57: 180-192 (1963).

Cohen, R.R. Anticoagulation, centrifugation time, and sample replicate number in the microhematocrit method for avian blood. Poult. Sci. 46: 214-218 (1967a).

Cohen, R.R. An estimation of percentage trapped plasma in normal chicken microhematocrit, using Cr^{51}. Poult. Sci. 46: 219-223 (1967b).

Cohen, R.R. Total circulating erythrocyte and plasma volumes of ducks measured simultaneously with Cr^{51}. Poult. Sci. 46: 1539-1544 (1967c).

Cope, O. and S.B. Litwin. Contribution of the lymphatic system to the replenishment of the plasma volume following a hemorrhage. Ann. Surg. 156: 655-667 (1962).

Cort, J.H., M.F. Jeanjean, A.E. Thompson and M. Nickerson. Effects of "hormonogen" forms of neurohypophysial peptides in hemorrhagic shock in dogs. Amer. J. Physiol. 214: 455-462 (1968).

Courtice, F.C. and R.W. Gunton. Effect of nembutal anaesthesia on restoration of plasma volume after hemorrhage in dogs, cats and rabbits. J. Physiol. 108: 418-426 (1949).

Crile, G.W. and W.E. Lower. Anoci-Association. W.B. Saunders Co., Philadelphia, 1915.

Critz, J.B. and A.W. Merrick. Serum electrolyte and hematocrit changes in young rabbits following hemorrhage. Amer. J. Physiol. 196: 173-175 (1959).

Crowell, J.W. and W.L. Read. In vivo coagulation; a probable cause of irreversible shock. Amer. J. Physiol. 183: 565-569 (1955).

Deavers, S., E.L. Smith and R.A. Huggins. Critical role of arterial pressure during hemorrhage in the dog on release of fluid into the circulation and trapping of red cells. Amer. J. Physiol. 195: 73-76 (1958).

Deavers, S., E.L. Smith and R.A. Huggins. Movement of fluid, albumin, and globulins with overtransfusion and hemorrhage. Amer. J. Physiol. 205: 995-999 (1963).

Djojosugito, A.M., B. Folkow and A.G.B. Kovách. The mechanisms behind the rapid blood volume restoration after hemorrhage in birds. Acta Physiol. Scand. 74: 114-122 (1968).

Dow, P. Dimensional relationships in dye-dilution curves from humans and dogs, with an empirical formula for certain troublesome curves. J. Appl. Physiol. 7: 399-408 (1955).

Ebert, R.V., E.A. Stead Jr. and J.G. Gibson. Response of normal subjects to acute blood loss with special reference to the mechanism of restoration of blood volume. Arch. Int. Med. 68: 578-590 (1941).

Eckstein, R.W., I.M. Liebow and C.J. Wiggers. Limb blood flow and vascu-

lar resistance changes in dogs during hemorrhagic hypotension and shock. Amer. J. Physiol. 147: 685-694 (1946).

Ehrlich, E.W., S. Gollub and A.W. Ulin. Effects of graded hypovolemia on the coagulation mechanism. Ann. N.Y. Acad. Sci. 115: 97-98 (1964).

Einheber, A. Discussion of paper by Dr. Fine. Fed. Proc. 20: 170-172 (1961).

Einheber, A. and G.J. Cerilli. Hemorrhagic shock in the monkey. Amer. J. Physiol. 202: 1183-1187 (1962).

Erlanger, J. and H.S. Gasser. Studies in secondary traumatic shock. III. Circulatory failure due to adrenalin. Amer. J. Physiol. 49: 345-376 (1919).

Fell, C. Changes in distribution of blood flow in irreversible hemorrhagic shock. Amer. J. Physiol. 210: 863-868 (1966).

Fine, J. and A.M. Seligman. Traumatic shock: A study of the problem of the "lost plasma" in hemorrhagic shock by the use of radioactive plasma protein. J. Clin. Invest. 22: 285-303 (1943).

Fine, J. and A.M. Seligman. Traumatic shock: A study of the problem of the "lost plasma" in hemorrhagic, tourniquet and burn shock by the use of radioactive iodo-plasma protein. J. Clin. Invest. 23: 720-730 (1944).

Fine, J. Relation of bacteria to the failure of blood-volume therapy in traumatic shock. New Eng. J. Med. 250: 889-895 (1954).

Fine, J. Endotoxins in traumatic shock. Fed. Proc. 20: (suppl. 9) 166-170 (1961).

Fine, J. Comparison of various forms of experimental shock. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, pp. 25-39.

Fine, J., C. Palmerio and S. Rutenburg. New developments in therapy of refractory traumatic shock. Arch. Surg. 96: 163-175 (1968).

Folkow, B. Nervous adjustments of the vascular bed with special reference to patterns of vasoconstrictor fibre discharge. In, Shock: Pathogenesis and Therapy: An International Sympsoium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, pp. 61-75.

Folkow, B., N.J. Nilsson and L.R. Yonce. Effects of "diving" on cardiac output in ducks. Acta Physiol. Scand. 70: 347-361 (1967).

Fowler, N.O. and R. Franch. Mechanism of pressor response to l-norepin-

ephrine during hemorrhagic shock. Circ. Res. 5: 153-156 (1957).

Freeman, N.E. Decrease in blood volume after prolonged hyperactivity of the sympathetic nervous system. Amer. J. Physiol. 103: 185-202 (1933).

Freeman, N.E., S.A. Schaffer, A.E. Schecter and H.E. Holling. The effect of total sympathectomy on the occurrence of shock from hemorrhage. J. Clin. Invest. 17: 359-368 (1938).

Gesell, R. Studies on the submaxillary gland. IV. A comparison of the effects of hemorrhage and of tissue-abuse in relation to secondary shock. Amer. J. Physiol. 47: 468-506 (1918).

Gesell, R. and C.A. Moyle. On the relation of blood volume to tissue-nutrition. II. The effects of graded hemorrhage on the volume-flow of blood through the striated muscle of the dog. Amer. J. Physiol. 61: 412-419 (1922).

Gilbert, R.P. Possible role of endotoxin in the perpetuation of shock. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, pp. 40-49.

Gomez, O.A. and W.F. Hamilton. Functional cardiac deterioration during development of hemorrhagic circulatory deficiency. Circ. Res. 14: 327-336 (1964).

Gourzis, J.T. Studies on the Role of Adrenergic Vasoconstriction in the Development of Shock. Ph.D. Thesis, University of Manitoba, 1962.

Grant, R.T. In discussion. Fed. Proc. 20: (suppl. 9) 48-49 (1961).

Green, H.D. Physiology of peripheral circulation in shock. Fed. Proc. 20: (suppl. 9) 61-68 (1961).

Greever, C.J. and D.T. Watts. Epinephrine levels in the peripheral blood during irreversible hemorrhagic shock in dogs. Circ. Res. 7: 192-195 (1959).

Gregg, D.E. Hemodynamic factors in shock. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, pp. 50-60.

Groom, A.C., S. Rowlands and H.W. Thomas. Some circulatory responses to hemorrhage in the cat: A critical level of blood volume for the onset of hypotension. Quart. J. Exp. Physiol. 50: 385-405 (1965).

Guyton, A.C., H.M. Batson and C.M. Smith. Adjustments of the circulatory system following very rapid transfusion or hemorrhage. Amer. J. Physiol. 164: 351-359 (1951).

Guyton, A.C. and J.W. Crowell. Dynamics of the heart in shock. Fed. Proc. 20: (suppl. 9) 51-60 (1961).

Guyton, A.C. Concept of negative interstitial pressure based on pressures in implanted perforated capsules. Circ. Res. 12: 399-414 (1963).

Haddy, F.J., A.G. Richards, J.L. Alden and M.B. Visscher. Small vein and artery pressures in normal and edematous extremities of dogs under local and general anesthesia. Amer. J. Physiol. 176: 355-360 (1954).

Haddy, F.J., J.B. Scott and J.I. Molnar. Mechanism of volume replacement and vascular constriction following hemorrhage. Amer. J. Physiol. 208: 169-181 (1965).

Haddy, F.J., H.W. Overbeck and R.M. Daugherty Jr. Peripheral vascular resistance. Ann. Rev. Med. 19: 167-194 (1968).

Halmagyi, D.F.J. and D.J. Gillett. Dogs versus sheep as hemorrhagic shock models. J. Surg. Res. 7: 78-84 (1967).

Halpern, B.N. In discussion of a paper by Dr. Fine. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, p. 48

Hamilton, W.F., J.W. Moore, J.M. Kinsman and R.G. Spurling. Studies on the circulation. IV. Further analysis of the injection method, and of changes in hemodynamics under physiological and pathological conditions. Amer. J. Physiol. 99: 534-551 (1932).

Hardaway, R.M., W.H. Brune, E.F. Greever, J.W. Burns and H.P. Mock. Studies on the role of intravascular coagulation in irreversible hemorrhagic shock. Ann. Surg. 155: 241-250 (1962).

Hardaway, R.M. and J.W. Burns. Mechanism of action of fibrinolysin in the prevention of irreversible hemorrhagic shock. Ann. Surg. 157: 305-309 (1963).

Hardaway, R.M. and D.C. Drake. Prevention of "irreversible" hemorrhagic shock with fibrinolysin. Ann. Surg. 157: 39-47 (1963).

Hardaway, R.M. and D.G. Johnson. A new theory of shock. Milit. Med. 128: 198-208 (1963).

Hardaway, R.M. Syndromes of Disseminated Intravascular Coagulation, with Special Reference to Shock and Hemorrhage. Charles C. Thomas, Springfield, 1966.

Harvey, S.C. and M. Nickerson. Adrenergic mechanisms in the chicken. Fed. Proc. 10: 307 (1951).

Henry, J.N., A.H. McArdle, H.J. Scott and F.N. Gurd. A study of the acute

and chronic respiratory pathophysiology of hemorrhagic shock. J. Thorac. Cardiovasc. Surg. 54: 666-678 (1967).

Hollenberg, N.K. The Role of the Sympathetic Nervous System in the Development of Decompensation During Hemorrhagic Shock. Ph.D. Thesis, University of Manitoba, 1965.

Hopkins, R.W., I. Penn and F.A. Simeone. Studies of thoracic duct lymph and blood plasma in oligemic hypotension. J. Amer. Med. Ass. 187: 122-125 (1964).

Howard, J.M. Hemorrhagic and post-hemorrhagic shock. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, pp. 186-194.

Jones, C.E., J.W. Crowell and E.E. Smith. A cause-effect relationship between oxygen deficit and irreversible hemorrhagic shock. Surg. Gynec. Obstet. 127: 93-96 (1968).

Kotula, A.W. and N.V. Helbacka. Blood volume of live chickens and influence of slaughter technique on blood loss. Poult. Sci. 45: 684-688 (1966).

Kovach, A.G.B. Importance of nervous and metabolic changes in the development of irreversibility in experimental shock. Fed. Proc. 20: 122-137 (1961).

Lang, D.W. and H.H. Borgestedt. Rate of pulmonary excretion of paraldehyde in cats. Toxic. Appl. Pharmacol. 13: 24-29 (1968).

Lansing, A.M. and J.A. Stevenson. The standardization of stress in the production of hemorrhagic shock; the relation of the duration of strain and the resistance of the animal to the onset of irreversible shock. Canad. J. Biochem. Physiol. 35: 1085-1092 (1957).

Lefer, A.M., G.B. Craddock, R. Cowgill and E.D. Brand. Performance of papillary muscles isolated from cats in postoligemic shock. Amer. J. Physiol. 211: 687-692 (1966).

Lefer, A.M., R. Cowgill, F.F. Marshall, L.M. Hall and E.D. Brand. Characterization of a myocardial depressant factor present in hemorrhagic shock. Amer. J. Physiol. 213: 492-498 (1967).

Lewis, D.H. and S. Mellander. Competitive effects of sympathetic control and tissue metabolites on resistance and capacitance vessels and capillary filtration in skeletal muscle. Acta Physiol. Scand. 56: 162-188 (1962).

Lillehei, R.C. The intestinal factor in irreversible hemorrhagic shock in dogs. Surgery 42: 1043-1054 (1957).

Lillehei, R.C. Relationship of appearance of abnormal plasma hemin pigment to development of irreversible hemorrhagic shock in dogs. Circ. Res. 6: 438-441 (1958).

Lillehei, R.C., J.K. Longerbeam and J.C. Rosenberg. The nature of irreversible shock: Its relationship to intestinal changes. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, pp. 106-133.

Lillehei, R.C., J.K. Longerbeam, J.H. Bloch and W.G. Manax. The modern treatment of shock based on physiologic principles. Clin. Pharmacol. Ther. 5: 63-101 (1964).

Lin, Y.C. and P.D. Sturkie. Effect of environmental temperatures on the catecholamines of chickens. Amer. J. Physiol. 214: 237-240 (1968).

Lister, J., I.F. McNeill, V.C. Marshall, L.F. Plzak Jr., F.J. Dagher and F.D. Moore. Transcapillary refilling after hemorrhage in normal man: Basal rates and volumes; effect of norepinephrine. Ann. Surg. 158: 698-709 (1963).

Lundsgaard-Hansen, P., C. Meyer and H. Riedwyl. Transmural gradients of glycolytic enzyme activities in left ventricular myocardium. II. Prolonged hemorrhagic hypotension. Pflüger Arch. Ges. Physiol. 301: 144-161 (1968).

Lundgren, O., J. Lundwall and S. Mellander. Range of sympathetic discharge and reflex vascular adjustments in skeletal muscle during hemorrhagic hypotension. Acta Physiol. Scand. 62: 380-390 (1964).

McGinnis, C.H., Jr. and R.K. Ringer. Carotid sinus reflex in the chicken. Poult. Sci. 45: 402-404 (1966).

McGinnis, C.H., Jr. and R.K. Ringer. Arterial occlusion and cephalic baroreceptors in the chicken. Amer. J. Vet. Res. 28: 1117-1124 (1967).

McKay, D.G. Disseminated Intravascular Coagulation; an Intermediary Mechanism of Disease. Harper & Row, New York, 1965.

McNulty, W.P., Jr. and R. Linares. Hemorrhagic shock in germ-free rats. Amer. J. Physiol. 198: 141-144 (1960).

Maling, H.M., B. Highman and E.C. Thompson. Some similar effects after large doses of catecholamines and myocardial infraction in dogs. Amer. J. Cardiol. 5: 628-633 (1960).

Matsumoto, T., R.M. Hardaway, J.E. McClain and P.M. Margetis. Microcirculation of dogs in hemorrhagic shock and after treatment. Arch. Surg. 96: 179-183 (1968).

Medway, W. and M. Kare. Blood and plasma volume, hematocrit, blood specific gravity and serum protein electrophoresis of the chicken. Poult. Sci. 38: 624-631 (1959).

Mellander, S. Comparative studies on the adrenergic neuro-hormonal control of resistance and capacitance blood vessels in the cat. Acta Physiol. Scand. 50: (suppl. 176) 1-86 (1960).

Mellander, S. Contribution of small vessel tone to the regulation of blood volume and formation of oedema. Proc. Roy. Soc. Med. 61: 55-61 (1968).

Mellander, S. and D.H. Lewis. Effect of hemorrhagic shock on the reactivity of resistance and capacitance vessels and on capillary filtration transfer in cat skeletal muscle. Circ. Res. 13: 105-118 (1963).

Mellander, S. and B. Johansson. Control of resistance, exchange, and capacitance functions in the peripheral circulation. Pharmacol. Rev. 20: 117-196 (1968).

Miles, A.A. Local and systemic factors in shock. Fed. Proc. 20: (suppl. 9) 141-157 (1961).

Millar, R.A. and B.G. Benfey. The fluorimetric estimation of adrenaline and noradrenaline during haemorrhagic hypotension. Brit. J. Anaesth. 30: 158-165 (1958).

Milnor, W.R. and A.D. Jose. Distortion of indicator-dilution curves by sampling systems. J. Appl. Physiol. 15: 177-180 (1960).

Moon, V.H. and P.J. Kennedy. Pathology of shock. Arch. Path. 14: 360-371 (1932).

Nickerson, M. Factors of vasoconstriction and vasodilation in shock. J. Mich. State Med. Soc. 54: 45-49 (1955).

Nickerson, M. In discussion. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, p. 205.

Nickerson, M. Vasoconstriction and vasodilatation in shock. Int. Anaesth. Clin. 2: 385-397 (1964).

Nickerson, M. and J.T. Gourzis. Blockade of sympathetic vasoconstriction in the treatment of shock. J. Trauma 2: 399-411 (1962).

Noble, R.P. and M.I. Gregersen. Blood volume in clinical shock. II. The extent and cause of blood volume reduction in traumatic, hemorrhagic, and burn shock. J. Clin. Invest. 25: 172-183 (1946).

Öberg, B. Effects of cardiovascular reflexes on net capillary fluid transfer. Acta Physiol. Scand. 62: (suppl. 229) 1-98 (1964).

Oriol, A., Sekelj and M. McGregor. Limitations of indicator-dilution methods in experimental shock. J. Appl. Physiol. 23: 605-608 (1967).

Oriol, A. and M. McGregor. Indicator-dilution methods in estimation of cardiac output in clinical shock. Amer. J. Cardiol. 20: 826-830 (1967).

Osborne, M.W., H. Rowe, R. Kaufman and M. Johnson. Effect of P286 on blood catecholamine levels and survival rate in dogs exposed to hemorrhagic shock. Arch. Int. Pharmacodyn. 174: 167-180 (1968).

Overman, R.R. and S.C. Wang. The contributory role of the afferent nervous factor in experimental shock: Sublethal hemorrhage and sciatic nerve stimulation. Amer. J. Physiol. 148: 289-295 (1947).

Page, I.H. Some neurohumoral and endocrine aspects of shock. Fed. Proc. 20: (suppl. 9) 75-98 (1961).

Pappenheimer, J.R. and A. Soto-Rivera. Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs. Amer. J. Physiol. 152: 471-491 (1948).

Pareira, M.D., K.D. Serkes and S. Lang. Plasma volume response to graded hemorrhage. Surgery 52: 378-381 (1962).

Pearl, G.J., J.W. Trank, F.N. Gurd and L.G. Hampson. Lymph flow patterns under the influence of pressor amines, ganglionic blocking agents and hemorrhagic shock. Surg. Forum 14: 14-16 (1963).

Peters, T., Jr. Proposals for standardization of total protein assays. Clin. Chem. 14: 1147-1159 (1968).

Phemister, D.B., L. Eichelberger and C.H. Laestar. Early effects on dogs of section of the eighth cervical segment of the spinal cord and their bearing on shock. Arch. Surg. 51: 32-41 (1945).

Raškova, H. and J. Vaněcek. Pharmacology of bacterial toxins. Pharmacol. Rev. 16: 1-45 (1964).

Remington, J.W., W.F. Hamilton, G.H. Boyd Jr., W.F. Hamilton Jr. and H.M. Caddell. Role of vasoconstriction in the response of the dog to hemorrhage. Amer. J. Physiol. 161: 116-124 (1950).

Reynell, P.C., P.A. Marks, C. Chidsey and S.E. Bradley. Changes in splanchnic blood volume and splanchnic blood flow in dogs after hemorrhage. Clin. Sci. 14: 407-419 (1955).

Rosenberg, J.C., R.C. Lillehei, J. Longerbeam and B. Zimmermann. Studies on hemorrhagic and endotoxin shock in relation to vasomotor changes and endogenous circulating epinephrine, norepinephrine and serotonin. Ann. Surg. 154: 611-628 (1961).

Rothe, C.F., F.C. Schwendenmann and E.E. Selkurt. Neurogenic control of skeletal muscle vascular resistance in hemorrhagic shock. Amer. J. Physiol. 204: 925-932 (1963).

Rothe, C.F. and E.E. Selkurt. Cardiac and peripheral failure in hemorrhagic shock in the dog. Amer. J. Physiol. 207: 203-214 (1964).

Rushmer, R.F., R.L. van Citters and D.L. Franklin. Shock: A semantic enigma. Circulation 26: 445-459 (1962a).

Rushmer, R.F., R.L. van Citters and D.L. Franklin. Definition and classification of various forms of shock. In, Shock: Pathogenesis and Therapy: An International Symposium (Ciba)., edited by K.D. Bock, Springer-Verlag, Berlin, 1962, pp. 1-24.

Salzman, E.W. Does intravascular coagulation occur in hemorrhagic shock in man? J. Trauma 8: 867-871 (1968).

Sanford, J.P. and H.E. Noyes. Studies on the absorption of E. coli endotoxin from the gastrointestinal tract of dogs in the pathogenesis of irreversible hemorrhagic shock. J. Clin. Invest. 37: 1425-1435 (1958).

Sarnoff, S.J., R.B. Case, P.E. Waithe and J.P. Issacs. Insufficient coronary flow and myocardial failure as a complication factor in later hemorrhagic shock. Amer. J. Physiol. 176: 439-444 (1954).

Schmidt, H.D. and J. Schmier. Kontraktilitätsschädigung des Herzens im frühen hämorrhgischen Schock. Pflügers Arch. Ges. Physiol. 285: 241-252 (1965).

Sekelj, P., G.R. Tait and M.M. Nathanson. Studies on dye dilution curves using a digital computer. IEEE Trans. Biomed. Engin. 13: 32-37 (1966).

Selkurt, E.E., R.S. Alexander and M.B. Patterson. The role of the mesenteric circulation in the irreversibility of hemorrhagic shock. Amer. J. Physiol. 149: 732-743 (1947).

Selkurt, E.E. and C.F. Rothe. Critical analysis of experimental hemorrhagic shock models. Fed. Proc. 20: (suppl. 9) 30-37 (1961).

Shoemaker, W.C. Measurement of rapidly and slowly circulating red cell volumes in hemorrhagic shock. Amer. J. Physiol. 202: 1179-1182 (1962).

Simeone, F.A. Some issues in the problem of shock. Fed. Proc. 20: (suppl. 9) 3-11 (1961).

Skillman, J.J., H.K. Awwad and F.D. Moore. Plasma protein kinetics of the early transcapillary refill after hemorrhage in man. Surg. Gynec. Obstet. 125: 983-996 (1967).

Smith, E.E. and J.W. Crowell. Effect of hemorrhagic hypotension on oxygen consumption of dogs. Amer. J. Physiol. 207: 647-649 (1964).

Smith, E.L., R.A. Huggins and S. Deavers. Effect of blood volume on movement of protein and volume distribution of albumin in the dog. Amer. J. Vet. Res. 26: 829-836 (1965).

Smith, J.J. and R.A. Grace. Influence of differential aortic perfusion pressures in experimental shock. Amer. J. Physiol. 191: 135-139 (1957).

Starling, E.H. On the absorption of fluids from the connective tissue spaces. J. Physiol. 19: 312-326 (1896).

Sturkie, P.D. Cardiovascular effects of acclimatization to heat and cold in chickens. J. Appl. Physiol. 22: 13-15 (1967).

Swan, H. Experimental acute hemorrhage: The relation of blood pressure change to plasma dilution. Arch. Surg. 91: 390-406 (1965).

Szakacs, J.E. and A. Cannon. l-Norepinephrine myocarditis. Amer. J. Clin. Path. 30: 425-434 (1958).

Szakacs, J.E. and B. Mehlman. Pathologic changes induced by l-norepinephrine; quantitative aspects. Amer. J. Cardiol. 5: 619-627 (1960).

Taylor, P.H., D.K. Heydinger, J.D. Bowers and G.W. Callendine Jr. Evaluation of small acute blood loss in man. Amer. J. Surg. 114: 913-916 (1967).

Tummons, J. and P.D. Sturkie. Cardio-accelerator nerve stimulation in chickens. Life Sci. 7: 377-380 (1968).

Vogel, J.A. and P.D. Sturkie. Cardiovascular responses of the chicken to seasonal and induced temperature changes. Science 140: 1404-1406 (1963).

Walcott, W.W. Blood volume in experimental hemorrhagic shock. Amer. J. Physiol. 143: 247-253 (1945).

Waldhausen, J.A., F.L. Abel and W.L. Pearce. Portal-systemic venous shunts in hemorrhagic shock in the dog and monkey. Ann. Surg. 166: 183-189 (1967).

Walton, R.P., J.A. Richardson, R.P. Walton Jr. and W.L. Thompson. Sympathetic influences during hemorrhagic hypotension. Amer. J. Physiol. 197: 223-230 (1959).

Wang, S.C., R.R. Overman, J.W. Fertig, W.S. Root and M. Gregersen. The relation of blood volume reduction to mortality rate in hemorrhagic and traumatic shock in the dog. Amer. J. Physiol. 148: 164-173 (1947).

Webb-Peploe, M.M. and J.T. Shepherd. Response of large hindlimb veins of the dog to sympathetic nerve stimulation. Amer. J. Physiol. 215: 299-307 (1968).

Weidner, M.G., Jr., L. Roth and F.A. Simeone. Myocardial response to prolonged acute oligemic hypotension. Surgery 50: 75-81 (1961).

Wessely, J. Lymph circulation of dogs in experimental thermal, hemorrhagic and tourniquet shock. Acta Physiol. Acad. Sci. Hung. 14: 327-351 (1958).

Wiederhielm, C.A. Dynamics of transcapillary fluid exchange. J. Gen. Physiol. 52: 29s-63s (1968).

Wiggers, C.J. The present status of the shock problem. Physiol. Rev. 22: 74-123 (1942).

Wiggers, C.J. and J.M. Werle. Cardiac and peripheral resistance factors as determinants of circulatory failure in hemorrhagic shock. Amer. J. Physiol. 136: 421-432 (1942).

Wiggers, C.J. Physiology of Shock. The Commonwealth Fund, New York, 1950.

Wiggers, H.C., R.C. Ingraham, F. Roemhild and H. Goldberg. Vasoconstriction and the development of irreversible hemorrhagic shock. Amer. J. Physiol. 153: 511-520 (1948).

Whigham, H. and M.H. Weil. A model for the study of hemorrhagic shock in the rat: Development of the method. J. Appl. Physiol. 21: 1860-1863 (1966).

Yard, A.C. and M. Nickerson. Shock produced in dogs by infusions of norepinephrine. Fed. Proc. 15: 502 (1956).

Zweifach, B.W. Aspects of comparative physiology of laboratory animals relative to the problem of experimental shock. Fed. Proc. 20: (suppl. 9) 18-29 (1961).

Zweifach, B.W., R.E. Lee, C. Hyman and R. Chambers. Omental circulation in morphinized dogs subjected to graded hemorrhage. Ann. Surg. 120: 232-250 (1944).

Zweifach, B.W., R. Chambers, R.E. Lee and C. Hyman. Reactions of peripheral blood vessels in experimental hemorrhage. Ann. N.Y. Acad. Sci. 49: 553-570 (1948).

Zweifach, B.W. and S.G. Hershey. Predisposing action of anesthetic agents on the vascular responses in hemorrhagic shock. Surg. Gynec. Obstet. 89: 469-477 (1949).