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EFFECT OF HEMORRHAGE, VASOACTIVE AGENTS, ASPHYXIA AND EXERCISE ON THE VASCULATURE OF THE CHICKEN

By

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ABSTRACT

THE EFFECT OF HEMORRHAGE, VASOACTIVE AGENTS, ASPHYXIA AND EXERCISE ON THE VASCULATURE OF THE CHICKEN

By

James Mattes Ploucha

This study examines the vascular response of the chicken to hemorrhage, vasoactive agents, asphyxia, and exercise. Three studies (using a total of 118 birds) were conducted in domestic chickens (Gallus domesticus). The first study (n=62) examined the effect of hemorrhage to a mean arterial blood pressure (MABP) of 50 mm Hg on various hemotological and vascular parameters. Total peripheral resistance fell slightly or was unaffected and skeletal muscle vascular resistance, judged from changes in perfusion pressure (Pp) in the constantly-perfused hindlimb, was unchanged. Plasma protein concentration was significantly reduced within 30 minutes of hemorrhage indicating that fluid mobilization was immediate and rapid. Plasma osmolality was unchanged by hemorrhage.

Secondly, hemorrhage to a MABP of 25 mm Hg (n=28) produced a significant rise in Pp which was unaffected by severence of the sciatic nerve truck or bilateral cervical vagotomy. This vasoconstriction could be completely eliminated by intra-arterial infusion of phentolamine or by pump-perfusing the head with arterial blood during the hypotensive interval. Furthermore, concentrations of serotonin, dopamine, and norepinephrine in plasma were significantly elevated only when the rise in Pp was evident. The vasoconstrictor response to severe hemorrhagic hypotension in the chicken is apparently mediated primarily by an

increase in circulating catecholamines due to cerebral ischemia, rather than a baroreflex.

Finally, the change in Pp induced by either a bolus or continuous infusion of vasoactive substances into the extracorporeal perfusion circuit was monitored (n=28). Prostaglandin E_1 (0.5 ug, bolus) produced arteriolar vasodilatation lasting ten minutes, as indicated by a fall in Pp. Histamine (10 ug diphosphate, bolus) or adenosine (5 and 10 ug, bolus) produced vasodilatation of less than 2 min duration. Theophylline infusion (5 mM infused at 1 ml/min, ia) blocked the vasodilatory effect of adenosine. Norepinephrine (1 ug, bolus) produced vasoconstriction which was reduced 60% by systemic alpha-adrenergic blockade with phenoxybenzamine (7.5-10 mg/kg, iv). Tracheal occlusion produced intense vasoconstriction which was reduced 70% by alpha-adrenergic blockade. Electrical stimulation of the peripheral end of the cut sciatic nerve (6 Hz) produced an immediate vasodilatation lasting several minutes. These data indicate that the hindlimb vasculature of the chicken responds to vasoactive substances, exercise, and asphyxia in a manner similiar to mammals.

To Lael

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LIST OF ABBREVIATIONS

ACh	Acetylcholine chloride
ACTH	Adrenocorticotrophic hormone
ADO	Adenosine
ANOVA	Analysis-of-variance
CFC	Capillary filtration coefficient in ml/min/mm Hg/100 gm
CPp	Carotid perfusion pressure in mm Hg
DA	Dopamine
HCT	Hematocrit
HIST	Histamine diphosphate
HPp	Hindlimb perfusion pressure in mm Hg
HR	Heart rate in beats/min
hr	Hour
Hz	Hertz in cycles/sec
ia	Intra-arterial
iv	Intravenous
kg	Kilogram
MABP	Mean arterial blood pressure in mm Hg
MDF	Myocardial depressant factor
min	Minute
ml	Milliliter
msec	Millisecond
NE	Norepinephrine
Pa	Arterial blood pressure in mm Hg
PBZ	Phenoxybenzamine
Pc	Capillary hydrostatic pressure in mm Hg
Pc_i	Isogravimetric capillary pressure in mm Hg
PE	Polyethylene
PGA2	Thromboxane
PGE1	Prostaglandin E1
PGE2	Prostaglandin E2
PGI2	Prostacyclin
pH	Inverse log of hydrogen ion concentration
phentol	Phentolamine
pO_2	Oxygen partial pressure in mm Hg
Pp	Hindlimb perfusion pressure in mm Hg
PPC	Plasma protein concentration in gm%
PROP	Propranolol
sec	Second
SEM	Standard error of the mean
SER	Serotonin
TPR	Total peripheral resistance in p.r.u.
V	Volt
Vs	Ventricular stroke volume in ml/beat

INTRODUCTION

The term "shock" describes a condition in which the circulatory system fails to fulfill its basic function, i.e. to provide the various organs of the body with sufficient blood flow to meet their metabolic demands. This definition implies that shock may occur as a result of either an inappropriate cardiac output and/or peripheral resistance. The latter may be due to widespread peripheral vasodilatation (anaphylactic shock) or vasoconstriction (catecholamine shock) and the former may result from blood loss (hemorrhagic shock), pooling of the blood within the vasculature (septic shock) or from a defective myocardium (cardiogenic shock). Regardless of the etiology, if there is prolonged inadequate perfusion of the major organs of the body (brain, heart, splancnic bed and kidney) irreversible cellular damage occurs which eventually leads to the demise of the organism (irreversible shock).

The domestic chicken (Gallus domesticus), although intolerant to a large acute blood loss, can withstand a large slow removal of blood, and reportedly does not exhibit irreversible hemorrhagic shock. The ability of the chicken to tolerate a large slow bleed appears to be related to its ability to rapidly mobilize large volumes of extravascular fluids, whereas its inability to withstand a rapid large blood loss suggests a relatively inefficient sympathico-adrenal system. The latter might help to explain why the chicken does not enter into irreversible hemorrhagic shock, i.e. it is spared the deleterious effects of prolonged vasoconstriction.

Furthermore, most of the drugs and hormones that alter arterial blood

pressure in mammals have the same effect in avian species. Previous studies in chickens measured heart rate, arterial blood pressure, and cardiac output during drug administration and made assumptions concerning the peripheral vasculature. Likewise, the direct effect of exercise or asphyxia on the hindlimb vasculature has not been reported in the chicken.

This study is an attempt to elucidate some of the peculiarities of the response of the chicken to hemorrhage using research techniques and protocols which have been employed for years in mammalian research, but which have not been applied to fowl. These techniques are also utilized to determine the effect of asphyxia, exercise, and vasoactive agents on hindlimb vascular resistance in chickens.

LITERATURE REVIEW

I. Hemorrhage in Mammals

Hypovolemic hypotension in mammals sustained beyond a given duration of time results in myocardial and/or peripheral circulatory failure despite reinfusion of all shed, and even additional blood. Investigations involving the duration of time until the onset of irreversibility are of clinical importance. Certain sympathomimetic agents coupled with volume repletion and correction of the acid/base inbalance are the standard clinical treatment for hemorrhage (Carey, Lowery, and Cloutier, 1971). It is generally understood that primates withstand shock better, vasoconstrict less, and survive longer than dogs (Abel et al., 1967). Yet, primate tolerance to hemorrhage apparently does not compare to that of avian species where irreversible hemorrhagic shock may not occur. Furthermore, the rate of post-hemorrhagic fluid mobilization in avian species is much greater than that of mammals.

Shock is a condition where the circulatory system fails to provide the various organs of the body with sufficient blood flow to meet their metabolic demands. The cardiovascular response of mammals to hemorrhagic hypotension sustained by continuous bleeding is marked by two distinct phases; vascular compensation and vascular decompensation. The initial phase, i.e. compensated shock, involves activation of cardiovascular mechanisms which maintain the mean arterial blood pressure (MABP) in an attempt to maintain blood flow to high priority tissues like the heart and brain. The most prominent and immediate compensatory response is reflex activation of the autonomic nervous system (Chien, 1967; Djojosugito, Folkow and Kovach, 1969; Jacobson, 1968; Scott and Eyster,

1979; Shoemaker, 1964). It is this system which elevates vascular resistance, produces positive chronotropic and inotropic cardiac effects, and indirectly promotes the absorption of interstitial fluids (Djojosugito et al., 1969; Haddy, Scott and Molnar, 1965; Jacobson, 1968; Scott and Eyster, 1979; Shoemaker, 1964; Zweifach, 1974). During compensated hemorrhagic shock small volumes of blood must be continuously removed from the animal to prevent the MABP from rising above a given level of experimental hypotension. The importance of the autonomic system in hemorrhage is attested to by the fact that the ability of an organism to withstand a large acute blood loss is greatly dependent on the effiency of the sympathicoadrenal system (Chien, 1967; Shoemaker, 1964). However, prolonged activation of the latter system has been suggested as one factor that contributes to the phenomenon of "irreversible shock" (Irving, 1968; Zweifach and Fronek, 1975).

The compensatory mechanisms begin to fail following several hours of prolonged tissue hypoperfusion due to various metabolic, central nervous, cardiac, and microvascular alterations, and the animal is said to enter "decompensated shock". The MABP begins to wain due to myocardial depression and/or failure of the peripheral vasculature, and shed blood must be returned to the animal to maintain the MABP at a given level of experimental hypotension. The animal is considered to have entered a terminal condition termed "irreversible hemorrhagic shock" when approximately one-third of the shed volume has been returned to the animal. The return of all the shed blood, and even additional blood, at this time has only a transient and ever diminishing effect on the MABP. The MABP continues to fall until the animal lapses into peripheral circulatory collapse and death.

There are several reports supporting myocardial depression as the cause of decompensated shock. The prolonged intense vasoconstriction produces tissue ischemia and an acidosis which can depress the myocardial response to catecholamines (Darby et al., 1960). The myocardium can also be depressed by the hyperkalemia which results from cellular exchange of potassium ions for hydrogen ions, from inactivation of the electrogenic pump, by the increase in the plasma concentration of circulating gastrointestinal toxins due to the breakdown of the reticulo-endothelial system (Rothe and Selkurt, 1961), and by the release of a myocardial depressant factor (MDF) from the ischemic pancreas (Lefer and Martin, 1970). This combination of effects may produce the myocardial depression indicated by the rightward shift in the ventricular function curve in late shock (Crowell and Guyton, 1962). Other researchers have stated, conversely, that myocardial contractility is enhanced in late shock (Downing, Talner, and Gardner, 1965).

Other researchers suggest failure of the peripheral vasculature is the major cause of decompensated shock. When the MABP of the dog is maintained at 50 mm Hg, total peripheral resistance (TPR) (Rothe and Selkurt, 1964) or perfusion pressure in the constantly-perfused hindlimb (Bond et al., 1981) rises initially, then falls significantly within 2 hr. Inadequate cerebral perfusion can depress medullary vasomotor centers resulting in decreased sympathetic vascular tone, which again decreases venous return and cardiac output. The microcirculation may become damaged by the accumulation of vasoactive substances such as histamine (Galvin, Bunce and Reichard, 1977; Grega, Kinnard and Buckley, 1967), prostaglandins (Bond et al., 1981), bradykinin, and others. The blood tends to become hyperosmotic (Jarhult, 1975) and this can depress

vascular smooth muscle (Hollenberg and Nickerson, 1970). Cellular swelling and deformaties, and hemoconcentration may reduce the flow velocity resulting in intravascular thrombi causing the blood to demonstrate hypercoagulability (Hardaway et al., 1962; Shoemaker et al., 1961). Furthermore, terminal arterioles and precapillary sphincters may lose reactivity to constrictor stimuli (Rothe and Selkurt, 1961).

The increase in precapillary resistance immediately following blood loss is important, not only because it maintains the MABP, but also because it tends to shift the Starling equilibrium toward fluid absorption. This raises blood volume, and ultimately, the cardiac output. Interestingly, the plasma oncotic pressure of the chicken is only 11 mm Hg due to a high albumin/globulin ratio. Fluid mobilization after hemorrhage, primarily from the skeletal muscle interstitium, is thought to be brought about in most species by an increase in the pre/post-capillary resistance ratio which effectively lowers the capillary hydrostatic pressure (Pc) (Djojosugito et al., 1969; Hollenberg and Nickerson, 1970). This is attested to by the fact that the administration of an alpha-adrenergic blocking agent, which prevents the vasoconstriction, results in a reduced rate of post-hemorrhagic fluid mobilization (Grega et al., 1967; Hollenberg et al., 1970). Fluid mobilization is enhanced by the action of catecholamines on the liver and a decrease in pancreatic insulin secretion, both of which tend to cause an increase in the osmolarity of the plasma which, in turn, favors fluid absorption (Hinshaw, 1976; Strawitz et al., 1961; Jarhult, 1975). The reabsorptive process is self-limiting and forces favoring net reabsorption progressively diminish with prolonged hemorrhagic hypotension. The initial stage of fluid mobilization lasts several hours

and is capable of replacing about one-half of the shed blood.

The subsequent stage of fluid mobilization is slow, requiring 6 to 24 hr for complete blood volume restitution. Renal mechanisms are partially responsible via the renin-angiotensin-aldosterone mechanism. Interestingly, in the chicken angiotensin II produces hypotension followed by hypertension. The initial transient hypotension is thought to be due to vasopressin release, a vasodilator in birds (Moore, Strong, and Buckely, 1981a,b). In mammals, a cortisol mediated mechanism may also be necessary for complete blood volume restitution (Pirkle and Gann, 1975; Swingle and Swingle, 1965). Cortisol facilitates the transfer of cellular water to the interstitium and thereby promotes capillary absorption. It has been postulated that cortisol causes this cellular water loss by stimulating active transport of certain electrolytes, presumably sodium, from the cell.

II. Hemorrhage in Aves

The avian response to hemorrhage differs considerably from the mammalian response in three ways. First, avian species do not demonstrate a phase of vascular decompensation after prolonged hemmorhagic hypotension. Second, avian species have a greater rate of posthemorrhagic fluid mobilization than mammals. Finally, chickens apparently do not demonstrate a phase of shock irreversible to transfusion. Articles published concerning the avian response to hemorrhage are reviewed in the subsequent paragraphs.

The first four papers which examined the avian response to hemorrhage were published by the Scandanavian group of physiologists at the University of Goteborg, Sweden, in the late 1960's. The first

publication, which examined the vascular and hematologic response of the pigeon to hemorrhage (Kovach and Szasz, 1968), concluded that a large hemorrhage produces only a small fall in MABP and there is immediate, intense, and continuous hemodilution following hemorrhage with no terminal trend towards hemoconcentration.

The next publication (Kovach, Szasz and Pilmayer, 1969) examined the effect of graded hemorrhages, i.e. 1% of body weight blood removed/hr, on the mortality of various avian and mammalian species. The study found that flying (pigeons) and diving (ducks) birds survived much longer than mammals. In fact, 30% of the pigeons could survive the loss of 100% of its initial blood volume if bled over a 8 hr duration. Chickens were intermediate in their survival rates during graded blood loss.

The third paper (Kovach and Balint, 1969) compared the vascular and hemotologic response of the rat and pigeon to hemorrhage. Rapid hemodilution occurred in the pigeon following hemorrhage (48% fall in hematocrit (HCT), 74% fall in plasma protein concentration (PPC)) compared to the rat (18% fall in HCT, 12% fall in PPC). When 40% of the blood volume was removed in two successive 10 min hemorrhages, separated by 30 min, the MABP of the pigeon fell by only 30 mm Hg. The pigeon demonstrated a distinct pressor response due to cerebral ischemia when both carotid arteries were ligated and the MABP was reduced to 30 mm Hg by hemorrhage. A significant hyperkalemia occurred in both species. Whereas the hemodilution ceased rapidly in the rat (within 30 min), the process continued many hr in the pigeon.

The final paper examined the effect of hemorrhage on MABP, cardiac output, and capillary filtration coefficient (CFC) in the duck (Djojosugito et al., 1969). Mean arterial blood pressure was unchanged

by a hemorrhage of 25% of the blood volume. As in the pigeon, this maintainance of the MABP was due to a reflex increase in vascular resistance, i.e. TPR was elevated. It is well documented that the duck, being a natural diver, is capable of demonstrating intense peripheral vasoconstriction (particularly in the skeletal muscle vasculature). The rise in vascular resistance in the duck following hemorrhage was, at least in part, due to sympathetic nerves inasmuch as sciatic nerve block with lidocaine, or treatment with an alpha-adrenergic antagonist, would reduce the rate of fluid mobilization. The nerve block prevented the hemorrhage-induced rise in CFC, i.e. 0.05 to 0.13 ml/min/mm Hg/100 gm. Knowing the isovolumetric capillary pressure, the investigators calculated that this rise in the pre/post-capillary resistance ratio lowered Pc by 4 mm Hg and the nerve block reduced the fall in Pc by 70-75%. The remaining fluid absorption was due simply to a lowering of the MABP which was transmitted to the capillaries. The vascular resistance remained elevated even though the limb became isovolumetric, i.e. it stopped filtering.

Djojosugito et al. (1969) offered two reasons for the rapid rate of post hemorrhagic fluid mobilization in the duck. First, the intense reflex increase in precapillary resistance lowered the mean Pc sufficiently to shift the Starling equilibrium toward fluid absorption. Secondly, the capillary surface area that is available for fluid absorption is reportedly thrice (Folkow et al., 1966) that of the cat (Kjellmer, 1965) and, hence, identical changes in Pc would result in correspondingly greater changes in the rate of blood volume restitution. Folkow et al. (1966) did not state how capillary surface area was extracted from the CFC. Up to this point in time, most avian hemorrhagic

research had used flying and diving species and little had been learned about the responses of the chicken.

Wyse and Nickerson (1971) examined the effect of prolonged hemorrhagic hypotension on chickens using the standardized hemorrhagic protocol of holding the MABP at 50 mm Hg by continuous small hemorrhages. Plasma volume (via radio-iodinated serum albumin), PPC, HCT, and hemoglobin were determined and vital signs were monitored during a 5 hr hypotensive interval. The results were similiar in some respects to those of flying or diving birds and were considerably different in other respects. The chicken, like the pigeon and duck, demonstrated a high rate of fluid mobilization, i.e. twice that reported for mammals (Hollenberg and Nickerson, 1970). The chickens did not demonstrate a phase of shock irreversible to transfusion following reinfusion of the shed blood after 4 - 5 hr of hypotension. The chickens would survive after being reinfused at the onset of circulatory collapse, as indicated by a sudden fall in the MABP. Unlike the duck or pigeon, the chicken was very sensitive to small hemorrhages. A 4 ml/kg blood loss produced a 20 mm Hg fall in MABP, and only a 25% reduction in blood volume was required to drop the MABP to 50 mm Hg. This may suggest a reduced capacity for sympathetic activation in this species. Folkow et al. (1966) has demonstrated more dense adrenergic innervation in the adventitia of the duck femoral arterial vasculature than that occurring in the turkey or cat. Recent research has also indicated that reactivity of mesenteric and skeletal muscle vasculature of ducklings to exogenous norepinephrine or electrical stimulation is considerably greater than in chicks (Gooden, 1978).

Recent research has indicated that following hemorrhage in the hen

the intraerythrocytic concentration of 1,3,4,5,6 myoinsitol pentophosphate concentratioon increased, shifting the oxygen dissociation curve far right and enhanced oxygen delivery to the tissues (Jones, Smith and Board, 1978). This is different from mammals, where the main controller of oxygen transport is intracellular concentrations of 2,3 diphosphoglycerate. These researchers also found the rate of erythrogenesis in the hen was comparable to that of the dog.

Further research concerning the response of the chicken to systemic hypotension was given by Ploucha (1979) and Ploucha, Scott and Ringer (1981). In these studies, chickens were held at a MABP of 50 mm Hg for 225 min by continuous small bleedings while various hemodynamic and hematological parameters were measured. A hemorrhage of approximately 25% of the initial blood volume would reduce the MABP to 50 mm Hg (similiar to Wyse and Nickerson, 1971), and this was followed by intense, immediate, and continuing hemodilution with no terminal trend toward hemoconcentration. A progressive hyperkalemia and hyperglycemia occurred. The chickens were not acidotic after 4 hr of sustained hemorrhagic hypotension. Interestingly, cardiac output (measured by dye dilution) indicated that TPR fell with hemorrhage.

It is possible that the chicken is sensitive to a small blood loss due to a lack of peripheral baroreceptor activity. Although avian researchers have recorded afferent impulse traffic along the cardiac depressor nerve of ducks (Jones and West, 1978) and chickens (Estravillo and Burger, 1974a,b) which correspond with arterial systole, little physiological evidence exists demonstrating the existance of functional peripheral-vascular baroreceptors in the chicken. Harvey et al. (1954) reported that during the course of pharmacological studies in the chicken

11 birds were used before one would demonstrate a rise in MABP greater than 10 mm Hg in response to bilateral carotid occlusion in the cervical area, a response readily seen in mammalian studies. Durfee (1964) also found no pressure reflexogenic areas in association with the carotid arteries in the chicken. McGinnis (1964) and McGinnis and Ringer (1965, 1967) found that bilateral occlusion of the carotid and vertebral arteries of the chicken did not produce a baroreceptor-induced rise in MABP. The chicken did demonstrate a general cerebral ischemic response when cerebral perfusion pressure, measured through a carotid cannula introduced in a cranial direction, fell to about 26 mm Hg following bilateral carotid and vertebral artery occlusion (McGinnis, 1964).

Two recent morphological reports have been published dealing with birds which site the existence of possible cardiac stretch receptors in the conducting system of the avian heart (Bogusch, 1974a,b) and possible baroreceptor-like endings exist in the subendocardium of the pigeon (Mather and Mather, 1974). Other researchers have suggested that circulating adrenal-medullary hormones, rather than sympathetic vascular innervation, play a major role in regulating cardiac performance and blood pressure in the chicken (Karg and Scrams, 1966; DeSantis et al., 1975). The simple stress of being hand-held will double plasma corticosterone concentration of a chicken, presumably due to the release of adrenocorticotrophic hormone (ACTH) (Beuving and Vonder, 1978). Interestingly, in chickens ACTH stimulates not only adrenal cortical tissue, but adrenal mudullary tissue as well (Newcomer, Gephardt, and Hurst, 1972).

III. Vasoactive Agents in Aves

Most of the drugs and hormones that alter blood pressure in mammals are reported to have the same effect in avian species. The effect of various sympathomimetics (Akers and Peiss, 1963; Szeto et al., 1977), parasympathomimetics (Peterson and Ringer, 1968; Rodbard and Fink, 1948), histamine (Natoff and Lockett, 1957; El Ackad, 1972; Knight and McGreggor, 1974), angiotensin (Moore, Strong and Buckley, 1981a,b) and prostaglandins (Horton, 1971; Bult et al., 1981) have been examined in chickens. The direct action of the drugs in these studies is difficult to ascertain since they all employed intravenous drug administration.

Recent research has indicated that PGE_1 and PGE_2 strongly inhibit thrombocyte aggregation in whole chicken blood. Prostacyclin (PGI_2) does not exhibit anti-aggregratory activity in chicken blood, nor is it produced by aortic tissue (Claeys et al., 1981a; Bult et al., 1981). Furthermore, the metabolism of arachadonic acid in chicken aortic tissue is geared mainly toward the formation of PGE_2 and, in contrast to mammals, virtually no prostacyclin synthetase is present. However, the capacity of chicken thrombocytes to generate thromboxane (PGA_2) is similiar to that observed for mammalian platelets (Claeys et al., 1981b). In birds, it appears that PGE_2, not PGI_2, is the antithrombotic factor. Since E type prostaglandins are formed in the chicken vascular tissue, a possible role of PGE_1 or PGE_2 in avian hemostasis and/or in the developement of spontaneous avian atherosclerotic vascular lesions deserves further investigation.

Knight and McGreggor (1974) and McGreggor (1979) pump perfused the amputated feet of chickens and ducks with a Krebs solution and monitored perfusion pressure upon intra-arterial (ia) administration of vasoactive

drugs and exercise. They reported that there is a noncholinergic nonadrenergic vasodilator released in the feet of the bird. They were perfusing amputated feet, which consist mostly of bone, skin, and connective tissue, with very little skeletal muscle.

IV. Vascular Effect of Asphyxia in Aves

The mammalian response to asphyxia is intense vasoconstriction (Weissman, Sonnenschein and Rubinstein, 1978). Asphyxia in the chicken, induced by tracheal occlusion, produces hypertension subsequent to a transient period of hypotension (Harvey et al., 1954; Richards and Sykes, 1967). The initial hypotension is likely due to the local effect of systemic hypoxia, since hypoxia has been shown to produce a 35% fall in TPR and systemic hypotension in chickens (Besche and Kadono, 1978). Asphyxia, induced by submersion, is poorly tolerated by the chicken compared to a natural avian diver like the duck (Bond, Douglas and Gilbert, 1961). Furthermore, the chicken myocardium is depressed more by hypoxia than the duck heart (Sturkie and Abati, 1978) and levels of reduced nicotinamide-adenine dinucleotide accumulate much more quickly in the chicken brain during asphyxia than in the duck (Jones and West, 1978).

V. Vascular Effect of Exercise in the Chicken

Skeletal muscle activity produces a local vasodilatation, i.e. active hyperemia, in mammalian skeletal muscle vasculature. Perfusion pressure in the constantly-perfused canine hindlimb will fall with the onset of muscular activity and will remain lowered for several min following cessation of muscular activity (Tabaie, Scott and Haddy, 1977).

This is a local phenomenon which may be mediated by the release of local vasodilator metabolites, including potassium ion, hydrogen ion, adenosine-diphosphate, adenosine-triphosphate, blood gas tension, adenosine, and/or others (Haddy and Scott, 1968). This local vasodilatation in mammals will overide a neurogenic (remote) vasoconstriction induced by hemorrhage (Kjellmer, 1965). Active hyperemia in the duck will not "break through" an intense hemorrhage-induced neurogenic vasoconstriction (Folkow et al., 1966). The effect of exercise on blood flow through the skeletal muscle vasculature of the chicken has not been investigated. However, ischemic (reactive) hyperemia has been demonstrated in the skeletelal muscle vasculature of the chicken (Klabunde and Johnson, 1977) and an active hyperemia occurs in the duck, turkey (Folkow et al., 1966), and amputated Krebs-perfused chicken foot (McGreggor, 1979).

OBJECTIVES

1. To determine the effect of various degrees of hemorrhagic hypotension on hematologic and hemodynamic parameters in the chicken, particularly in regard to activation of the sympathico-adrenal axis.

2. To determine the response of the skeletal muscle vasculature to vasoactive agents, asphyxia, and exercise.

MATERIALS AND METHODS

I. VASCULAR RESPONSE TO HEMORRHAGE TO MEAN ARTERIAL BLOOD PRESSURE OF 50 mm Hg

Unless otherwise stated, the MABP was measured in all chickens (1.5 - 3.9 kg) used in this study via a cannula inserted into a brachial or carotid artery connected to a Statham (PA-23AC) pressure transducer and either a Grass (7A) or Hewlett-Packard (956-100W) polygraph. Various breeds were used. All animals were tracheotomized and heparinized systemically (390 IU/kg, iv) and, unless otherwise indicated, were anesthetized with sodium pentobarbital (25 mg/kg, iv). The animals were artificially ventilated at a tidal volume of 35 cc and a rate of 25 strokes per min (Harvard small animal respirator). Unless otherwise indicated, all statistical analysis was via a one-way analysis-of-variance (ANOVA) with a Dunnett test, a $P<0.05$ was considered significant. All values are given as mean $\pm$SEM.

A. Plasma Osmolality and Protein Concentrations

This experiment investigated the effect of hemorrhage on plasma osmolality and total protein in 12 phenobarbital anesthetized male birds (1.91 $\pm$0.08 kg) bled via a cannula (PE-90) in the right ischiadic artery. The MABP was held at 50 mm Hg for 150 min by continuous small bleedings, after this time the shed blood (41^{o}C) was returned to the animal via an ischiadic vein. The birds were monitored for 1 hr after reinfusion. Plasma osmolality (Advanced instruments) and plasma protein concentration (PPC) (Acustat, Clay Adams) were measured initially, every 30 min after hemorrhage, and then 30 min after reinfusion of shed blood.

B. Thermodilution Studies During Selective Autonomic Blockade

This experiment used birds anesthetized with sodium pentobarbital (25 mg/kg, iv) and cardiac output was determined by the thermodilution technique during hemorrhagic hypotension. This cardiac output was determined by advancing a pediatric (4 French) Swan-Ganz catheter down the right jugular vein such that the thermistor was either in or near the pulmonary outflow tract. The position of the catheter was determined at autopsy. A 1 ml bolus of cold saline (0.1^{o}C) was rapidly injected into the vena cava through the catheter side port. The resultant temperature deflection curve (sensed by a catheter-tip thermistor) was indicated on an analog meter and integrated by a Cardiotherm-500 computer (Columbus Instruments) giving a digital display of cardiac output. A minimum of four cardiac output determinations were obtained at each sampling time. Cardiac output was converted to cardiac index by dividing cardiac output by the body weight in kg raised to the 0.734 power (Speckmann and Ringer, 1963).

Twenty-six (3.22 $\pm$0.06) male chickens were divided into three unequal groups. Eight were untreated, eight received the alpha-adrenergic antagonist, phenoxybenzamine (PBZ, 5 mg/kg, iv), and ten received the beta-adrenergic antagonist, propranolol (PROP, 0.25 mg/kg iv bolus followed by 5 ug/kg/min infusion into the brachial vein). The cardiac output was determined initially and the blocking agents were then administered. Cardiac output was determined 30 min later. The animal was then immediately hemorrhaged to a MABP of 50 mm Hg and was held at that level of hypotension by continuous small bleedings. Cardiac output was then determined at 5, 30, 60, 90, and 120 min after the onset of hemorrhage.

C. Hindlimb Perfusion Studies

The blood supply to the gastrocnemius muscle was isolated in male chickens anesthetized with sodium phenobarbital or sodium pentobarbital and the muscle was pump perfused (Masterflex pump) with arterial blood at a constant flow (Figure 1). Since flow was held constant, the perfusion pressure will vary directly with vascular resistance. Hence, these experiments permitted the direct determination of the response of the skeletal muscle vasculature.

Vascular isolation of this muscle was accomplished by ligating the external iliac artery and placing a tourniquet above the tibial-metatarsus junction. The muscle was then pump perfused via the ischiadic artery. The tourniquet prevented blood from flowing to the lower leg and toes through the anterior tibialis artery, thus shunting the perfusing blood to the femoral caudalis artery and medial and posterior tibialis arteries. The medial tibialis artery supplies the gastrocnemius muscle and skin (Koch, 1973; Nishida, 1963; Westpfahl, 1961). The posterior tibialis artery supplies the gastrocnemius muscle and also sends poorly developed branches to the flexor perforans muscles and skin. The caudal femoralis artery supplies blood primarily to skin. Thus, this is not an entirely isolated muscle preparation but the amount of skin perfused relative to muscle is small.

To perfuse the muscle, a polyethylene cannula (PE-160) was inserted midthigh in a cranial direction several centimeters into the left ischiadic artery. Blood was then shunted to the perfusion pump and returned to the same artery through a cannula (PE-160) inserted caudally. The perfusion pressure (Pp) was measured in the pump outflow line with a Statham pressure transducer (PA-23AC). Blood flow was adjusted to

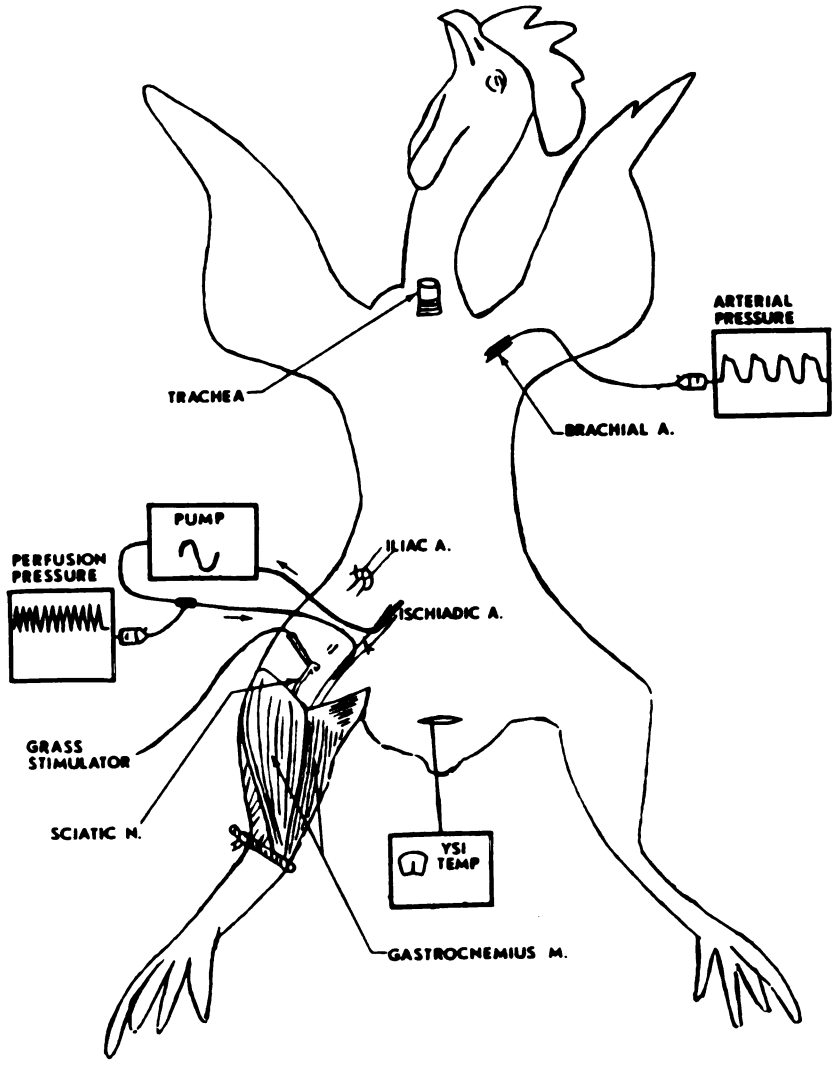


Figure 1. Diagramatic illustration of the isolated hindlimb perfusion technique.

produce a control Pp approximately equal to systemic pressure and then flow was held constant throughout the experiment. The pressure waveform generated by the perfusion pump had a pulse pressure similiar to that of the bird, while the pump rate was somewhat less than the bird (see Figures 5,6,9).

Prior to hemorrhage, steps were taken to assure that the perfused muscle was a valid assay organ. First, the perfusion pump was turned off and a Pp less than 20 mm Hg indicated adequate vascular isolation. A Pp greater that this with the pump off would indicate collateral circulation to the leg. Next, 1.0 ug acetylcholine (ACh) and then 1.0 ug norepinephrine (NE) were injected into the extracorporal perfusion line prior to the pump to determine if the vascular bed would respond.

Statistical analysis in study IC was via a Students t test, and a $P<0.05$ was considered significant.

1. Acute Bleed - Phenobarbital Anesthesia

Six male chickens (2.50 $\pm$0.17 kg) were anesthetized with phenobarbital and blood was removed rapidly (2 min) from the right ischiadic artery until the peak systolic arterial pressure fell to approximately 50 mm Hg. The Pp was monitored during a 15 min hypovolemic interval after which the shed blood was reinfused and the bird monitored for 30 min. The experiment was then repeated yielding two sets of data for each animal. Flow averaged 8.3 $\pm$0.9 ml/min/100 gm leg weight.

2. Chronic Bleed - Phenobarbital Anesthesia

Nine male chickens (2.80 $\pm$0.12 kg) were anesthetized with phenobarbital (100 mg/kg), bled at a rate of 2 ml/kg/min to a MABP of 50

mm Hg, and maintained at that level of hypotension for 60 min by subequent bleeding. The hindlimb Pp was continuously monitored.

3. Acute Bleed - Pentobarbital Anesthesia

Nine male chickens (2.80 ±0.14 kg) were anesthetized with sodium pentobarbital (25 mg/kg) and then blood was removed rapidly (2 min) from the right ischiadic artery until systolic pressure fell to approximately 50 mm Hg. Hindlimb Pp was monitored before and during a 10 to 15 min hypovolemic interval after which the shed blood was returned to the animal. This series of experiments was designed to determine if phenobarbital influenced the responses seen under phenobarbital anesthesia. Pentobarbital was selected because perfusion studies have demonstrated that this agent does not preclude vascular constriction in the dog skeletal muscle during hemorrhage (Haddy et al., 1965).

4. Isogravimetric Hindlimb Studies - Pentobarbital Anesthesia

The effect of hemorrhagic hypotension on capillary filtration coefficient (CFC) was determined in three experiments using 18 male chickens (2.65 ±0.05 kg). A schematic illustration of the experimental preparation is shown in Figure 2. The birds were anesthetized with pentobarbital (25 mg/kg), tracheotomized, and artificially ventilated (Harvard small animal respirator). Body temperature was maintained at 41°C with a heating pad placed under the animal. Arterial blood pressure was monitored via a cannula inserted into a carotid artery attached to a Statham transducer (PA-23AC) and a Grass 7A polygraph. A cannula (PE-160) was inserted into the left ischiadic artery in a cranial direction for hemorrhaging the animal into an elevated and pressurized

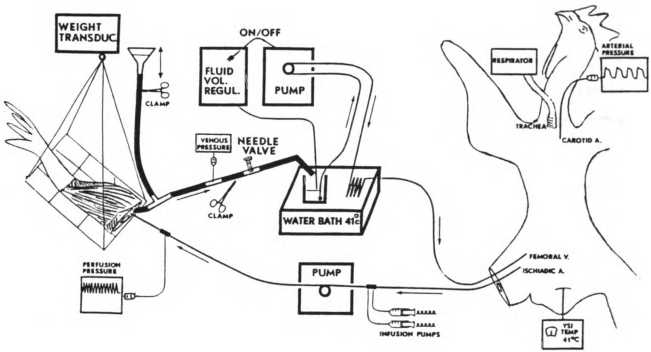


Figure 2. Diagramatic illustration of the isogravimetric isolated hindlimb perfusion technique.

glass reservior.

Skin was removed from the leg below the thigh by electrocautery. Three heavy string tourniquets were applied to the mid-thigh musculature and the muscle was then severed by electrocautery proximal to the tourniquets. The first tourniquet bound the gracillis, adductor, and quadricepts femoris muscles. The second tourniquet bound the semimembranosus and semitendonosis muscles. The third tourniquet bound the sartorium with glutaeus superficial muscle, and the tensor fasciae latae with the glutaeus superficial and bicepts femoris muscles. The animals were then heparinized systemically (390 IU/kg).

The limb was pump perfused (Masterflex pump) at constant flow via the ischiadic artery with autologous blood drawn from the same artery. The limb venous outflow was directed via a cannula (PE-240) through a 1/4 inch needle valve to a reservoir (40 ml beaker) in a 41^{o}C water bath. Venous pressure was measured in this cannula immediately in front of the needle valve via a Statham transducer (PA-23BC). The reservoir volume was held constant at approximately 20 ml by a fluid volume regulator which activated the venous return pump (Holter roller pump). Blood in the reservoir was then returned to the animal through a long cannula which was coiled in the water bath before entering the ischiadic vein. The femur was severed mid-thigh and sealed with bone wax, the limb was placed on a wire mesh, and suspended from a sensitive strain gauge (Unimeasure/80 force displacement transducer). The sensitivity of the strain gauge was adjusted so that a 2 gm weight on the grid would produce a 20 mm pen deflection on the recording paper.

Capillary filtration coefficient was calculated as filtration rate, i.e. the rate of gain in leg weight with a given rise in venous pressure

in the leg (in gm/min/100 gm), divided by the change in venous pressure (in mm Hg). Isogravimetric capillary pressure (Pc_i) was estimated by the stop flow technique described by Johnson (1965). This technique involves shutting off the perfusion pump, clamping venous outflow, and then elevating venous outflow pressure (by raising and lowering a static fluid column connected to the venous outflow cannula) such that the limb remains isogravimetric. The venous pressure and Pp would then equilibrate at the Pc_i.

Eighteen animals were divided into three equal groups. In all three groups, CFC was determined at 5, 15, 30, 40, 50, and 60 min. The Pc_i was determined immediately after the 5, 30, and 50 min CFC determinations in all groups. Group 1 was a control (non-hemorrhaged, nerve cut) group to determine the effect of time on CFC and Pc_i. The animals in groups 2 and 3 were hemorrhaged to a MABP of 50 mmHg immediately after the 15 min CFC determination and were maintained at that level of hypotension by continuous small hemorrhages from the contralateral ischiadic artery. The sciatic nerve trunk was cut initially in groups 1 and 2, whereas the nerve remained intact in group 3 by suspending the limb in close juxtaposition to the body of the animal.

II. VASCULAR RESPONSE TO HEMORRHAGE TO A MEAN ARTERIAL BLOOD PRESSURE OF 25 mm Hg

A. Hindlimb Perfusion Studies

Four experimental series were performed using 26 chickens of both sexes. The chickens were anesthetized with sodium pentobarbital (25 mg/kg, iv), tracheotomized, artificially ventilated, and heparinized systemically (390 IU/kg). Arterial blood pressure was monitored from a

cannula inserted into a carotid or brachial artery. Body temperature was maintained at 41°C by a heating pad under the animal. The right ischiadic artery was cannulated for hemorrhaging and reinfusing the animals. In some animals, the sciatic nerve of the perfused limb or the vagi were isolated and looped with suture for subsequent sectioning. In study IIA, the blood supply to the left leg was isolated, and the limb was pump perfused as described previously. All statistical analysis in study IIA was by a Students t test, and a $P<0.05$ was considered significant.

1. Effect of Sciatic Nerve Severance and Alpha-Adrenergic Blockade

Six female chickens (1.84 ±0.07 kg) were hemorrhaged to a MABP of 50 mm Hg and the Pp was recorded. The MABP was held at 50 mm Hg by continuous small bleedings. When all values had stabilized the animal was further hemorrhaged to a MABP of 25 mm Hg and was maintained at that level of hypotension by subsequent small bleedings and the Pp was again recorded. When the Pp stabilized the sciatic nerve trunk was severed and the Pp was monitored. Next, the alpha-adrenergic antagonist phentolamine was infused into the perfusion line prior to the perfusion pump (50 ug/min). The Pp was monitored until it stabilized at which time the phentolamine infusion was discontinued and the shed blood was returned to the animal.

2. Effect of Bilateral Vagotomy

Seven female chickens (1.82 ±0.07 kg) were hemorrhaged to a MABP of 50 mm Hg and then were further hemorrhaged to a MABP of 25 mm Hg while Pp was monitored. The shed blood was returned, a bilateral cervical

vagotomy was performed and the animals were again hemorrhaged to a MABP of 25 mm Hg while the Pp was recorded.

3. Head Perfusion Studies

Eight male chickens (2.33 ±0.06 kg) were hemorrhaged and the hindlimb Pp was monitored as in the previous studies. However, in this study, the head of the bird was also pump (Sigma motor pump) perfused. This was done by pumping blood from the contralateral ischiadic artery through a bifurcated cannula (PE-90) inserted in a cranial direction into both carotid arteries. The carotid perfusion pressure was monitored in the pump outflow line. Mean arterial blood pressure was monitored from a brachial artery and the animal was hemorrhaged from a cannula inserted caudally into a carotid artery. The shed blood was reinfused through a cannula inserted caudally into an ischiadic artery. The carotid perfusion pump was turned off and the animal was hemorrhaged to a MABP of about 35 mm Hg and was held at that pressure several minutes by continuous small bleedings. The shed blood was then reinfused, the carotid perfusion pump was turned back on, and the animal was allowed to stabilize. The animal was then again hemorrhaged to the same MABP as in the initial hemorrhage while the carotid perfusion pump continued to perfuse the head. The carotid perfusion pressure and hindlimb Pp were monitored during both hemorrhages.

B. Effect on Concentration of Serotonin, Dopamine, and Norepinephrine in Plasma

Five male chickens (1.96 ±0.14 kg) were held at a MABP of 50 mm Hg for 30 min and then at a MABP of 25 mm Hg for 30 min by continuous small

bleedings. Arterial blood samples (1 ml) were drawn prior to hemorrhage, at 5, 15, and 30 min of each level of hypotension, and at 10 min following reinfusion of the bled volume. The blood was centrifuged and the plasma was frozen for subsequent analysis for serotonin, dopamine, and norepinephrine concentrations by a modification of the method of Jacobowitz and Richardson (1978). Instead of brain tissue and an amount of 0.01N HCl which is dependent on tissue weight being added to 5 ml of butanol in the first step of the assay, 0.5 ml plasma and 0.5 ml 0.01N HCl were added to 5 ml butanol. The rest of the assay was performed according to Jacobowitz and Richardson (1978). The changes in plasma hormone concentration over time were compared with the initial value by a one-way ANOVA with a Student-Newman-Keuls test.

III. HINDLIMB VASCULAR RESPONSE TO VASOACTIVE AGENTS, ASPHYXIA, AND EXERCISE

A. Log Dose-response Curves for Vasoactive Agents

In eight male chickens (2.51 $\pm$0.11 kg) the perfusion pump flow rate was adjusted to produce a Pp of 125 mm Hg and flow was maintained constant for the duration of the experiment. Acetylcholine (ACh, 50 ug/ml), adenosine (ADO, 500 ug/ml), histamine diphosphate (HIST, 55 ug/ml), prostaglandin E_1 (PGE_1, 5 ug/ml), and norepinephrine (NE, 10 and 100 ug/ml) were individually infused into the extracorporeal perfusion circuit prior to the perfusion pump by a infusion/withdrawal pump (Harvard Model 950). The infusion rate of each drug was incrementally increased starting from a subminimal rate until a further increase in infusion rate failed to produce a change in Pp. This technique produced 5 - 10 points per dose-response curve per animal. The saline vehicle was

also infused so that Pp could be corrected for dilution effects. The effective blood concentration of each agent could be calculated knowing the infusion rate, the concentration of the infusate, and the limb blood flow. Log dose-response curves were drawn from these data.

B. Response to Bolus Administration of Vasoactive Agents; Blockade of Adenosine with Theophylline; the Response to Asphyxia

In nine male chickens (2.80 ±0.14 kg), vasopressor and depressor agents were delivered in a 0.1 ml saline vehicle via bolus administration into the extracorporal muscle perfusion circuit prior to the pump. A control 0.1 ml bolus of physiological saline was administered at the start of each experiment. The Pp was monitored following each drug injection until it returned to the control level at which time the next drug was administered. The maximal change in Pp, the duration of change, and the integral of the curve were recorded. The curves were integrated with a digitizer. The drugs tested were ACh (1 ug), ADO (5 and 10 ug), PGE_1 (0.5 ug), and NE (1 ug). Five of these animals also received HIST (10 ug). The effect of two blocking agents was then determined. Adenosine (5 and 10 ug, bolus) was readministered during a local ia infusion of theophylline (5 mM infused at 1 ml/min), a competitive inhibitor of adenosine (Bunger, Haddy, and Gerlach, 1975). The effect of saline vehicle infusion was also determined.

In 11 additional animals (2.84 ±0.06 kg), NE (1 and 5 ug, bolus) was administered before and 20 min after systemic alpha-adrenergic blockade with phenoxybenzamine (PBZ, 7.5 - 10 mg/kg, iv). Significance of the differences between means during the control and experimental periods was evaluated by paired Students t test, and a $P < 0.05$ was considered

significant.

C. Effect of Alpha-adrenergic Blockade on the Vascular Response to Norepinephrine and Asphyxia; the Effect of Exercise

The effect of asphyxia induced by tracheal occlusion was examined in the above mentioned 11 birds before and after PBZ administration. Finally, the effects of exercise and asphyxia were examined. Exercise was produced in the perfused leg in 5 of the 11 animals by electrical stimulation of the peripheral end of the cut sciatic nerve (1.6 msec duration, 6V, and 6 Hz for 15 sec). The maximal change in Pp, the duration of change, and the integral (via digitization) of the curves were recorded. Significance of the differences between means during the control and experimental periods was evaluated by paired Students t test, and a $P < 0.05$ was considered significant.

RESULTS

I. VASCULAR RESPONSE TO HEMORRHAGE TO MEAN ARTERIAL BLOOD PRESSURE OF 50 mm Hg

A. Plasma Osmolality and Protein Concentration

The effects of hemorrhage on plasma osmolality and plasma protein concentration (PPC) are shown in Figure 3. While there was a tendency for osmolality to increase during hemorrhage, osmolality never was significantly elevated above the prehemorrhage value. Of 11 birds studied, osmolality rose in 9, remained unchanged in one, and fell in one. The PPC was determined in seven of the 11 roosters. There was a significant fall in PPC within 30 min of hemorrhage and PPC fell to 54% of the initial value after 150 min.

B. Thermodilution Studies During Selective Autonomic Blockade

The effects of acute blood loss sufficient to reduce and maintain MABP at approximately 50 mm Hg for 120 min on cardiac index, stroke volume (Vs), heart rate (HR), and TPR are presented in Figure 4. Cardiac index in the untreated (control) birds was significantly reduced only at 5 and 120 min of hemorrhage and TPR was reduced only at the 30 min of hemorrhage. Heart rate was not affected by hemorrhage in the control group. Propranolol (PROP), *per se*, significantly reduced MABP from 156 $\pm$9 to 130 $\pm$9 mm Hg, HR from 294 $\pm$21 to 201 $\pm$23 beats/min, and cardiac index from 297 $\pm$34 to 177 $\pm$20 ml/min/kg$^{0.734}$ ($P<0.05$, analysis by a paired Students t test). Total peripheral resistance and Vs were not altered by PROP. During hemorrhage the only cardiovascular parameter that changed in the PROP group was TPR, which was significantly reduced

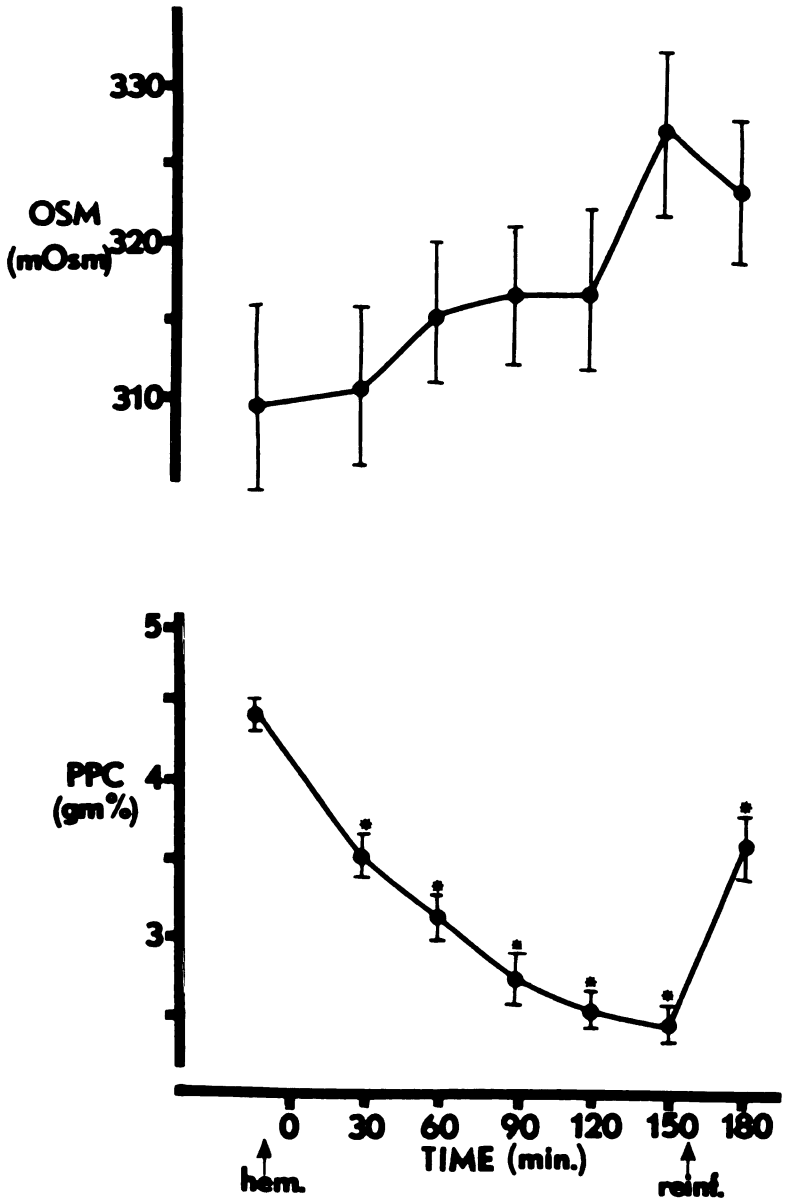


Figure 3. The effect of hemorrhage on plasma osmolality (OSM) (N=11) and plasma protein (PPC) (N=7) concentrations in male chickens. hem: mean arterial blood pressure reduced to, and sustained at, 50 mm Hg. reinf: reinfusion of the shed blood. * indicates significant change from initial value ($P<0.05$).

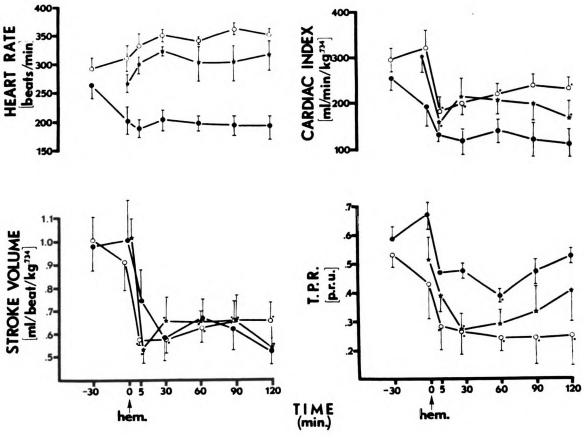


Figure 4. The effect of hemorrhage on heart rate, cardiac index, stroke volume, and total peripheral resistance in untreated, n=5 (★ ---- ★), alpha-blocked, n=6 (O ---- O), and beta-blocked, N=5 (● ---- ●), male chickens. Pentobarbital anesthesia. The drugs were administered 30 min prior to hemorrhage and the first cardiac output determination was taken immediately prior to drug administration. hem: mean arterial blood pressure reduced to, and sustained at, 50 mm Hg. Small star designates significant change from the value at time = 0 ($P<0.05$).

at 60 min of hemorrhage (Figure 4) (statistics via one-way ANOVA with a Dunnett test). However, of the five PROP-treated chickens in which results are shown in Figure 4 cardiac index and Vs were reduced below the 0-time value at 5 and 30 min of hemorrhage and both were reduced in four of the five birds throughout the hemorrhagic period. Phenoxybenzamine (PBZ), per se, did not affect any measured or calculated parameter, however there was a tendency for the MABP to fall. The birds pretreated with PBZ responded to hemorrhage similiarly to the untreated birds except TPR remained significantly reduced throughout the hemorrhagic period (Figure 4). The results shown in Figure 4 are only from birds that survived the entire experiment. This study concurs with the results of Ploucha (1979) which showed that TPR in the chicken is unaffected by hemorrhage.

C. Hindlimb Perfusion Studies

1. Acute Bleed - Phenobarbital Anesthesia

In six male chickens (total of 12 separate hemorrhages) Pp averaged 146 ±4 mm Hg initially, 159 ±7 mm Hg at 5 min, 147 ±7 mm Hg at 10 min, and 148 ±5 mm Hg at 15 min of hemorrhage. None of the pressures during hemorrhage were significantly different from the prehemorrhage value. Corresponding values for MABP were 113 ±6 (initially), 38 ±4 (5 min), 55 ±4 (10 min), and 64 ±4 mm Hg (15 min). Typical vascular responses of the in situ constantly-perfused chicken hindlimb to acute volume depletion are shown in Figure 5. Panel A of Figure 5 is a tracing from a chicken in which the estimated blood volume was rapidly reduced by 26%. This was associated with an immediate drop in arterial blood pressure (Pa in Figure 5) from 149 mm Hg to about 50 mm Hg. Over the subsequent 15 min,

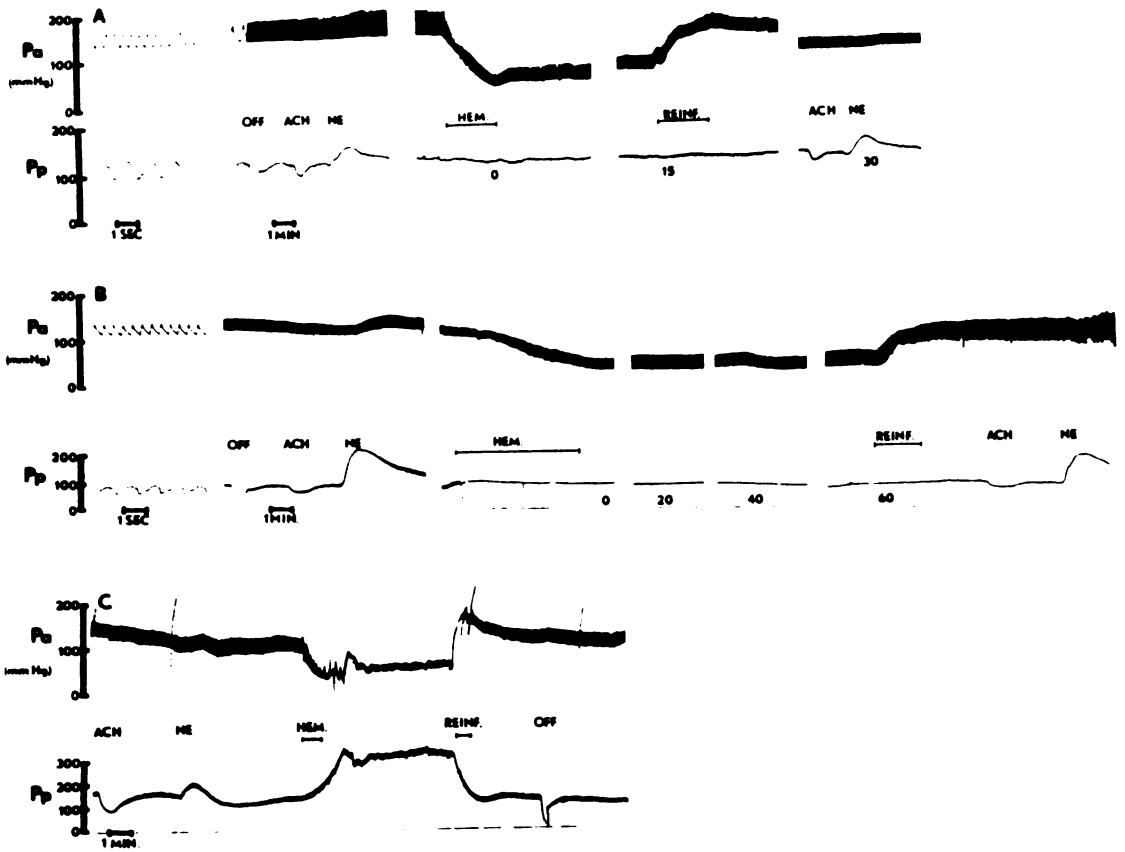


Figure 5. Tracing of the effect of hemorrhage on arterial blood pressure (Pa) and perfusion pressure (Pp) in the constantly-perfused hindlimb of a male chicken (panels A and B) and the constantly-perfused breast muscle of a female mallard (panel C). Phenobarbital anesthesia. Panel A shows the effect of a rapid hemorrhage and reinfusion after a 15 min hypovolemic interval (NE=1.25 ug norepinephrine, ACH=1.0 ug acetylcholine, OFF=perfusion pump off). Panel B shows the effects of sustained hemorrhagic hypotension (MABP = 50 mm Hg) for one hour followed by reinfusion. Numbers indicate minutes after hemorrhage. Panel C shows the effect of vasoactive agents (NE=0.25 ug) and rapid hemorrhage on Pa and Pp in the mallard.

arterial pressure rose by approximately 25 mm Hg and returned to near the prehemorrhage level after reinfusion of the shed blood. In contrast, Pp did not change during the entire period of hypotension nor was it affected by reinfusion of the bled volume. The hindlimb vasculature responded to NE and ACh before and after hemorrhage and Pp fell to approximately 10 mm Hg when the perfusion pump was turned off. The latter indicates adequate vascular isolation, i.e. no collateral circulation to the assay limb. Reactive dilatation, as indicated by the slow return of Pp on restarting flow, was seen in the majority of experiments. These results demonstrate that an acute hemorrhage to a MABP of 50 mm Hg in the phenobarbitalized chicken does not produce a rise in hindlimb vascular resistance.

2. Chronic Bleed - Phenobarbital Anesthesia

In this series, consisting of nine male chickens, the initial Pp was 114 $\pm$4 mm Hg and it reached a maximal value of 121 $\pm$6 mm Hg (nonsignificant change) at 40 min of hemorrhage. Panel B of Figure 5 is a tracing from an experiment in which blood was withdrawn to lower and maintain MABP at 50 mm Hg for 60 min. Again, there was no evidence of vasoconstriction at any time during the hypovolemic period. Panel C of Figure 5 shows the vascular response in an isolated constantly-perfused breast muscle of a phenobarbital anesthetized mallard duck during hemorrhage and reinfusion of the shed blood. Mean arterial blood pressure was reduced by hemorrhage from 110 to 50 mm Hg. The bottom tracing of Pp shows that the vasculature responded to NE (0.25 ug) and ACh (1.0 ug) and fell to less than 20 mm Hg when the pump was turned off, again indicating adequate vascular isolation. Also, there is evidence of

reactive dilatation. Perfusion pressure began to rise immediately following the onset of bleeding, remained elevated during the hypotensive period, and returned to the prehemorrhage level after reinfusion of the shed blood. This increase in skeletal muscle vascular resistance is similiar to that seen by Jones and West (1978) in the constantly-perfused duck hindlimb during submersion. These results demonstrate that holding the MABP at 50 mm Hg for an hour in the phenobarbitalized chicken does not produce a rise in hindlimb vascular resistance. However, an acute hemorrhage in mallards may produce a sharp rise in hindlimb vascular resistance.

3. Acute Bleed - Pentobarbital Anesthesia

Perfusion pressure in nine males did not change significantly during a 10 to 15 min hemorrhagic period. The initial MABP in this group was 175 $\pm$7 mm Hg and initial Pp was 165 $\pm$6 mm Hg. The latter increased to 171 $\pm$8 mm Hg during the hemorrhagic period. A tracing from a typical experiment is shown in Figure 6. These results demonstrate that acute hemorrhage to a MABP of 50 mm Hg in the pentobarbitalized chicken does not produce a rise in hindlimb vascular resistance.

4. Isogravimetric Studies - Pentobarbital Anesthesia

The MABP immediately prior to hemorrhage, leg weight, and leg blood flow did not differ significantly between the three group. The overall mean values (n = 18) were; MABP = 95.8 $\pm$1.2 mm Hg, leg weight = 217 $\pm$12 gm, and leg blood flow = 14.9 $\pm$0.6 ml/min/100gm. Figure 7 summarizes the results of the first three experimental series. The mean CFC values of the three groups were compared statistically at each determination time

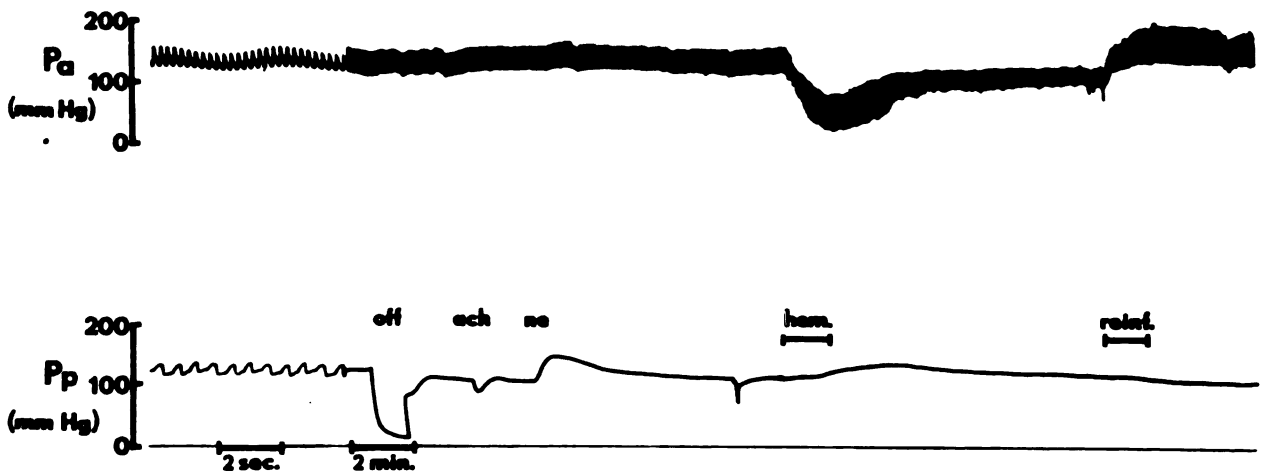
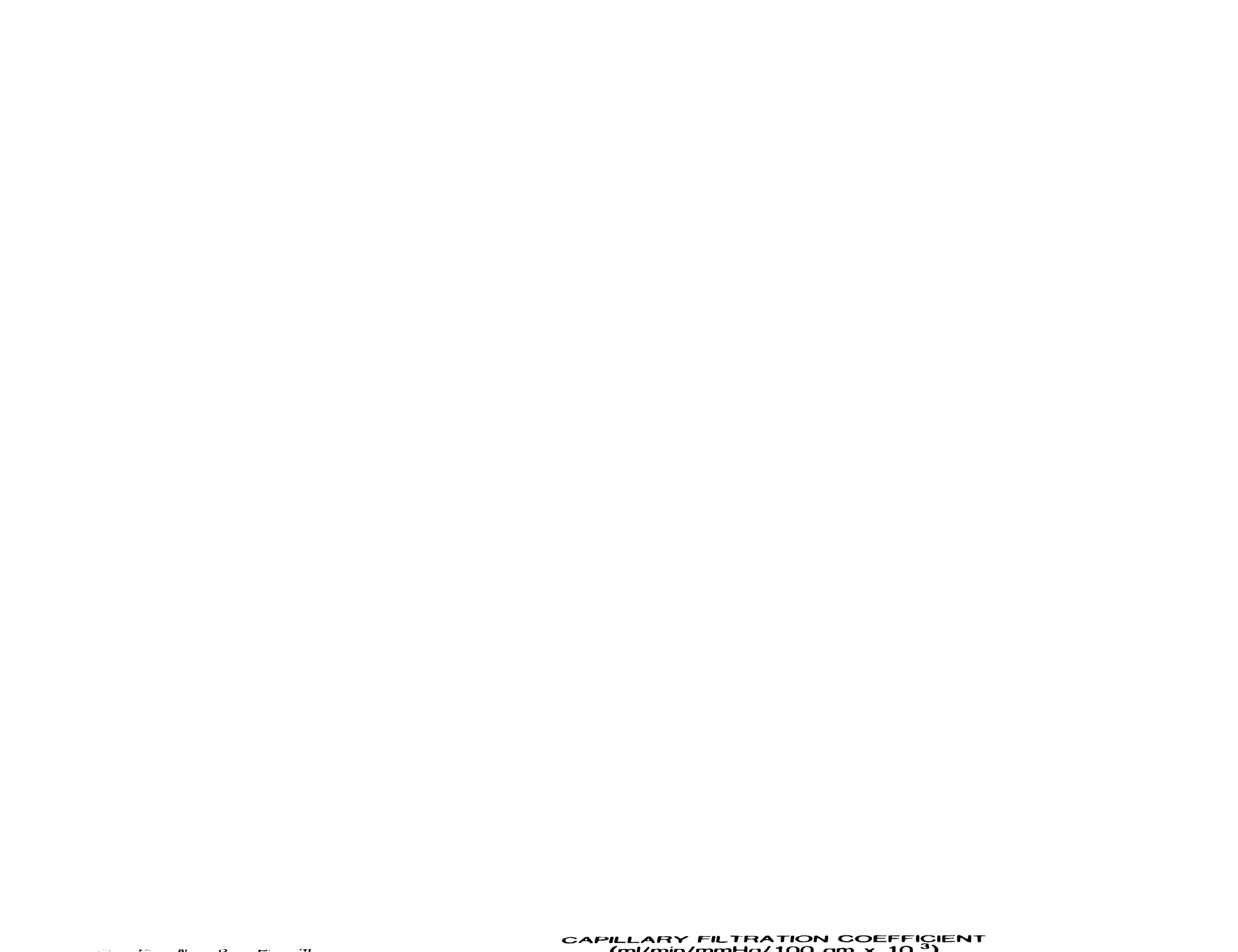


Figure 6. Tracing of the effect of hemorrhage on arterial blood pressure (Pa) and perfusion pressure (Pp) in the constantly-perfused hindlimb of a male chicken. Pentobarbital anesthesia. off = 1 minute perfusion pump off; ach = 1.0 ug acetylcholine delivered behind the pump; ne = 1.0 ug norepinephrine; hem.= rapid removal of about 50 ml of blood; reinf. = reinfusion of the shed blood.


CAPILLARY FILTRATION COEFFICIENT
(ml/min/mmHg/100 gm x 10^{-3})

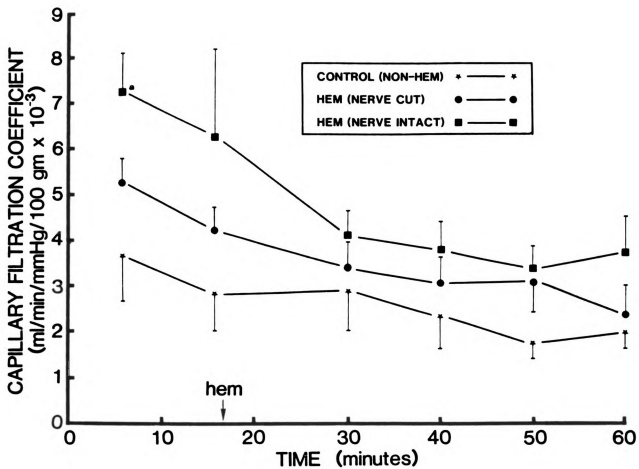


Figure 7. The effect of hemorrhage on capillary filtration coefficient in the isogravimetric isolated constantly-perfused hindlimb of male chickens. Pentobarbital anesthesia. Six animals per group. hem: mean arterial blood pressure reduced to, and sustained at, 50 mm Hg. "a" indicates significantly different from the control group at that time ($P<0.05$).

by a one-way ANOVA using a Dunnett test. The initial (5 min) CFC determination in the nerve intact group was significantly different from the other two groups at that time. This was the only instance of a significant difference between the three experimental groups. The 5 min CFC of each group was not significantly different from the 60 min CFC (Students t test).

A representative tracing from this study is shown in Figure 8. As the venous pressure is increased to approximately 40 mm Hg, there was a rapid increase in limb weight due to distention of the capacitance vessels followed by a steady slope which represents capillary filtration. The Pp increased due to the increase in venous outflow resistance and the MABP (Pa in Figure 8) fell due to the volume shift into the limb. The Pc_i values ranged from 13.2 $\pm$2.8 to 18.3 $\pm$1.5, and were unchanged by hemorrhage. This study demonstrates that hemorrhage in the chicken does not affect CFC, whether the sciatic nerve trunk is intact or severed.

II. VASCUALAR RESPONSE TO HEMORRHAGE TO MEAN ARTERIAL BLOOD PRESSURE OF 25 mm Hg

A. Hindlimb Perfusion Studies

In all animals, a hemorrhage to a MABP of 50 mm Hg did not produce a change in Pp, whereas a further hemorrhage to 25 mm Hg produced a significant increase in Pp. The Pp immediately began to increase as the MABP fell near 25 mm Hg and it remained elevated until the shed blood was reinfused. During reinfusion of the shed blood, the Pp would decrease to the control value following the return of enough blood to raise the MABP to approximately 50 mm Hg.

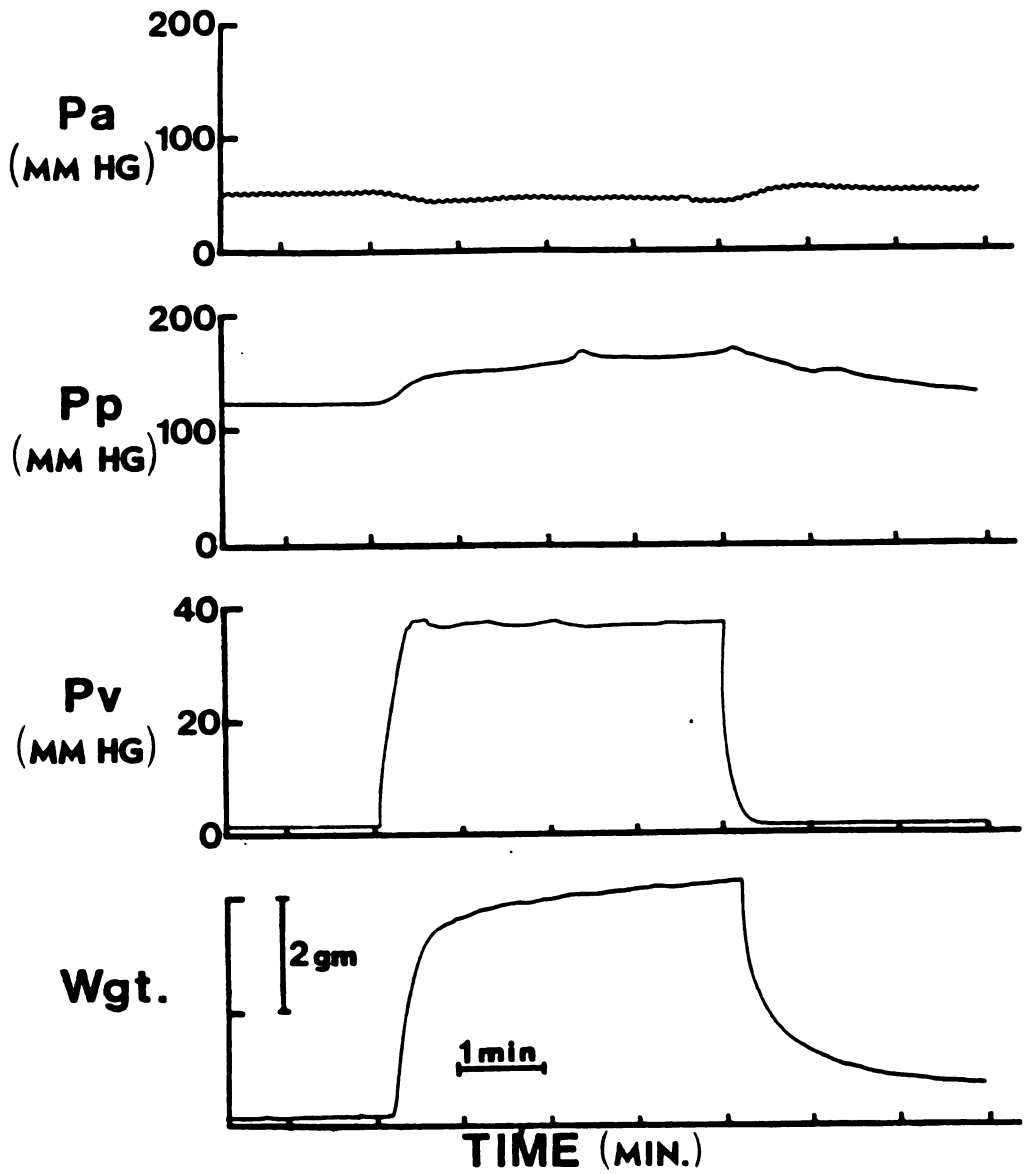


Figure 8. Tracing of mean arterial blood pressure (Pa), perfusion pressure (Pp), venous pressure (Pv), and leg weight (Wgt.) in the isogravimetric isolated constantly-perfused hindlimb of a male chicken. Pentobarbital anesthesia.

1. Effect of Sciatic Nerve Severance and Alpha-adrenergic Blockade

Severance of the sciatic nerve during the elevated Pp would generally produce only a transient (30 sec) 25 - 30 mm Hg fall in Pp, other than this the Pp was unaffected (Table 1). In contrast, an intra-arterial infusion of phentolamine would promptly return the Pp to the prehemorrhage level, where it was maintained as long as the alpha-blocker was infused (Figure 9). This study demonstrates that severence of the sciatic nerve trunk does not attenuate the rise in hindlimb vascular reisitance seen during severe hemorrhagic hypotension.

2. Effect of Bilateral Vagotomy

The Pp again was unaffected by a hemorrhage to 50 mm Hg and increased significantly as the MABP was reduced to 25 mm Hg (Table 1). Mean arterial blood pressure and Pp returned to control values following reinfusion of the shed blood. The rise in Pp in response to severe hemorrhage was unaltered by bilateral vagotomy. This study demonstrates that the rise in hindlimb vascular resistance during severe hemorrhagic hypotension is not mediated through the vagi.

3. Head Perfusion Studies

A hemorrhage to a MABP of 25 mm Hg was not required to produce a rise in limb Pp in this study (Table 1). Some birds demonstrated intense vasoconstriction in the limb at a MABP of 45 mm Hg, however, at this time carotid perfusion pressure was less than 25 mm Hg. When head blood flow was artificially maintained during hemorrhage, no rise in hindlimb Pp occurred (Table 1). Figure 10 is a representative tracing showing the effect of hemorrhage on limb Pp with and without head perfusion. It is

Table 1. The effect of hemorrhage on mean arterial blood pressure (MABP), hindlimb perfusion pressure (HPp), and carotid perfusion pressure (CPp) in chickens following severence of the sciatic nerve, bilateral vagotomy, intra-arterial phentolamine, or artificial perfusion of the head. All values expressed as mean ±SEM. * designates significant change in HPp from the preceding control ($P<0.05$).

CONDITION	MABP (mmHg)	HPp (mmHg)	CPp (mmHg)
SERIES 1 (n = 6)			
control	124 ±6	97 ±5	---[a]
hem.[b]	50 ±2	97 ±5	---
hem.	25 ±2	174 ±10*	---
hem. + sciatic[c]	25 ±2	172 ±11*	---
hem. + phentol.[d]	25 ±2	92 ±10	---
SERIES 2 (n = 7)			
control	109 ±6	77 ±8	---
hem.	50 ±2	77 ±8	---
hem.	25 ±2	189 ±9*	---
control	111 ±4	79 ±9	---
hem. + vagot.[e]	25 ±2	184 ±10*	---
SERIES 3 (n = 8)			
control	109 ±6	144 ±7	141 ±7
hem. + CPP[f] off	37 ±2	335 ±20*	19 ±2
control	121 ±9	153 ±12	157 ±8
hem. + CPP on	35 ±2	174 ±15	96 ±12

a CPp not measured in series 1 and 2.
b rapid hemorrhage
c severance of the sciatic nerve trunk during hemorrhage
d intra-arterial infusion of phentolamine (50 ug/min) during hemorrhage
e bilateral cervical vagotomy prior to hemorrhage
f CPP designates carotid perfusion pump

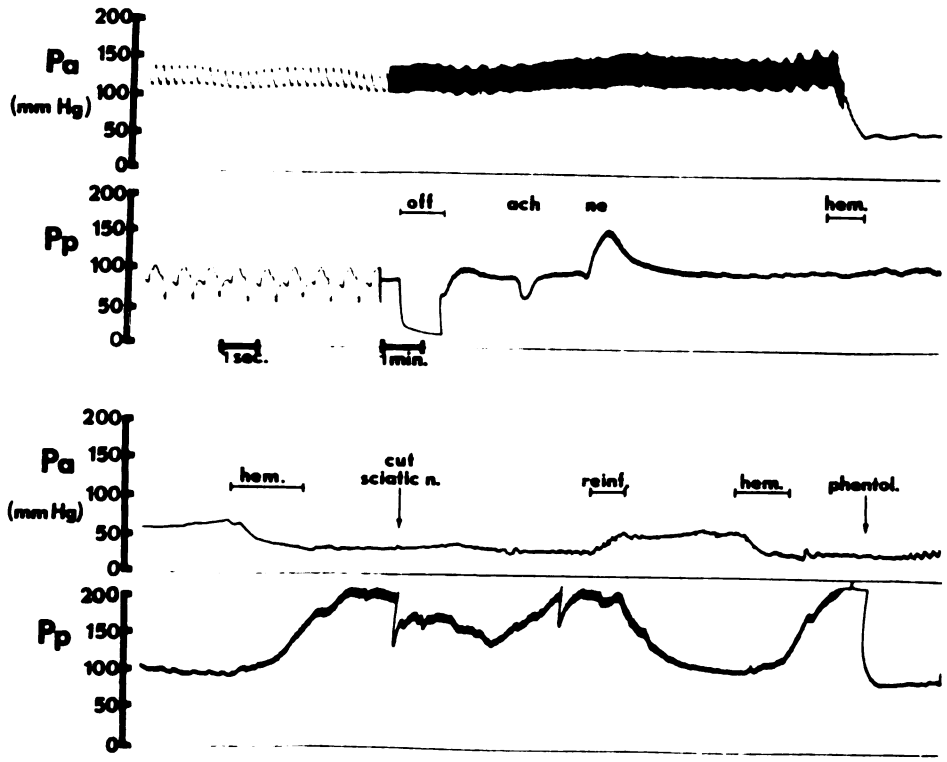


Figure 9. Continuous tracing of the effect of severence of the sciatic nerve and intra-arterial phentolamine infusion on mean arterial blood pressure (Pa) and hindlimb perfusion pressure (Pp) in the chicken after stepwise hemorrhage. off, perfusion pump off; ach, 1.0 ug acetylcholine; ne, 1.0 ug norepinephrine; hem., hemorrhage; reinf., reinfusion of 10 ml shed blood; phentol., phentolamine infused at 50 ug/min, ia.

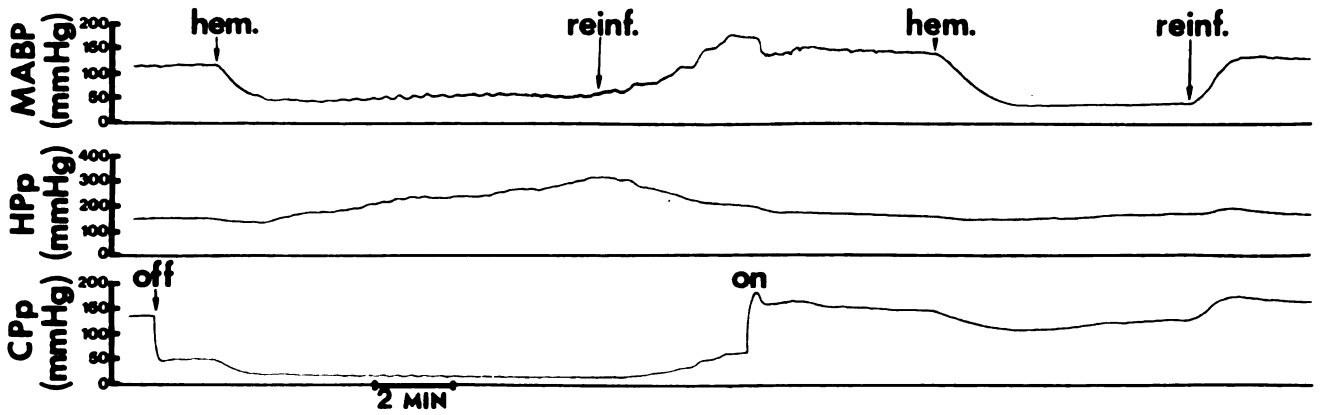


Figure 10. Tracing of the effect of hemorrhage on mean arterial blood pressure (MABP) and perfusion pressure (HPp) in the constantly-perfused hindlimb of a male chicken with and without artificial perfusion of the head via the carotid arteries. Pentobarbital anesthesia. CPp, carotid perfusion pressure; off, carotid perfusion pump turned off; hem, MABP reduced to, and sustained at, 50 mm Hg; reinf, reinfusion of shed blood; on, carotid perfusion pump turned on.

evident that Pp increased markedly, i.e. 150 mm Hg, when carotid perfusion pressure fell below 25 mm Hg even though MABP was 40 mm Hg. However, when head flow was maintained, the limb Pp rose only 20 mm Hg during the hypotensive period. This study demonstrates that the rise in hindlimb vascular resistance during severe hemorrhagic hypotension can be completely eliminated by artificially maintaining blood flow to the head.

B. Effect on Concentration of Serotonin, Norepinephrine and Dopamine in Plasma

The initial MABP in this series was 127 ±15 mm Hg. While a hemorrhage to a MABP of 50 mm Hg did not affect the concentration of serotonin (SER), dopamine (DA), or NE in plasma, the concentrations of all three hormones increased significantly when the MABP was reduced 25 mm Hg (Figure 11). This study demonstrates that a moderate hemorrhage (MABP = 50 mm Hg) does not significantly effect the concentration of SER, DA, and NE in plasma, while a more severe hemorrhage (MABP = 25 mm Hg) produces a significant increase in the concentration of all three hormones in plasma.

III. HINDLIMB VASCULAR RESPONSE TO VASOACTIVE AGENTS, ASPHYXIA, AND EXERCISE

A. Vasoactive Agents

1. Log Dose-response Curves for Vasoactive Agents

The initial MABP and Pp for the eight animmals in this study were 134 ±11 and 125 mm Hg, respectively. Figures 12 and 13 show log dose-response curves for the agents tested. The drug infusion rate in this series ranged from 0.002 to 2.0 ml/min. Though five dose-response

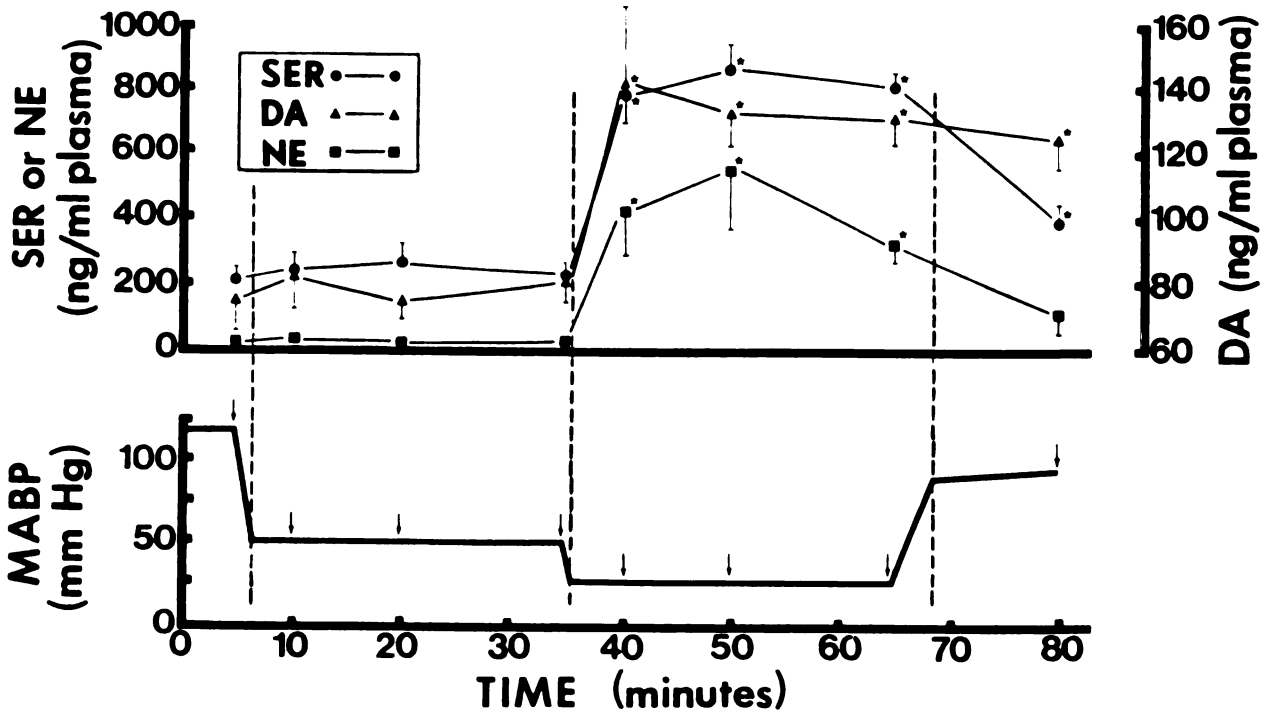
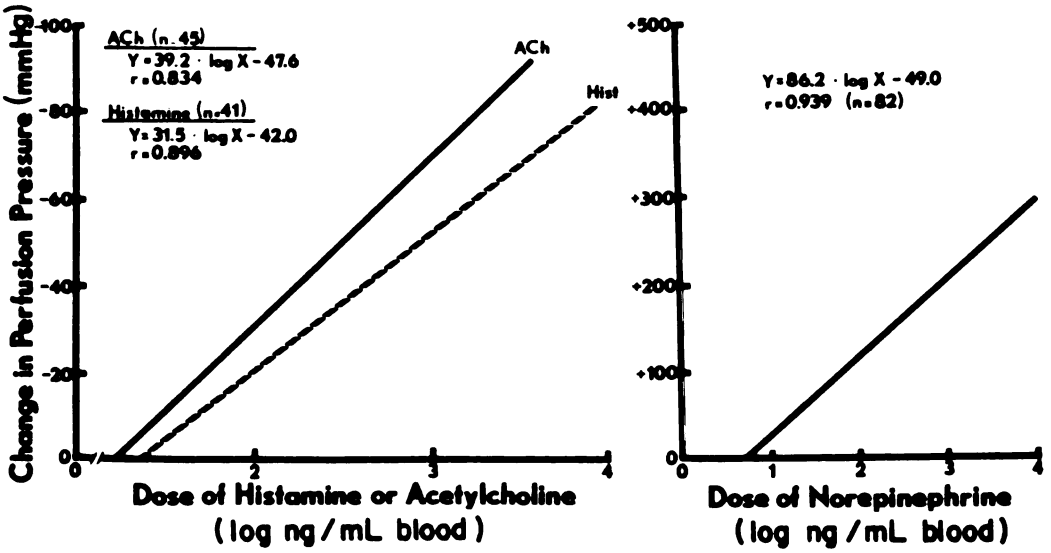
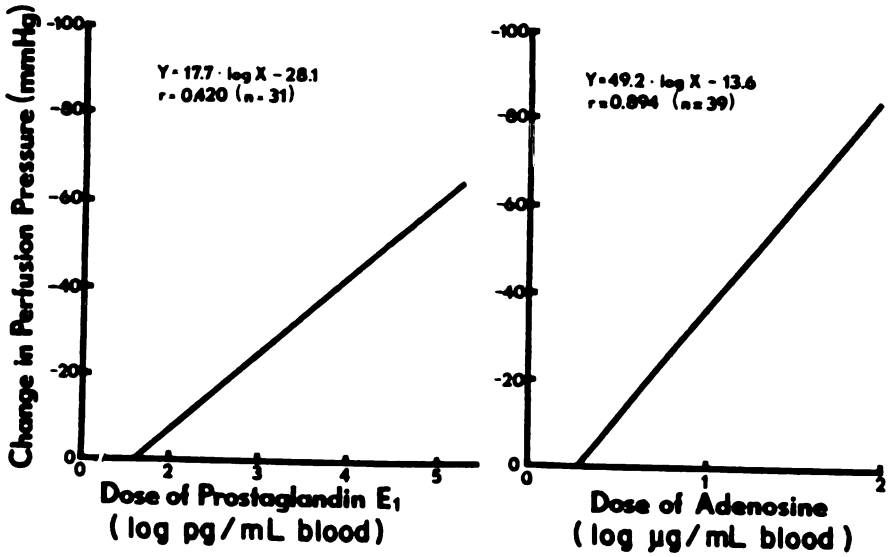


Figure 11. The effect of stepwise hemorrhage on the concentration of norepinephrine (NE), serotonin (SER), and dopamine (DA) in plasma of male chickens (n=5). Pentobarbital anesthesia. * indicates significant change from control ($P<0.05$).

Figure 12. The effect of local intra-arterial infusion of histamine (diphosphate), acetylcholine, or norepinephrine on hindlimb perfusion pressure in the constantly-perfused hindlimb of male chickens (n=8). Pentobarbital anesthesia.

Figure 13. The effect of local intra-arterial infusion of prostaglandin E_1 or adenosine on perfusion pressure in the constantly-perfused hindlimb of male chickens (n=8). Pentobarbital anesthesia.


ACh (n=45)
Y=39.2 · log X - 47.6
r=0.834
Histamine (n=41)
Y=31.5 · log X - 42.0
r=0.896
ACh
Hist
Change in Perfusion Pressure (mmHg)
Dose of Histamine or Acetylcholine
(log ng/mL blood)
Y=86.2 · log X - 49.0
r=0.939 (n=82)
Dose of Norepinephrine
(log ng/mL blood)


Y=17.7 · log X - 28.1
r=0.420 (n=31)
Change in Perfusion Pressure (mmHg)
Dose of Prostaglandin E1
(log pg/mL blood)
Y=49.2 · log X - 13.6
r=0.894 (n=39)
Dose of Adenosine
(log µg/mL blood)

curves were obtained from each animal, drug interaction was not likely a problem inasmuch as the concentrations were quite small and the Pp would return within 10 mm Hg of control following discontinuation of the drug infusion. This return to control level occurred within 3 min for all drugs except PGE_1 which required approximately 10 min. Norepinephrine (beginning at approximately 7 ug/ml blood) was the only drug which produced a significant change in MABP. The perfusion pump flow rate in this series averaged 18.1 $\pm$2.0 ml/min. This study generates log-dose response curves for PGE_1, ADO, HIST, ACh, and NE which are similiar to the mammal.

2. Response to Bolus Administration of Vasoactive Agents

a. Vasodilators

Table 2 summarizes the magnitude, duration and the integral of the change in Pp for bolus injections causing vasodilation. Injection of the saline vehicle (0.1 ml ia) did not affect Pp. Prostaglandin E_1 produced an immediate 54 mm Hg fall in Pp which gradually returned to the control value in 10 min. Histamine produced a fall in Pp which lasted on the average about 2 min. Intra-arterial administration of PGE_1 or histamine produced a 20 to 40 mm Hg fall in the MABP at approximately 15 sec following injection, with the MABP generally returning to control within 3 min. Acetylcholine produced a fall in Pp lasting about 20 sec. Adenosine at both 5 and 10 ug dosage produced a fall in Pp, with the higher dose having a significantly larger effect. This study demonstrates that the effect of bolus administration of various vasoactive agents on hindlimb vascular resistance in the chicken are similiar to the mammal.

Table 2. Effect of exercise, prostaglandin E_1 (PGE_1), histamine (diphoshpate), exercise, acetylcholine, phenoxybenzamine, adenosine, and theophylline on perfusion pressure in the constantly-perfused hindlimb of the chicken (n=9).

TREATMENT	MAXIMAL CHANGE IN PERFUSION PRESSURE (mm Hg)	DURATION OF CHANGE (min)	AREA[a] (mm Hg min)
Exercise[b,c]	-52 ±7[d]	4.3 ±0.3	-107.1 ±5.8
PGE_1 (0.5 ug)	-54 ±9[d]	10.1 ±1.4	-270.0 ±52.2
Histamine[b] (5 ug)	-55 ±11[d]	1.9 ±0.4	-58.1 ±5.0
Acetylcholine (1ug)	-46 ±5[d]	0.4 ±0.0	-11.0 ±1.8
plus phenoxybenzamine (7.5 - 10 mg/kg, iv)	-44 ±8[d]	0.4 ±0.1	-11.7 ±2.7
Adenosine (5 ug)	-19 ±5[d]	0.4 ±0.1	-2.8 ±0.6
plus theophylline (5 mM at 1 ml/min, ia)	-3 ±1[e]	0.1 ±0.0[e]	-0.4 ±0.2[e]
Adenosine (10 ug)	-30 ±6[d]	0.6 ±0.1	-5.2 ±0.8
plus theophylline (5 mM at 1 ml/min, ia)	-7 ±2[d,e]	0.2 ±0.1[e]	-1.7 ±0.4[e]

a area represents the product of duration (min) and time-averaged change in Pp (mm Hg)

b n = 9 for all groups except Exercise and Histamine

c 15 sec nerve stimulation at 1.6 msec duration, 6V, and 6 Hz

d values (x ±SEM) represent a significant change from baseline ($P<0.05$)

e response significantly changed from prior to blockade ($P<0.05$)

b. Vasoconstrictors

The data in Table 3 summarizes the magnitude, duration, and area of the change in Pp for treatments causing vasoconstriction. The NE (1 and 5 ug) produced a significant dose-related rise in Pp.

3. Inhibition of Adenosine with Theophylline

Theophylline infusion significantly reduced the maximal change in Pp induced by 5 and 10 ug adenosine by 85 and 76%, respectively (Table 2). The duration and area also appear attenuated. The theophylline infusion significantly reduced the Pp by 32 $\pm$4 mm Hg. A 1 ml/min infusion of the saline vehicle, per se, reduced the Pp by 10 - 15 mm Hg due to a reduction in blood viscosity. Acetylcholine administered to the birds during the theophylline infusion produced a fall in Pp indicating that the vascular bed was not maximally dilated and, therefore, had the capacity to undergo vasodilation. This study demonstrates that theophylline effectively inhibits the vasodilator effect of adenosine on hindlimb vasculature of the chicken.

4. Inhibition of Norepinephrine with Phenoxybenzamine

Phenoxybenzamine did not significantly alter the maximal change in Pp, duration, or area of the vascular response induced by ACh (Table 2). The PBZ dosage level (5 mg/kg) which Szeto et al. (1977) reported produced a 75% inhibition of a phenylephrine-induced rise in diastolic blood pressure in chickens did not prevent a 1 ug NE-induced rise in Pp. However, 7.5-10.0 mg/kg of intravenous PBZ significantly attenuated the 1 and 5 ug NE induced rise in Pp by 56 and 59%, respectively (Table 3). The duration and area of the NE response were also significantly

Table 3. Effect of alpha-adrenergic blockade on the vascular response to norepinephrine and asphyxia in the constantly-perfused hindlimbs perfused of the chicken (n=9).

TREATMENT	MAXIMAL CHANGE IN PERFUSION PRESSURE (mm Hg)	DURATION OF CHANGE (min)	AREA[a] (mm Hg min)
Norepinephrine (1 ug)	+39 ±7[d]	1.3 ±0.0	+26.2 ±4.3
plus phenoxybenzamine[b]	+17 ±4[d,e]	0.4 ±0.2[e]	+7.6 ±1.8[e]
Norepinephrine (5 ug)	+69 ±8[d]	2.2 ±0.3	+61.9 ±10.6
plus phenoxybenzamine	+28 ±4[d,e]	1.1 ±0.2[e]	+21.1 ±3.9[e]
Asphyxia (2 - 3 min)[c]	+122 ±9[d]	2.0 ±0.3	+146.0 ±25.3
plus phenoxybenzamine	+40 ±8[d,e]	0.7 ±0.2[e]	+28.1 ±7.2[e]

a area represents the product of duration (min) and time-averaged change in Pp (mm Hg)
b phenoxybenzamine, 5.0 - 7.5 mg/kg, iv
c asphyxia, 2 - 3 min tracheal occlusion
d values (x ±SEM) represent a significant change from baseline ($P<0.05$)
e response significantly changed from prior to blockade ($P<0.05$)

attenuated following PBZ. Phenoxybenzamine did not significantly reduce the Pp or the MABP. This study indicates that 5 - 10 mg/kg phenoxybenzamine produces about a 60% blockade of the increase in hindlimb vascular resistance induced by intra-arterial NE.

B. Asphyxia

Tracheal occlusion (2 - 3 min) produced a transient fall in MABP generally followed by a secondary increase in MABP. Tracheal occlusion generally produced a transient fall in Pp followed by a secondary increase in Pp of 121 $\pm$8 mm Hg (n=18). This asphyxia-induced maximal change in Pp was reduced about 67% by systemic alpha-adrenergic blockade with PBZ, 7.5 - 10.0 mg/kg (Table 3). The duration and area of the asphyxic response were also significantly attenuated by PBZ. The asphyxia-induced rise in Pp following PBZ was transient, and often returned to the baseline prior to deocclusion of the trachea. This study indicates that acute asphyxia produces a sharp rise in hindlimb vascular resistance which can be blocked 60% with phenoxybenzamine.

C. Exercise

Fifteen sec of high frequency exercise (6 Hz) produced an immediate fall in Pp which gradually returned to control after several min (Table 2). These results indicate that simulated exercise in the chicken produces a fall in hindlimb vascular resistance of a magnitude and duration similiar to mammals.

DISCUSSION

I. Hemorrhage to Mean Arterial Blood Pressure of 50 mm Hg

Previous studies have demonstrated that small reductions in blood volume in the chicken are associated with large reductions in blood pressure (Wyse and Nickerson, 1971; Ploucha et al., 1981). Concomitant with a hemorrhage to a MABP of 50 mm Hg is a reduction in cardiac index which is mediated solely by a reduction in Vs, inasmuch as HR increases or does not change (Figure 4). Total peripheral resistance either falls or is unchanged at this level of hypotension and resistance to flow through skeletal muscle is not affected (Figures 4 and 5). Blood compositional changes during hemorrhage in the chicken include progressive hyperkalemia, hyperglycemia, and hemodilution, as indicated by linear falls in hematocrit, hemoglobin (Ploucha, 1979) and plasma protein concentration (Figure 3). Plasma sodium, plasma osmolality, arterial pH and pO_2 are unchanged but there is a significant fall in arterial pCO_2 due to hyperventilation (Ploucha, Scott and Ringer, 1981).

The finding that small reductions in blood volume in the chicken are associated with marked hypotension is in marked contrast to the duck, pigeon, and dog. For example, a blood loss of about 9% of the estimated initial blood volume of the chicken over a 225 min duration reduced the MABP by 26% and a 25% reduction in blood volume generally reduced the MABP to 50 mm Hg (Wyse and Nickerson, 1970; Ploucha et al., 1979). Wyse and Nickerson (1971) reported that removal of only 4 - 5 ml/kg (less than 10% of the initial blood volume) reduced the MABP by 20 mm Hg. This is in stark contrast to other avian and mammalian species. For instance, Kovach and Balint (1969) reported that 54% of the blood volume must be

removed from the pigeon to produce a 30 mm Hg fall in MABP, while in the duck a 25% reduction in blood volume did not affect blood pressure (Djojosugito et al., 1969). Approximately 50% of the blood volume of a dog must be removed to lower the MABP to 50 mm Hg (Halmagyi, Gillett, and Irving, 1967; Hollenberg et al., 1970; Steikel et al., 1967) and even more than this must be removed from primates. It is interesting to note that pretreatment of the dog with an alpha-blocker reduces the amount of blood that must be removed to lower the pressure 20 mm Hg by about 75%, i.e. from 19 ml/kg to 4 ml/kg (Hollenberg et al, 1970) and that the latter value is similiar to that which lowers pressure by 20 mm Hg in the untreated chicken.

The ability of many species to withstand a relatively large rapid blood loss with a minimal reduction in MABP is largely attributed to reflex activation of the sympathico-adrenal system with a rise in TPR (Figure 5, panel C; Haddy et al., 1965; Djojosugito et al., 1969). Total peripheral resistance is elevated following hemorrhage in man, dogs, cats, rats (Chien, 1967) and ducks (Djojosugito et al., 1969). The present studies indicate this is not the case in the chicken. Hemorrhage to a MABP of 50 mm Hg was not associated with a rise in TPR, in fact calculated resistance fell, and skeletal muscle resistance was unafffected during hemorrhage. Moreover, PBZ did not reduce bleeding volumes as in the dog (Halmagyi et al., 1967; Hollenberg et al., 1970; Steikel et al., 1967; Grega et al., 1967). Thus, there is no evidence to support vasoconstriction in the chicken during hemorrhage to a MABP of 50 mm Hg. The slight fall in TPR (Figure 4) may well be the result of vasodilation in beds other than skeletal muscle or due to a decrease in blood viscosity, since it occurred at the time that the HCT was

significantly reduced. It has been shown in the dog that a viscosity, and hence vascular resistance, is reduced by a fall in HCT (Chien et al., 1973). This may have occurred in the chickens because there was a sustained decrease in TPR during hemorrhage with PBZ which would suggest that a slight vasoconstriction was being masked in the other groups by a lowered blood viscosity (Figure 4). The reason for the lack of sympathetic involvement is not completely clear, however, the sympathetic branch of the autonomic nervous system may be of minor importance in the control of peripheral resistance in this species.

For example, PBZ did not significantly alter the TPR in the anesthetized chicken indicating little resting peripheral sympathetic activity (Figure 4). Also, TPR was not elevated during beta-blockade with PROP even though arterial pressure fell. This indicates lack of reflex activation of the sympathetic system, a response seen in other species (Dunlop and Shanks, 1968). Moreover, simultaneous bilateral carotid and vertebral arterial occlusion in the chicken, which lowers carotid back pressure by 65%, does not initiate a reflex increase in MABP (McGinnis and Ringer, 1967). Finally, the slight rise in plasma glucose in the chicken (Ploucha et al., 1981) relative to the dog (Chien et al., 1973) would also support minor sympathetic activation. On the other hand, the present studies show that the vasculature of skeletal muscle does respond to alpha-agonists with a marked constriction (Figure 5 and Table 3). Thus, while alpha-receptors are present in the vasculature they are seemingly not normally activated and are apparently not activated to any great extent during the stress of hemorrhage to a MABP of 50 mm Hg. This is further supported by the finding that alpha-blockade did not greatly alter the overall cardiovascular response

to hemorrhage (Figure 4). However, a more severe stress can stimulate vasoconstriction in the chicken. For instance, a pressor response to cerebral ischemia has been shown in some chickens (particularly when the carotid perfusion pressure falls below 26 mm Hg) following bilateral occlusion of the carotid and vertebral arteries (McGinnis and Ringer, 1967) and constriction in skeletal muscle vasculature occurs in chickens following tracheal occlusion (Table 3) or a more severe hemorrhage which capable of producing cerebral ischemia (Table 1, Figure 9). Therefore, while a moderate hemorrhage will not initiate sympathetic activation, a more severe hemorrhage does cause intense activation of the sympathico-adrenal axis.

In the present study the PPC fell significantly within 30 min of hemorrhage indicating that hemodilution is occurring (Figure 3). The progressive hemodilution seen during the hypotensive period indicates marked microvascular fluid influx and, in fact, the rate of fluid mobilization is much greater than mammals. The rate of post-hemorrhagic fluid mobilization in dogs is approximately equal to 6.7% of the initial blood volume per hour, and about 13% of the initial blood volume is restored (Hollenberg et al., 1970). The chicken, on the other hand, mobilizes fluids at a rate of 13 - 17% of the initial blood volume per hour, ultimately restoring 40 - 52% of the initial blood volume (Wyse and Nickerson, 1971). While the mechanism(s) involved in this fluid movement are not clear they apparently are not exactly the same ones that are operant in other species. The initial transcapillary fluid influx in hemorrhage has classically been ascribed to a reduction in Pc subsequent to a fall in arterial blood pressure and a rise in the pre/postcapillary resistance ratio (Djojosugito et al., 1969; Hollenberg and Nickerson,

1970). The latter supposedly plays a major role in the reduction in Pc in most species and prevention of its occurrence by alpha-blockade in the dog is associated with a slower rate of fluid absorption (Steikel et al., 1967; Grega et al., 1967). Likewise, constrictor nerve fiber blockade with lidocaine also reduces the rate of fluid mobilization in the duck after hemorrhage (Djojosugito et al., 1969). Interestingly, in the chicken there is little evidence to indicate that this ratio changes during hemorrhage and yet fluid influx is apparently immediate and rapid (Figure 3 and Wyse and Nickerson, 1971; Ploucha et al., 1981). While there must be a reduction in Pc associated with the fall in arterial pressure it seems unlikely that it would be of the same magnitude as occurs in other species where the pre/postcapillary resistance ratio increases. Thus, there may be other forces operating in the chicken to facilitate transcapillary fluid mobilization.

Studies conducted in the cat suggest that during hemorrhagic hypotension fluid enters the vascular system from cells as a result of an osmotic force generated by glucose release from the liver (Jarhult, 1975). Plasma glucose is increased by hemorrhage in the chicken (Ploucha et al., 1981) and plasma osmolality was unchanged (Figure 3) during the course of the hypotension. The importance of this mechanism in volume repletion is difficult to assess by examining plasma concentrations because they, in turn, are continuously affected by dilution and loss of solute (Haddy et al., 1976). Pirkle and Gann (1975) have proposed that restitution of plasma volume following hemorrhage in the dog is partly due to the action of cortisol to transfer cellular water to the interstitium which increases interstitial pressure and thereby promotes capillary absorption. It has been postulated that cortisol causes

cellular water loss indirectly by stimulating active transport of certain electrolytes, presumably sodium, from the cell (Swingle and Swingle, 1965). While the chicken does not have cortisol it does have corticosterone and the levels of this hormone are greatly increased by mild stress (Beuving and Vonder, 1978). Finally, the duck has been reported to have three times the capillary surface area of the cat or turkey (Folkow et al., 1966). Hence, reflex cardiovascular adjustments producing identical changes in Pc would result in a correspondingly more rapid fluid transfer in the duck. It is possible that the chicken also has a large capillary surface area which could enhance fluid absorption at a given reduction in capillary pressure.

The fact that prolonged periods of moderate hypotension do not lead to irreversible shock in the chicken coupled with the observations that the chickens are neither acidotic nor hypoglycemic (Ploucha et al., 1981) after four hours of sustained hypotension deserve further comment. Other species, that develop irreversible hemorrhagic shock show acidosis and normal or reduced plasma glucose at this time (Bond, Manning, and Peissner, 1977; Chien et al., 1973; Strawitz et al., 1961). It is generally accepted that the acidosis is largely due to inadequate tissue perfusion with a resultant anaerobic metabolism and the development of lactic-acidosis (Bond et al., 1977; Chien et al., 1973; Scott and Eyster, 1979; Shoemaker, 1964). Other studies support the concept that the hypoglycemia associated with irreversible hemorrhagic shock may well result from cellular hypoxia (Raymond, Harkema and Emerson, 1979; Strawitz et al., 1961). Consequently, it appears that the chicken, probably by virtue of the fact that it does not exhibit intense vasoconstriction, is spared some of the detrimental effects produced by

inadequate tissue perfusion. If indeed this is the case it would tend to incriminate the peripheral actions of the sympathetic nervous system either directly (Irving, 1968) or indirectly as a major contributor to the development of irreversibility in other species.

II. Hemorrhage to a Mean Arterial Blood Pressure of 25 mm Hg

Although alpha-adrenergic receptors are present in the vasculature of the chicken, they are seemingly not activated to a measurable extent by the stress of a hemorrhage to a MABP of 50 mm Hg. In this study, "severe" hemorrhage to a MABP of 25 mm Hg produced a vasoconstriction similiar to that which occurs during asphyxia (Table 3), again mediated through activation of the alpha-receptors (blocked by the alpha-antagonist phentolamine) (Table 1, Figure 9). The chicken, unlike the mammal, does not have a functional carotid sinus baroreceptor (McGinnis and Ringer, 1969). The fact that the vasoconstriction during severe hypotension was not eliminated, or even attenuated, by bilateral vagotomy (which would eliminate afferent baroreceptor information) suggests that the constrictor response is not likely mediated via the vagi. Inasmuch as the vasoconstriction could be eliminated by artificially maintaining carotid blood flow during the systemic hypotension (FIgure 10), the constrictor response is due to cerebral ischemia.

The vasoconstrictor response occurs only when the carotid perfusion pressure reaches a sharply delineated lower critical limit ($\sim$25 mm Hg). McGinnis (1964) noted that the pressor response due to the cerebral ischemia occurred when the cerebral perfusion pressure fell below 26 mm Hg. The values of cerebral perfusion pressure at which the

vasoconstriction occurred in the chicken (both in this study and in the McGinnis (1964) study) agree with the levels reported by Guyton (1976) which produce an ischemic response in mammals. Kovach et al. (1969) also reported that the pigeon would demonstrate a distinct cerebral ischemic pressor response when both carotids were ligated (as in the present carotid perfusion study) at a MABP of 30 mm Hg. The vasoconstrictor response in chickens may be due to an adrenal catecholamine release inasmuch as severance of the sciatic nerve, which most likely contains some sympathetic efferents to the skeletal muscle vasculature under study, only transiently attenuated the vasoconstriction (Table 1).

The control concentrations of NE and SER are similiar to those previously reported in the chicken (Desantis et al., 1975, Newcomer, Gephardt and Hurst, 1972; Meyer and Sturkie, 1974). The dramatic increase in the concentration of catecholamines (Figure 11), which only occurs at the time of the rise in Pp (Figure 11) also supports the hypothesis that the vasoconstriction is predominantly the result of humoral pressor substances. Researchers have suggested that, in the chicken, adrenal medullary hormones play an important role in regulating cardiac performance and blood pressure (Desantis et al., 1975; Karg and Schrams, 1966) and that release of adrenal medullary hormones may be under the influence of adrenocorticotrophic hormone (Newcomer et al., 1972). Serotonin has been shown to be a potent vasoconstrictor in the isolated constantly-perfused chicken and duck foot (McGreggor, 1979). During the head perfusion studies, the vasoconstrictor response in the limb was independent of MABP (Figure 10, Table 1) suggesting it was not initiated via aortic baroreceptors. In fact, the present studies would suggest that the chicken does not have functional aortic baroreceptors in

addition to not having functional carotid baroreceptors. The conclusion is drawn that the vasoconstriction during severe hypotension is not part of an autonomic baroreflex, but rather, it is due to the release of hormones, presumably from the adrenal medulla, in response to cerebral ischemia.

III. Vasoactive Agents, Asphyxia, and Exercise

These data indicate that the skeletal muscle vasculature of the chicken responds to vasoactive substances and exercise in a manner similiar to mammals. While the response to systemic hypoxia in the chicken reportedly differs somewhat from mammals, the response to asphyxia is similiar. Prostaglandin E_1 is reported to produce arteriolar vasodilatation in mammals (Messina, Weiner, and Kaley, 1976; Greenberg and Sparks, 1969) and marked hypotension in the chicken (Horton, 1971). Inasmuch as PGE_1 had no effect on the Locke-perfused chicken heart the hypotension is likely due to changes in peripheral vasculature. Because of the large fall in vascular resistance during PGE_1, it seems likely that it is mainly acting on resistanace blood vessels in the skeletal muscle vasculature of the chicken. The peak response is similiar to that reported in the dog (Cassin et al., 1979). The duration of action is considerably longer than for the other agents employed in the present study, and this is in agreement with canine literature (Greenberg and Sparks, 1969).

The hypotensive effect of HIST in the chicken is well documented. As little as 3.23 ug HIST (active base)/kg will decrease the MABP by 20 mm Hg (Matoff and Lockett, 1957). El Ackad (1972) reported that HIST (active base, 5 ug/kg) decreased the MABP by 40 mm Hg within 15 sec for 1

- 2 min in the chicken. This effect was blocked by trypelennamine or mepyramine. The level of systemic hypotension reported is similiar to that seen in our experiments following 10 ug, i.e. 3.53 ug/kg ia, HIST (diphosphate). McGreggor (1979) also found histamine to produce vasodilatation in the isolated Krebs-perfused chicken foot. Again, because of the magnitude of the response it is likely mediated by vasodilatation in the skeletal muscles in a manner quantitatively similiar to mammals (Daugherty et al., 1968).

Avian species are often used in studies of cardiovascular disease since they are hypertensive and exhibit spontaneous atherosclerosis (Roberts and Straus, 1965). A great deal of mammalian cardiovascular research centers on the role of adenosine in the regulation of blood flow to the myocardium (Berne, 1980). Adenosine is known to be a potent vasodilator in the mammalian skeletal muscle vascular bed and may participate in the genesis of exercise (active) hyperemia (Tabaie et al., 1977). Neither the vascular effects of ADO or theophylline, nor the development of exercise hyperemia, have been examined in the skeletal muscle vasculature of the live chicken. However, ischemic (reactive) hyperemia has been demonstrated in the chicken (Klabunde and Johnson, 1977) and an exercise hyperemia occurs in the duck, turkey (Folkow et al., 1966), and amputated Krebs-perfused chicken foot (McGreggor, 1979). The present data indicate that the vasodilatation induced by ia ADO in the chicken, and the inhibition of this response by theophylline, is quantitatively similiar to the mammalian response (Tabaie et al., 1977). Likewise, an exercise hyperemia occurs in the chicken which is quantitatively similiar in magnitude and duration to that reported in mammals (Tabaie et al., 1977). The role of ADO in the genesis of this

hyperemia has yet to be determined.

Exercise hyperemia (local control) in the duck reportedly will not "break through" the more intense neurogenic vasoconstriction (remote control) induced by hemorrhage (Folkow et al., 1966). The chicken, on the other hand, apparently does not vasoconstrict in response to moderate hemorrhage (Ploucha et al., 1981) and actually vasodilates in response to systemic hypoxia (Richards and Sykes, 1967; Besche and Kadono, 1978). The transient initial hypotension following tracheal occlusion previously reported in the chicken (Harvey et al., 1954; Richards and Sykes, 1967) probably resulted from the local action of hypoxia and hypercapnia on the vasculature. The subsequent severe anoxemia/hypercapnia was then sufficient stimulus to activate an intense sympathetico-adrenal discharge and a concomitant alpha-adrenergic-mediated vasoconstriction in the skeletal muscle vascular bed as demonstrated in the present study.

In summary, the chicken skeletal muscle vasculature responds to vasoactive agents in a manner similiar to mammals. Exercise produces an active hyperemia in the chicken hindlimb vasculature. While alpha-adrenergic receptors are present in the vasculature of the chicken they do not normally appear to be activated to any extent under resting conditions, i.e. PBZ did not reduce TPR or Pp, and are not activated by the stress of moderate hemorrhage or hypoxia, conditions which do elicit activation in flying and diving birds. This observation concurs with those of other avian researchers who have shown that the demand for oxygen and the tolerance to oxygen deficiency differ greatly between flying, diving, and terrestrial birds (Atland, 1961; Bond et al., 1961; Richards and Sykes, 1967; Sturkie and Abati, 1978). However, these results have shown that asphyxia can produce an intense vasoconstriction

mediated through the alpha-receptors, similiar to that which occurs during cerebral ischemia.

SUMMARY

I. **Hemorrhage to a Mean Arterial Blood Pressure of 50 mm Hg**

A. Produced a significant fall in plasma protein concentration (within 30 min) while plasma osmolality was unchanged after 120 min of hypotension.

B. Total peripheral resistance was reduced or unchanged by hemorrhage.

C. Hindlimb vascular resistance in the constantly-perfused hindlimb was unaffected by acute or chronic hemorrhage with either phenobarbital or pentobarbital anesthesia.

D. Capillary filtration coefficient with and without the sciatic nerve innervation was unchanged by hemorrhage.

E. This level of hypotension was not associated with a change in the concentrations of dopamine, serotonin, or norepinephrine in plasma.

II. **Hemorrhage to a Mean Arterial Blood Pressure of 25 mm Hg**

A. This level of hypotension produced an intense and significant increase in hindlimb vascular resistance. This response was:

1. Unaffected by bilateral cervical vagotomy
2. Unaffected by severance of the sciatic nerve
3. Completely eliminated by alpha-adrenergic blockade with phentolamine
4. Completely eliminated by artificial perfusion of the head with arterial blood
5. Associated with a significant rise in the concentration of norepinephrine, dopamine, and serotonin in plasma

III. **Vascular response to vasoactive agents, asphyxia, and exercise**

A. Vasoactive agents

1. Log dose-response curves were drawn for prostaglandin E_1, histamine, adenosine, acetylcholine, and norepinephrine during hindlimb perfusion
2. Bolus administration of prostaglandin E_1, histamine, and acetylcholine produced vasodilatation
3. Norepinephrine produced vasoconstriction which was reduced 70% by systemic administration of phenoxybenzamine
4. Adenosine produced vasodilatation which was reduced 85% by intra-arterial administration of theophylline

B. Asphyxia

1. Produced a transient fall in mean arterial blood pressure followed by hypertension
2. Produced a transient fall followed by an intense rise in hindlimb vascular resistance
3. Phenoxybenzamine reduced the rise in hindlimb vascular resistance by about 70%

C. Exercise produced an immediate fall in hindlimb vascular resistance which lasted several min following cessation of muscular activity

CONCLUSIONS

This research indicates that a hemorrhage to a mean arterial blood pressure of 50 mm Hg in the chicken does not elicit activation of the sympathico-adrenal axis, i.e. a rise in total peripheral resistance or skeletal muscle vascular resistance. This may help to explain why the chicken does not exhibit irreversible hemorrhagic shock, i.e. it is spared the deleterious effects of prolonged tissue hypoperfusion. These findings tend to incriminate the peripheral actions of the sympathetic nervous system as a major contributor to the development of irreversibility in other species. However, hemorrhage to a mean arterial blood pressure of 25 mm Hg in the chicken does elicit a large rise in skeletal muscle vascular resistance. While the response is unaffected by bilateral vagotomy or severence of the main nerve to the assay limb, the response is completely eliminated by alpha-adrenergic blockade or artificial perfusion of the head with arterial blood. Furthermore, levels of circulating catecholamines are significantly elevated only when the vasoconstriction is apparent. Therefore, the vasoconstriction during severe hemorrhagic hypotension in the chicken appears to be primarily mediated by an increase in circulating catecholamines due to to cerebral ischemia, rather than a baroreflex. It also appears the chicken skeletal muscle vasculature responds to vasoactive agents and exercise in a manner similiar to mammals.

These results indicate that while alpha-adrenergic receptors are present in the vasculature of the chicken, they do not normally appear to be activated by the stress of a moderate hemorrhage, a condition which does elicit activation in mammalian and flying or diving avian species.

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However, severe stress, such as asphyxia (induced by tracheal occlusion) or cerebral ischemia (induced by severe hemorrhagic hypotension), can produce intense vasoconstriction mediated through the alpha-receptors.

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APPENDIX 1

Full length publications:

1. Ploucha, J.M., J.B. Scott, and R.K. Ringer, 1980. Vascular and hematologic effects of hemorrhage in the chicken. Amer. J. Physiol. 240(9): 9-17.

2. R.J. Aulerich, M.R. Bleavins, A.L. Napolitano, J.M. Ploucha, R.K. Ringer, W.V. Stoffs, and S. Tonsager, 1981. A study of urinary incontinence and "wet belly". MSU Progress Report to the Mink Farmers Research Foundation

3. Ploucha, J.M., and R.K. Ringer, 1981. Aortic pulse-wave velocity in chickens and ducks. Poultry Sci. 60: 2337-2341.

4. Ploucha, J.M., R.K. Ringer, and J.B. Scott, 1981. Vascular response of the chicken hindlimb to vasoactive agents, asphyxia, and exercise. Canad. J. Physiol. Pharmacol. 59: 1228-1233.

5. Ploucha, J.M., and R.J. Aulerich, 1981. Autonomic control of heart rate during diving in mink and ferrets. Scientifur (submitted 1/82)

6. Ploucha, J.M., W.V. Stoffs, and R.J. Aulerich, 1981. Hematologic parameters in normal mink, ferrets, and mink with "wet belly" disease. MSU Progress Report to the Michigan Mink Farmers Research Foundation.

7. Ploucha, J.M., R.K. Ringer, and J.B. Scott, 1982. Effects of severe hemorrhagic hypotension on the vasculature of the chicken. Proc. Soc Exp. Biol. Med. 170: 160-164.

Abstracts:

1. Ploucha, J.M., R.K. Ringer, and J.B,. Scott, 1980. Vascular and hemodynamic effects of hemorrhage in the chicken. Fed. Proc. 39: 974.

2. Ploucha, J.M. and R.K. Ringer, 1980. Effect of vasoactive substances and asphyxia on skeletal muscle vascular resistance in chickens. Poultry Science 59: 1651.

3. Ploucha, J.M. and R,K. Ringer, 1980. Aortic compliance and pulse-wave velocity in chickens and ducks. Physiologist 23(4): 153.

4. Ploucha, J.M., R.K. Ringer, and J.B. Scott, 1981. Effect of severe hemorrhagic hypotension on the skeletal muscle vasculature of the chicken. Physiologist 24(4): 94.