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THE EFFECT OF SUSTAINED HEMORRHAGIC HYPOTENSION IN CHICKENS FOLLOWING ADRENERGIC BLOCKADE

By

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ABSTRACT

THE EFFECT OF SUSTAINED HEMORRHAGIC HYPOTENSION
IN THE CHICKEN FOLLOWING ADRENERGIC BLOCKADE

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Research concerning hemorrhagic shock has been extensive in mammals and all studies demonstrate vascular decompensation after large blood losses, i.e. irreversible shock. Studies designed for other purposes suggest that avian species possess a unique tolerance to the deleterious effects of hemorrhage, i.e. they do not enter vascular decompensation. Many researchers feel that sympatholytic agents may reduce the mortality of hemorrhagic shock. This study was undertaken to determine the effects of alpha- and beta-adrenergic blockade on survival time in the hemorrhaged domestic fowl (Gallus domesticus), and also to evaluate cardiovascular performance and several hematological parameters, including plasma electrolytes, plasma glucose, and blood gases and pH. Large deviations from the established mammalian physiological responses might help to explain why avian species apparently do not enter vascular decompensation.

Thirty adult Single Comb White Leghorn (SCWL) hens were divided into five equal groups. The birds were anesthetized with 100 milligrams of sodium phenobarbital per kilogram of bodyweight. The birds were in laying condition and weighed between 1.5 and 2.2 kilograms. The carotid artery was cannulated for measuring the arterial blood pressure, obtaining blood samples, and hemorrhaging. Following a control period, blood was removed to lower the arterial pressure to 50 mm Hg and blood was periodically removed to maintain the arterial pressure at 50 mm Hg. Two groups served as non-hemorrhaged controls. The remaining groups

were hemorrhaged. Two of these received a 30 minute drug pretreatment, one with phenoxybenzamine, an alpha-adrenergic antagonist, and the other with propranolol, a beta-adrenergic antagonist. The latter was infused continuously via a cannula in the brachial vein.

The propranolol had a considerable negative chronotropic effect upon the myocardium. Consequently, the survival time was less in these birds than in the other hemorrhaged groups. Phenoxybenzamine reduced the total peripheral resistance without positive inotropic effects on the myocardium. Both of the drugs reduced the mean arterial pressure, the propranolol having the larger effect.

Hemorrhage reduced the cardiac index and the total peripheral resistance, whereas mammals show intense vasoconstriction. Bleeding volumes were not affected by either drug treatment. Alpha-adrenergic blockade has a large effect on the bleeding volumes in mammals.

Hematrocrit and hemoglobin levels fell continuously up to the time of death in the hemorrhaged groups. Mammals experience hemoconcentration in late shock. Plasma magnesium and plasma sodium levels did not change in any of the groups. Plasma potassium and plasma glucose increased progressively throughout the duration of the experiment. Mammals experience severe hypoglycemia in late hemorrhagic shock. All of the hens became progressively alkalotic throughout the duration of the experiment. Mammals enter a severe metabolic acidosis. The phenoxybenzamine reduced the magnitude of the hyperglycemia and hyperkalemia and did not increase the survival time.

The results of this study indicate that following hemorrhage the chicken, unlike the mammal, does not vasoconstrict, enter vascular decompensation, experience hypoglycemia, or become acidotic. The

chicken homeostatic mechanisms act predominantly to maintain blood volume, not blood pressure.

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Abbreviations Used:

ADH	antidiuretic hormone.
ANG II	angiotensin II.
ANOVA	analysis of variance.
cAMP	adenosine 3'5' - cyclic phosphate.
CI	cardiac index, ml/min/$kg^{.734}$.
CO	cardiac output, ml/min.
CNS	central nervous system.
CVS	cardiovascular system.
DP	diastolic pressure in mm Hg.
Hb	hemoglobin, gm% or mg/dl.
HCO_3^-	bicarbonate anion.
HCT	hematocrit, %.
HR	heart rate in beats/min.
JGA	juxtaglomerular apparatus.
LVEDP	left ventricular end diastolic pressure in mm Hg.
MABP	mean arterial blood pressure in mm Hg.
MDF	myocardial depressant factor.
NADH	reduced nicotinamide-adenine dinucleotide.
PBZ	phenoxybenzamine.
Pc	capillary hydrostatic pressure in mm Hg.
pCO_2	arterial partial pressure of carbon dioxide in mm Hg.
pO_2	arterial partial pressure of oxygen in mm Hg.
ppm	parts per million.
PROP	propranolol.
RES	reticuloendothelial system.

SCWL single comb white leghorn.

T= time of a given sample in minutes.

TPR total peripheral resistance.

INTRODUCTION

The poultry industry is growing worldwide at a tremendous pace. Any research providing insight into the chicken not only benefits comparative physiology, but the poultry industry as well. Many avian diseases involve hemodilution and/or hemmorhage, including coccidiosis, fowl cholera, Marek's disease, aplastic anemia, leukosis, fatty liver hemorrhagic syndrome, aortic aneurysm rupture, hemorrhagic arteries, and cannabalism. Therefore, avian research concerning hemodilution or hemorrhage has an application to the industry.

Perhaps more important is the role of such research in shedding additional light, from a new perspective, on the shock phenomenon in man.

Shock, in man, is a descriptive term characterized by hypotension, pallor, collapse of superficial veins, altered mental status, and diminished urine formation. Hypovolemic (hemorrhagic) shock is the most common of about seven types of shock. Cardiogenic shock, due to a pumping insufficiency, rates second in prevelance in man. Septic shock, due to a systemic gram negative endotoxin, and anaphylactisis, due to allergic reaction, tie for third in prevelance.

Voluminous research involving rats, rabbits, sheep, cats, dogs, and subhuman primates has provided much insight into the irreversible shock phenomenon. Observations during experiments designed for other purposes have suggested that the domestic fowl is quite resistant to the deleterious effects of hemorrhage. This prompted Wyse and Nickerson to provide the only research article on an avian species utilizing a standardized shock protocol.

It should be mentioned here that the most advanced avian

cardiovascular (CVS) research has been conducted in the diving duck by Scandanavian physiologists. Vascular responses in the chicken which are thought to be quite different from the duck have not been documented. The chicken is hypertensive, is bipedal, has a CVS anatomy similar to mammals, is prone to spontaneous atherosclerosis, and is of a convenient size and availability, making it an interesting animal for CVS research.

This study was an attempt to elucidate some of the hemodynamic and hematological events which occur while the hen was held in sustained hypovolemic hypotension. Adrenergic receptors, electrolyte, glucose, and blood gas alterations were of particular concern.

REVIEW OF LITERATURE

I. Circulatory Homeostasis

A. The Shock Phenomenon

Hypovolemic hypotension 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 great practical importance. Certain sympathomimetic agents coupled with volume repletion are the standard treatment for hemorrhage (Carey et al., 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 shock irreversible to transfusion may not occur.

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. This circulatory deficiency is the result of either an inappropriate cardiac output and/or peripheral resistance. Blood loss causes a fall in the cardiac output and immediately initiates remote neural reflex systems to increase the peripheral resistance in an attempt to raise the arterial pressure. Some vascular beds, i.e., the skeletal muscles, possess local control mechanisms which, upon sensing the fall in blood flow, initiate a local vasodilation which keeps the blood flow to metabolic rate ratio constant. Hence, the final degree of vascular tone is influenced by local and remote control systems.

The cardiovascular system makes several adjustments following acute

blood loss in an attempt to increase the cardiac output. Increases occur in cardiac contractility, venoconstriction (to increase venous return), arteriolar constriction, and transvascular fluid absorption (to increase blood volume).

The latter adjustment is due mainly to a decrease in capillary hydrostatic pressure (Pc), but is also due to hyperosmolarity of the plasma due to the action of cathecholamines on the liver and a decrease in pancreatic release of insulin. This reabsorptive process is self-limiting and forces favoring net reabsorption progressively diminish. The end result is a transvascular fluid efflux which results in hemoconcentration. This conversion from hemodilution to hemoconcentration is an indicator of the onset of irreversible hemorrhagic shock.

The shock syndrome will lapse into a terminal state called "irreversible shock" if these compensatory mechanisms are not able to restore an adequate cardiac output. This "irreversible shock" is a tremendously complex problem involving many interrelated systemic and cellular mechanisms only a few of which are recognized and understood. Metabolic, central nervous, cardiac, and microvascular alterations may all contribute to the irreversible shock phenomenon in mammals.

The acidosis resulting from tissue anaerobic metabolism can relax vascular smooth muscle and decrease its response to cathecholamines. A hyperkalemia results from both the cellular exchange of potassium ions for hydrogen ions and from the inactivation of the eletrogenic pump. A hyperkalemia not exceeding ten mEq/l. causes vasodilation which may be partially responsible for the vascular decompensation which leads to irreversibility to transfusion.

A heart is depressed during prolonged hemorrhagic hypotension by

cardiac hypoxia, acidosis, and the hyperkalemia. The ischemic pancreas releases a myocardial depressant factor which depresses cardiac performance. Sympathetic tone is also depressed which, in turn, depresses the inotropic state of the myocardium. Other researchers, however, have failed to demonstrate cardiac depression and have found that, in fact, cardiac performance is increased in late shock. The latter researchers attribute the circulatory collapse in late shock to a failure of peripheral resistance.

The medullary vasomotor centers which are responsible for the maintenance of the peripheral resistance may begin to fail due to inadequate cerebral perfusion. The resultant decrease in vascular tone causes venopooling and a reduced venous return. The inotropic state of the myocardium is also reduced for the same reason.

The microcirculation may become damaged by the accumulation of vasoactive metabolites such as histamine and bradykinin. Terminal arterioles and precapillary sphincters lose reactivity to constrictor stimuli. Blood is shunted from nutritional to non-nutritional vessels. A hyperosmolarity can relax the vascular smooth muscle. Intravascular thrombi may result from the now reduced flow velocity and hypercoagulability. Cellular swelling, cellular deformities, and hemoconcentration all reduce the flow velocity.

The reticuloendothelial system (RES) also becomes depressed allowing gastro-intestinal toxins to enter the blood stream. These toxins reduce vascular tone which ultimately reduces the cardiac output.

The onset of irreversibility is marked by a shift from hemodilution to hemoconcentration in the mammal. This hemoconcentration is thought to be due to myocardial depression (Crowell and Guyton, 1962)

and/or failure of some component of the vascular bed (Lansing and Stevenson, 1957; Lillehei et al., 1964; Rothe and Selkurt, 1964; Bond et al., 1977). Mortality increases as decompensation progresses.

The body maintains the mean arterial blood pressure (MABP) during blood loss by immediate (sympathetic) and delayed (renal) mechanisms. A dog can adequately compensate for a ten percent blood loss. A ten percent blood loss in the chicken will cause a large fall in the MABP which could reduce the Pc shifting the Starling equilibrium favoring fluid influx across the capillary wall. The resultant influx of low protein fluids, primarily from the skeletal muscles, increases the plasma volume. This is the cause of the hemodilution, i.e., the greatly reduced hematocrit (HCT) and hemoglobin (Hb), seen during early hemorrhage in bird and mammal.

Hollandberg and Nickerson (1970) found Pc began to rise in the dog at the onset of the decompensatory stage due to a fall in the pre/post capillary resistance ratio which facilitated the efflux of fluids across the capillary wall. This fluid loss into the surrounding tissues had to be replaced by reinfusion of the shed blood to maintain the MABP at a given level of hypotension. Once about 25 percent of the shed blood had been reinfused, reinfusion of all of the shed blood was insufficient to prevent the subsequent cardiovascular and circulatory collapse and death in irreversible hemorrhagic shock. Avian species may not experience this fall in pre/post capillary resistance ratio since reinfusion of the shed blood will almost always reinstate normal cardiovascular function and MABP.

The increase in Pc in a mammal for any given arterial or venous pressure during decompensation is thought by some researchers to involve an unchanged post capillary resistance and a decreased precapillary

resistance for a given flow rate. The fall in precapillary resistance may be due to neurogenic failure (Rothe and Selkurt, 1964) or may be linked to a hyperkalemia-acidosis induced dilatation of the vascular smooth muscle (Bond et al., 1977). Diana and Laughlin (1974) found that secondary and tertiary to the importance of increased Pc in influencing the transvascular fluid movement were increased capillary surface area and increased capillary porosity, respectively.

Djojosugito et al. (1968) found that the duck had about three times the capillary surface area of the cat. Folkow et al. (1967) found that submersion, in diving duck species, elicited an intense reduction in Pc. These findings may partially explain why Kovách and Balint (1968) and Wyse and Nickerson (1971) found a high rate of transcapillary fluid influx in avian species. It should be mentioned here that the diving duck and the chicken are quite dissimiliar in regard to certain hematological and cardiovascular parameters.

Folkow et al. (1967) have found that the hind limb hyperemia which can "break through" neurogenic vasoconstriction in mammals could not "break through" neurogenic vasoconstriction in the diving duck species. This lack of dilatation was due, in part, to the inherent differences in mammalian and avian extramuscular arteries. Such avian vessels are narrower, more numerous, and more densely adrenergically innervated. The adrenergic nerves run along the vessel inasmuch as sectioning of the sciatic nerve didnot prevent the constrictor response. The turkey behaves more like the mammal in regard to these responses.

Wyse and Nickerson (1971) found the post-hemorrhage transcapillary fluid influx in the hen to be twice the rate and four times the volume of the dog. They noted that small blood losses (4 to 5 ml/kg) lowered

the MABP by 20 mm Hg, and a 15 to 20 percent reduction in blood volume reduced the MABP to 50 mm Hg. In the dog about 20 ml/kg of blood must be removed to lower the MABP by 20 mm Hg (Grega et al., 1967). However, dogs in alpha-adrenergic blockade showed an initial bleeding volume (IBV) similar to a chicken. Half of a dog's blood volume must be removed to lower the MABP to 50 mm Hg. The IBV is the amount of blood removed to initially lower the MABP to 50 mm Hg and the secondary bleeding volume (SBV) is the volume removed subsequently. The maximal bleeding volume (MBV) is the total amount of blood removed from the animal i.e. the sum of the IBV and SBV. The SBV/IBV ratio for the dog was about 0.1 whereas the ratio was about 2.0 for the bird (Wyse and Nickerson, 1971). This suggested that avian regulatory mechanisms acted to maintain blood volume, whereas mammalian regulatory mechanisms acted to maintain the MABP.

Mammalian pressoreceptors communicate with medullary control centers through afferent tracts within the vagus and glossophyaryngeal nerves. These control centers then elicit the proper compensatory adjustments in central nervous system outflow to maintain a constant MABP. The primary mammalian baroreceptors are located at the bifurcation of the internal and external carotid arteries and on the aortic arch. Low pressure receptors exist in the atria, ventricles, veno-atrial junctions, lungs, and elsewhere within the thorax and abdomen. Compensation for decreased blood pressure is by way of vagal inhibition and an enhanced sympathetic outflow, i.e. a heightened cardiac inotropic state and peripheral vasoconstriction. Rothe and Selkurt (1964) found there was a failure of neurogenic control in late hemorrhagic shock characterized by a decrease in total peripheral resistance, respiratory rate, and HR. Blood chemistry

is also sensed by chemoreceptors located on the carotid arteries, aorta, lungs, and elsewhere.

Though avian species do have carotid bodies and pulmonary chemoreceptors homologous to mammalian species, the existance of carotid or aortic baroreceptors has not yet been documented. The avian homologue for the carotid sinus is within the thoracic cavity on the carotid artery proximal to the vertebral artery and distal to the subclavian artery. Avian baroreceptors have been identified histologically (Chowdhany, 1953) but not physiologically. McGinnis and Ringer (1967a) performed bilateral occlusion of the carotid and/or vertebral arteries in the hen and found no reflex tachycardia or pressor response. Prolonged bilateral ligation of carotid and/or vertebral arteries for two weeks produced no apparent brain damage and no cardiac hypertrophy (McGinnis and Ringer, 1965). Occlusion studies in mammals generally produce cardiac hypertrophy (Best and Taylor, 1961). Bilateral carotid and/or vertebral occlusion in the hen produced no effects typical of a baraceptor when the cerebral perfusion pressure, measured by a carotid cannula inserted craniad, decreased by 64 percent, i.e. to about 30 mm Hg (McGinnis and Ringer, 1967b). This all indicated that a functional baroreceptor does not exist in the head of the fowl. Results following bilateral vagotomy were similar. Avian baroreceptors will be elaborated upon subsequently in this review.

B. Alpha-Adrenergic Blockade

The alpha-adrenergic antagonist phenoxybenzamine, trade name Dibenzylene, behaves the same in mammals and birds. Phenoxybenzamine (PBZ) produces a noncompetitive blockade of alpha receptors, i.e. increasing the dosage of an alpha-agonist will not overcome the blockade.

This is due to the drug binding in a very stable manner to the receptors of other nearby structures in a persistant manner. Phentolamine, another alpha-adrenergic antagonist, causes a competitive blockade of alpha receptors that can usually be overcome by increasing the availability of an agonist.

The drug has a multitude of actions. It causes postural hypotension in humans. Phenoxybenzamine inhibits reflexogenic pressor responses by preventing transmission of nerve impulses to the blood vessel cells. The positive inotropic and chronotropic effects of catecholamines on heart muscle is not affected by PBZ, but it may reduce the arrhythmias caused by catecholamines.

Phenoxybenzamine, administered by slow i.v. infusion has been suggested as a treatment procedure for preventing ischemia of the organs and microcirculation during shock in animals.

Phenoxybenzamine has been shown to reduce the mortality of shock due to trauma, bacterial endotoxin, adrenaline infusion, and superior mesenteric artery occlusion (Gregerson and Root, 1947; Lillehei et al., 1961; Nickerson, 1961; Lillehei et al., 1964; and Hollandberg et al., 1970). The usefulness of treating hemorrhagic shock with PBZ is uncertain. PBZ has been shown to increase the blood volume (Nickerson, 1961) and prevent the severe vasoconstriction of shock (Clauss and Ray, 1968; Hollenberg and Nickerson, 1970; and Carlson et al., 1976). Hollenberg and Nickerson (1970) found PBZ pretreatment delayed the onset of decompensation and reduced the rate of reuptake of blood from the reservoir, and so prolonged survival.

However, Grega et al. (1967) found PBZ decreased the rate of transcapillary fluid efflux, but not at a rate sufficient to increase the

survival time when administered as an intermediate treatment. These researchers did find intermediate treatment with strong beta-adrenergic agonists to be beneficial. Chien (1967), in his comprehensive review, also held doubts about the merit of PBZ in treating shock. In view of this PBZ controversy, a brief review of shock related articles concerning PBZ is in order.

Nickerson (1961) found PBZ would increase the blood volume in normotensive dogs. Stekiel et al. (1967) found PBZ had no effect on plasma volume in normotensive dogs, but did increase the plasma volume in hypertensive dogs. Williams and Rodbard (1960) found PBZ caused a 26 percent increase in plasma volume in the normotensive chicken, producing an overall increase of 13 percent in blood volume.

Carlson et al. (1976) found a maldistribution of coronary blood flow during hemorrhagic shock in the dog. This involved a fall in the endocardial/epicardial flow ratio. Phenoxybenzamine, 5 mg/kg, delayed but did not prevent this decrease in subendocardial blood flow. The resultant subendocardial ischemia may lead to subendocardial hemorrhages, necrosis, and zonal contraction lesions sometimes seen after shock. The lesions may contribute to the disruption of the contractile machinery of the myocardium, and to the eventual cardiac failure that follows, although this has not been proven experimentally. Hackel et al. (1974) demonstrated that large exogenous or endogenous catecholamine concentrations can cause the lesions. Hyperbaric oxygen during the hypotensive phase (Ratcliff et al., 1963) or beta-adrenergic blockade (Entman et al., 1969) prevented the lesions. Phenoxybenzamine has no effect upon the lesions (Martin et al., 1969). Birinyi et al. (1977) have shown PBZ produced a 32 percent increase in myocardial blood flow in hemorrhaged

dogs indicating that the sympathetic nervous system limits the maximal coronary dilatation during shock.

Fitch et al. (1975) have shown PBZ decreased the vasoconstriction and increased the blood flow to the head of the hemorrhaged baboon by dilating the extraparenchymal cerebral arteries at the base of the skull. This indicated that the sympathetic nervous sytem can regulate the lower limit of cerebral autoregulation of blood flow. Fitch used the radiolabeled xenon gas technique which is not as accurate as other methods for measuring cerebral blood flow, i.e. the Repella-Green method.

Birds, like reptiles, amphibia, and fish, have a renal portal system. Located at the junction of the renal vein and the iliac artery is a valve that governs the flow of blood into the renal vein. Histamine and acetylcholine have been shown to close the value in vitro and epinephrine opens the valve. The avian kidney possesses nephrons with long and intermediate length loops of Henle, like the mammal, and also loopless reptilian-type nephrons confined to the cortex. Birds do possess a juxtaglomerular apparatus (JGA) and utilize the renin-ANG II-aldosterone-ADH mechanism as do mammals.

Plasma renin activity (PRA) increased tenfold in mammals in hemorrhagic shock (Jakschik et al., 1974). Du Charme and Beck (1971) found that the renal pressor system reduced the vascular capacity by about 60 percent that of the nervous system. Alpha-adrenergic blockade alone, therefore, does not overcome the severe vasoconstriction of shock, and ANG II antagonists may be beneficial. Errlington et al. (1973) found that the administration of ANG II converting-enzyme inhibitors increased the survival of dogs in hemorrhagic shock.

Feigen et al. (1977) reported that PBZ, 2.0 to 2.5 mg/kg, greatly

reduced the severe increase in renal vascular resistance seen in shock and so increased renal blood flow, glomerular filtration rate, and urine flow rate. Presumably PBZ dilated the renal afferent arterioles yielding a 50 percent increase in urine flow rate. Phenoxybenzamine increased the renal blood flow by 83 percent above control shock dogs. Therefore, PBZ and an ANG II inhibitor, e.g. cysteine - ANG II, given together may prevent the prolonged ischemia of organs, such as acute tubular necrosis, seen during shock.

Phenoxybenzamine caused a tenfold increase in urinary sodium concentration and did not affect urinary potassium levels in control dogs. Neither urinary electrolyte concentration was changed significantly in PBZ pretreated shocked dogs, however the urine flow rate increased by 50 percent, giving a twofold increase in potassium excretion rate. This potassium loss is beneficial in shock because it alleviates the hyperkalemia (Feigen et al., 1977).

Kashyap et al. (1975) found the arterial free fatty acid (FFA) concentration fell significantly in dogs and rabbits, while there were continuous increases in adipose FFA's until death, in hemorrhagic shock. Phenoxybenzamine pretreatment had no effect upon prehemorrhagic mean arterial or adipose FFA concentrations, but did cause significant post-hemorrhage increases in both of these parameters. Therefore, hypoperfusion of adipose may play an important role in the decreased supply of this major body fuel in shock. Avian plasma FFA levels are comparable to other vertebrates except for the laying hen whose plasma FFA concentration is very high (Christie and Moore, 1972).

Haggendal et al. (1976) found the dilating effect of PBZ was potentiated by supplemental hydrocortisone. Conversly, the dilating effect

of hydrocortisone was greater following PBZ treatment. This latter effect indicated that the vasodepressor effect of the steroid was not due to an alpha-blockade, but rather, it increased the concentration of neurotransmitter at the smooth muscle receptor site. This was accomplished by either inhibiting extraneuronal degradation mechanisms or by preventing reuptake into the neurone. Phenoxybenzamine is known to inhibit the extraneuronal uptake of neurotransmitters (Avakian and Gillespie, 1968). The lingering catecholamines may continue to stimulate beta-adrenergic receptors and initiate a further dilatation, which is supported by the fact that beta-adrenergic blockade has prevented the hydrocortisone-induced vasodilation after PBZ treatment.

Vargish et al. (1977), in their attempt to identify the most beneficial form of steroid therapy in hemorrhagic shock, found dexamethazone, 15 mg/kg, provided the best protection in purebred beagle dogs. Second best was methylprednisolone at 15 and 30 mg/kg.

Harvey et al. (1954), in their early studies with PBZ in the chicken, found the drug to produce vasomotor reversal when given at 20 to 60 mg/kg. Nickerson (1961) found the reversal phenomenon in mammals during alpha-blockade to be due to the unmasking of beta-effects initiated by a non-specific agonist, epinephrine in the case of Harvey. Bunag and Walaszek (1962a) found the dose administered by Harvey to be lethal to hens. Peterson and Ringer (1968) administered PBZ, 39 mg/kg, to adult hens while measuring feather intrafollicular pressure. The drug produced hypotension but no mention was made of the degree of inhibition produced. Kovách et al. (1969) found the pigeon capable of surviving a 100 percent blood volume depletion if the hemorrage was extended over a six hour period. Peripheral resistance increased in response to hemorrhage

(Kovách and Szász, 1968) although treatment with PBZ had no effect on survival (Kovách et al. 1969). The significance of this latter result is unclear since, in the pigeon, PBZ fails to block the vasoconstriction caused by epinephrine. Kovách and Balint (1969) noted that hemodilution occurred continuously until death in the pigeon, whereas in the rat, the hemodilution stopped after about fifteen minutes of hemorrhage. Blockade of alpha-receptors eliminated vasoconstriction in the skeletal muscles of the duck and led to a greatly retarded restoration of blood volume (Djojosugito et al., 1968). Alpha-adrenergic blockade also abolished the intense vasoconstriction in the sciatic vascular bed in the submerged duck (Butler and Jones, 1971). The duck showed a large postdive hyperemia in carotid blood flow which was likely due to the intense postdive tachycardia.

Szeto et al. (1977) produced accurate dose-response curves for the chicken for PBZ, PROP, and atropine. Doses of 5 mg/kg PBZ were found to produce 75 percent blockade of the phenylephrine-induced rise in arterial diastolic pressure. They also found PBZ to be effective within 15 minutes in the hen, unlike the very slow induction in mammals.

Harvey et al. (1954) and Peterson and Ringer (1968) both mentioned the extreme variation in various CVS parameters in birds as compared to the dog or cat.

C. Beta-Adrenergic Blockade

Propranolol, tradename Inderal, causes a competitive blockade of beta-adrenergic receptors. Therefore, large doses of beta-agonists can overcome the beta-blocking effects. The drug produces similar effects in mammals and birds. It prevents the positive chronotropic and inotropic

effects of catecholamines on the myocardium. Propranolol (PROP) has the "quinidinelike" effect of stabilizing cell membranes. It also causes a prolongation of the atrioventricular conduction time and decreased upstroke velocity and overshoot of the cardiac action potential. Its advent in the late sixties as an antiarrhythmatic and antihypertensive medication had tremendous impact upon the treatment of various CVS disorders in man. It has reduced the mortality in humans due to arrhythmias by 50 percent (Rowe, 1974).

Investigators advocating alpha-adrenergic blockade in shock seek to obviate the deleterious effects of prolonged vasoconstriction leading to the ischemic damage of vital organs. Conversely, other investigators recommend beta-adrenergic blockade based on the supposition that failure of peripheral vasoconstriction after prolonged hemorrhagic hypotension leads to death. Evidence supporting the latter hypothesis is scant.

Berk et al. (1967) found equal mortality (78 percent) in untreated dogs and dogs in beta-blockade subjected to hemorrhagic shock. Increased survival was observed only if the PROP was coupled with administration of atropine, oubain, hypertonic glucose, sodium bicarbonate and calcium chloride. Zierott et al. (1969) found PROP reduced the oligemic period and was not beneficial. Halmagyid et al. (1967) found that PROP and PBZ given together provided additional protection against shock in dogs. Later, Wood et al. (1974) found likewise and suggested that this protective action was the result of the combined drugs' suppression of the large endogenous catecholamine concentration during compensation.

It is generally conceded that PROP does not have any beneficial effects for the mammal in hemorrhagic shock. Beta-blockade has relieved the systolic reduction in coronary blood flow seen in hemorrhagic shock

and decreased the incidence of subendocardial lesions by relieving the severe beta-adrenergic stimulation (Rowe, 1974). These effects are far outweighed by the following deleterious effects: reduced absolute coronary blood flow, decreased cardiac output (CO), increased coronary vascular resistance, depressed general inotropic state of the myocardium, decreased heart rate and decreased stroke work.

Most birds have two main coronary arteries, while some avian species have three or four coronary arteries. The right coronary artery is always larger. There are four primary coronary veins. The avian heart is similar anatomically to the mammalian heart except the right AV valve consists only of a heavy muscular flap. The avian heart also represents a much higher percent of the body weight than in mammals. Basal resting heart rate in the chicken is about 300 b/minute. Myocardial beta-receptors in the chicken are associated with positive inotropic and chronotropic effects. The vagi exert a powerful tonic inhibiting influence on heart rate and the ventricular inotropic state. The avian myocardial tissue does not have t-tubules.

St. Petery et al. (1977) confirmed the existance of alpha- and beta-receptors in the three day chick embryo. Beta-adrenergic blockade was achieved in the chicken by Peterson and Ringer (1968) using dichloroisoproterenol. They found hypotension. Bulton and Bowmen (1969) found alpha-blockade inhibited the hypertension caused by catecholamines and that beta-blockade slightly increased the pressor response in hens. Therefore, the hen has alpha- and beta-adrenergic receptors, but the beta-receptors are dominated by the alpha-receptors. The beta-receptors do, however, have an inhibitory effect upon pulmonary arterial alpha-receptors. Here beta-blockade produced maximum isotonic contraction in vitro because

it removed the beta-adrenergic inhibition of alpha-receptors. Szeto et al. (1977) found PROP to have an effect of short duration in birds, as in mammals. They obtained an 82 percent blockade of isoproternol-induced tachycardia by administering a .25 mg/kg bolus of PROP followed by a 5 ug/kg/minute infusion.

Edens (1974) found the normally-occurring increase in plasma corticosterone in chicks in heat stress was nonexistant after alpha-blockade. He also found PROP, 4 mg/kg, or reserpine would prevent the fall in corticosterone which usually occurs after 80 minutes of heat stress.

When Kregenow et al. (1976) added norepinephrine to an isotonic medium containing duck erythrocytes it initiated a very rapid bidirectional movement of sodim and potassium ions into the erythrocyte. A hypertonic solution caused the erythrocytes to shrink and the cells re-adjusted their volumes by an adenosine 3'5'-cyclic physphate (cAMP) facilitated change in cation permeability. Isoprotenenol bound to beta-receptors and increased cAMP, but the binding was not necessary. PROP has been shown to be a potent inhibitor of adenyl cyclase activity, but a weak inhibitor of binding in the turkey erythrocyte (Bilezikian and Aurbach, 1973).

Beta-adrenergic blockade did not have any effect on the CVS response to submersion in the duck, but did reduce the immediate hyperemia and abolished the rise in MABP normally occurring upon emersion (Butler and Jones, 1971).

D. Normovolemic Anemia

Sustained hemorrhagic hypotension produced a 50 percent reduction in HCT and plasma protein concentrations in the fowl (Wyse and Nickerson,

1971), thus a brief discussion of normovolemic anemia is in order. Acute normovolemic anemia is accomplished by exchanging whole blood with six percent dextran-70.

In the dog a dextran exchange sufficient to reduce the HCT from 36 to 13 percent produced a 91 percent increase in CO. The HR, stroke volume, and LVEDP all increased, whereas the TPR decreased. Beta-adrenergic blockade at this time decreased the CO and HR and increased the TPR. Therefore, the sympathetic nervous system plays a large role in the physiological response to normovolemic anemia.

Nightengale (1976) produced acute normovolemic anemia in the chick by replacing one percent of the body weight, i.e. about one-sixth of the blood volume, with six percent dextran-70. This reduced the HCT by 50 percent. Tissue oxygen delivery was maintained by increased extraction of the oxygen coupled with increased stroke volume and CO. The TPR decreased. The HR, right atrial pressure and oxygen consumption ($\dot{V}O_2$) were not changed. Further decreases in HCT and Hb resulted in CVS collapse as indicated by a rapidly fallen CO, stroke volume and $\dot{V}O_2$. Wyse and Nickerson (1971) found that a similar CVS collapse, occurring at any point in time during the hypovolemic procedure, could be corrected by a rapid reinfusion of the shed blood.

A two percent dextran-70 exchange provided the maximum CVS adjustment in the chick. Exchanges of four to six percent of the body weight were required in the dog to produce the same hemodilution effect. Dextran-70 exchanges of one through three percent of the body weight produced progressive increases in the plasma sodium and potassium concentrations in the chick (Nightengale, 1976).

E. Other Factors in Shock

It has been well established that extracellular osmolarity and the concentrations of potassium, hydrogen and magnesium ions become elevated in far advanced hemorrhagic shock (Schwinghamer et al., 1970). These may be involved in the gradual diminution of the compensatory constriction since all of these electrolytes caused vasodilation in the systemic circulation in test systems. Bond et al. (1977) found that hyperkalemia in shocked dogs may be due to hypoxic inactivation of the electrogenic pump activity, but is more likely due to a passive movement of potassium ions out of the muscle cell with the bicarbonate anion in response to the intracellular accumulation of hydrogen ions. The direct inhibition of vascular smooth muscle by hyperkalemia and acidosis may be one of the causes of vascular decompensation during late shock in mammals.

Corticoids augmented intracellular sodium, which increased smooth muscle tone and contractile responsiveness which,in turn, increased the MABP (Clauss and Ray, 1968). The corticoids also caused a rapid extrusion of intracellular potassium, which diminished the transmembrane gradient of potassium, resulting in increased CVS contractility and MABP in mammals. Electrolyte changes during hemorrhage in avian species have not been documented.

Histamine, released from the mast cells during trauma, infection, exercise, etc., has been shown to be a potent vasodilator of arterioles and caused increased capillary permeability. Elevated histamine levels resulted in degeneration of the microvasculature. Trauma resistance has been obtained from the injection of spleen extracts prepared from trauma-conditioned rats, RES stimulation, and trauma conditioning. This may have been due to the RES which recognized high histamine levels and

elaborated mediators to prevent the histamine-induced destruction of the microvasculature beds (Galvin et al., 1977). Harvey et al. (1954) demonstrated that histamine was released in response to serotonin (5-HT) in avian species. Bunag and Walaszek (1962b) reported that histamine was found in high concentrations in avian plasma, was readily released, and was depressor in the chicken.

The humoral agent "myocardial depressant factor" (MDF) is another consideration in shock. Discovered in mammals, MDF is thought to be a small peptide produced by lysosomal hydrolases in the ischemic pancreas. MDF depresses both the inotropic state of the myocardium and the RES. Lefer and Martin (1970) found that the MDF titer correlated inversely with survival time in hemorrhaged dogs. Phenoxybenzamine may cause hyperperfusion of the pancreas and diminish MDF release. This factor has not been isolated, and may not exist, in avian species.

Rothe and Selkurt (1961) have demonstrated the existance of dilating agents of intestinal origin in the portal blood of the hypovolemic dog.

Splanchnic pooling is another consideration in irreversible hemorrhagic shock. Abel et al. (1965) reported the primate did not demonstrate the congestion and bowel necrosis encountered in the dog. Pulmonary pooling may be another site of blood loss from the circulating blood volume although Abel et al. (1967) have cast serious doubt on this theory.

Plasma glucose concentration increased initially in hemorrhagic and endotoxin shock in the mammal then quickly fell to acute hypoglycemia (Strawitz et al., 1961, Hinshaw et al., 1976). The hypoglycemia was coupled with a decreased sensitivity to insulin which decreased glucose transport. Moffat et al (1968) found that a glucose infusion late in

shock can prolong survival beyond that of control animals. Drucker et al. (1975) showed that a low protein/high carbohydrate diet decreased the tolerance to hemorrhagic shock. High protein diets did not increase the tolerance.

Plasma glucose concentrations in man and hen are 80 mg% and 180 mg%, respectively. The total plasma protein concentration of the chicken is about 5.3 gm%, whereas this value is 7.3 gm% in man. The colloid osmotic pressure of the hen is only 11.1 mm Hg (Albritton, 1952) compared to 28 mm Hg in man. The difference is due to the low albumen/globulin ratio in the chicken (0.8) as compared to that of man (2.0). Wyse and Nickerson (1971) and Kovách and Balint (1968) have reported that hemorrhage can reduce the plasma total protein concentration by as much as 35 percent in the fowl.

II. Respiratory Homeostasis

A. Avian Blood Gases and pH

Mammals in hypovolemic shock have generally maintained arterial pO_2 indicating an adequacy of oxygen exchange (Bond et al., 1977). A mild respiratory alkalosis in early shock in mammals resulted in a low pCO_2 in the arterial blood. This alkalosis soon converted into a severe acidosis and the arterial pH dropped as low as 7.00 (Bond et al., 1977). The changes in blood gases during hemorrhage in avian species have not been documented.

The measurement of avian pH with direct-reading radiometer electrodes must be corrected for the high avian body temperature, i.e. 40.5°C. If the radiometer bath is at mammalian body temperature, i.e. 37°C, the Rosenthal (1948) formula makes the correction as follows:

Blood pH = pH_t - .0147 (37 - t).

Shepard et al. (1959) reported arterial pCO_2 values of 40 mm Hg in the deeply anesthetized hen. This is similar to man at rest. However, numerous subsequent investigators found lower pCO_2 levels. Edens and Siegel (1974a) reported pCO_2 values of 26 mm Hg in lightly anesthetized hens. Choidi and Terrman (1965) may have been the most accurate in their assessment of blood gases from the locally anesthetized, lightly restrained hen. They found a pCO_2 of 32.8 mm Hg and an arterial pH of 7.49 by comparing samples to reference pH-pCO_2 curves obtained from two aliquots equilibrated at different known CO_2 levels.

This variation in reported values may be due to stress hyperventilation. Hyperthermia-induced panting in adult hens increased the arterial pH above 7.70 reducing the pCO_2 below 15 mm Hg and the CO_2 content to below 10 mEq/L (Calder and Schmidt-Nielson, 1968; Frankel and Franscella, 1968). This suggests a need for telemetric studies with in-dwelling cannulas for unstressed blood sampling.

Account should also be taken for wide swings in acid-base balance associated with age, nutrition and laying cycle. Helbacka et al. (1964) demonstrated significant effects of feeding and starvation on plasma pCO_2. Egg calcification over a 16 hour duration has been shown to decrease plasma bicarbonate content and to decrease plasma pH from 7.52 to 7.42 (Mongin and Lacassagne, 1966). Sodium phenobarbital anesthesia did not affect pH, pCO_2, or bicarbonate levels in avian species, but may have decreased the pO_2 slightly (Edens and Siegel, 1974b). Cohen and Horwitz (1974) have found a high sodium diet will increase plasma pH in hens.

B. Intrapulmonary Receptors in Aves

Early researchers thought that high CO_2 inhalation decreased

respirations in the hen, but this was only due to the stimulation of chemoreceptors in the nares, tongue, and pharynx.

Birds breathing a zero percent CO_2 gas mixture in air experienced apnea after hyperventilation (Ray and Fedde, 1969). A five percent CO_2 mixture produced normal respirations in deeply anesthetized hens (Fedde et al., 1963). A 20 percent CO_2 mixture produced an increase in MABP and a 200 to 300 percent increase in muscle vascular resistance reaching a maximum in three to five minutes in the diving duck species (Peterson and Fedde, 1968a). Attempts to create this vasoconstriction in the cat or turkey with a 20 percent CO_2 mixture were unsuccessful (Hiestand and Randall, 1941). Inspiratory minute volume, tidal volume and hydrogen ion concentration all increased as the inspired CO_2 increased (Osborne and Mitchell, 1978).

The existance of intrapulmonary chemoreceptors was demonstrated by Hiestand and Randall (1941). They found a rapid decrease in inspired CO_2 would produce a rapid fall in ventilation within 0.5 seconds even when the pulmonary veins and arteries were occluded. Conversely, the administration of high CO_2 concentrations via low trachae (Ray and Fedde, 1969) or humori (Jones and Purves, 1970) stimulated respirations. The maximal respiratory response occurred at an arterial pCO_2 of 40 to 55 mm Hg.

Avian intrapulmonary chemoreceptors sensitive to CO_2 play a major role in the control of breathing during normocapnic and hypocapnic conditions (Osborne et al., 1977). The impulse frequency in vagal afferent fibers from these intrapulmonary chemoreceptors increased when the intrapulmonary CO_2 was decreased. The receptors were not sensitive to hypoxia, hyperoxia (Tschorn and Fedde, 1974), lactic acid, sodium

cyanide, acetylcholine (Fedde and Peterson, 1970),or changes in intrapulmonary pressure.

Afferent fibers from intrapulmonary mechanoreceptors run centrally in the vagus nerve. Unlike the mammalian slow adapting stretch receptors which respond to changes in both CO_2 and mechanical stimuli, the avian receptors are not sensitive to changes in CO_2 concentrations (Fedde et al. 1974).

C. Intracardiac Receptors in Aves

Cardiac ventricular CO_2 receptors have been identified in the bird (Estravillo and Burger, 1973). Such chemoreceptors may extend into the aorta. The magnitude of the tidal volume is inversely related to the ventricular-receptor discharge frequency along the afferent fibers of the middle cardiac nerve. Electrical stimulation of this nerve depressed the respiratory rate in the same manner as low CO_2 inhalation.

A transient rise in MABP, which increased the rate of receptor activity, also produced a decreased respiratory rate for the duration of the pressure rise (Estravillo and Burger, 1978). Information supporting any functional description for ventricular mechanoreceptors is lacking. However, recent work has shown that inflation of a balloon in the left ventricle produced inhibition of spontaneous breathing in closed-chest dogs on cardiac bypass (Kostreva et al., 1977).

D. Other Receptors in Aves

Rodbard and Saiki (1952) hypothesized that an intracranial baroreceptor mechanism may control cerebral blood flow in the chicken.

Although nerve fibers from the nodose ganglion of the vagus nerve terminate in the region of the carotid sinus and aortic wall, no functional

baroreceptor has been identified in these areas.

The avian carotid body is innervated by a branch of the vagus nerve which arises from the nodose ganglion. Hollandberg and Uvnas (1963) presented evidence that indicated stimulation of the carotid bodies was responsible for the circulatory changes, i.e. bradycardia, increased blood pressure, decreased splanchnic and cutaneous blood flow and little change in skeletal muscle blood flow, which occurred in diving ducks during submersion asphyxia. Carotid body denervation abolished these responses. The carotid bodies of the chicken do not appear to play a role in respiratory control inasmuch as ventilatory sensitivity to inhaled CO_2 was not greatly affected by hypoxia (Jones and Purves, 1970).

Atland (1961) found that the hypoxic tolerance of the fowl was lower than other small animals. His birds died as a result of exposure to an inspired pO_2 of 46 to 73 mm Hg. In support of Atland, Richards and Sykes (1967) found that alterations in the avian electroencephlogram began at about 70 percent oxygen saturation. This was much higher than in mammals (Brechner et al., 1965). The hyperventilation alkalosis in hens produced vasoconstriction which may have offset the local dilation of the cerebral arterioles occurring in hypoxia (Paff and Boucek, 1958). This may have resulted in failure of the medullary cardiovascular and respiratory centers.

Much research by the Scandanavian group has been directed at determining the relative roles of chemoreceptors and baroreceptors in the CVS responses to diving in the duck (Blix et al., 1975). Jones (1973) found that the bradycardia, obtained during a 1 to 2 minute submergence in the chronically denervated duck, was identical to that in intact ducks, though the MABP fell greatly in the denervates due to a relatively lower vascular

resistance. Even so, the sciatic vascular resistance in the denervates was still half that of the intact ducks indicating that half of the increase in the TPR was achieved independently of baroreceptor stimulation. Jones and West (1978) performed a constant-flow hind limb perfusion on the duck during submergence and at the same time electrically stimulated the one intact depressor nerve innervating the baroreceptor. Submergence caused bradycardia and increased vascular resistance, and nerve stimulation caused a fall in HR, MABP, and perfusion pressure. Hind limb perfusion studies have not been reported for the chicken.

Mechanoreceptors may exist in many of the visceral organs of the bird (Duke et al., 1977). The function of these receptors is unknown.

Aortic bodies have been found in the connective tissue between the ascending aorta and the pulmonary artery of the chicken (Tcheng et al., 1963). These researchers suggested a possible chemoreceptor or baroreceptor function for these bodies in the bird. They are innervated by a branch of the vagus nerve.

E. Hypoxia in Aves

Whereas hypercarbia produced linear increases in MABP and HR in the duck, Ray and Fedde (1969) reported that hypoxia decreased the arterial diastolic blood pressure. Besche and Kadono (1978) have shown that the mean femoral blood pressure decreased linearly in progressive hypoxia. The hypoxia-induced hypotension involved an unchanged HR (Butler and Taylor, 1974), bradycardia (Sturkie, 1970), or tachycardia (Besche and Kadono, 1978). The latter have reported that an eight percent reduction in inspired oxygen, i.e. from 21 to 13 percent, caused a 35 percent reduction in TPR in the adult Leghorn-type hen. Recently, Grubb and Schmidt-Neilson (1978) employed the xenon clearance technique for measuring cerebral

blood flow in the duck during hypoxia. The hyperventilation-induced respiratory alkalosis and hypocapnia did not alter cerebral blood flow in the duck, whereas mammals vasoconstrict under similar conditions reducing the cerebral blood flow by 50 to 75 percent.

Although the fowl can adapt well to chronic hypoxia, it has little tolerance to acute hypoxia compared to mammals and other species of birds. The effects of acute hypoxia in the chicken include: decreased body temperature and oxygen consumption, increased respiratory rate, plasma volume, and HR. However, Richards and Sykes (1967) have reported a decreased respiratory frequency in the hen in acute hypoxia. The cardiac output did not change during acute hypoxia in the chicken (Besche and Kadono, 1978). The decreased blood pressure coupled with normal CO values suggests that marked changes may have occurred in the vascular beds, i.e. possibly a redistribution of blood flow.

Sturkie and Abati (1978) showed that a large drop in cardiac contractility occurred in various avian species' isolated hearts when made hypoxic by substituting 95 percent nitrogen for 95 percent oxygen in the perfusion fluid. The drop ranged from 15 percent of normal for the chicken to 52 percent of normal for deep-diving ducks. The pigeon was intermediate at 31 percent. This demonstrated how demand for, and utilization of, oxygen, and the tolerance to oxygen deficiency differ greatly between diving, flying, and terrestrial birds.

OBJECTIVES

1. To determine the effects of propranolol or phenoxybenzamine on heart rate, arterial blood pressure, cardiac index, stroke volume, stroke work, and total peripheral resistance in adult SCWL hens thirty minutes after treatment.

2. To determine the effect of sustained hypovolemic (hemorrhagic) hypotension without pretreatment and following pretreatment with phenoxybenzamine or propranolol in adult SCWL hens on heart rate, arterial blood pressure, cardiac index, stroke volume, stroke work, total peripheral resistance, bleeding volumes, survival times, and mortalities.

3. To determine the effect of sustained hypovolemic hypotension without pretreatment and following pretreatment with phenoxybenzamine or propranolol in adult SCWL hens on hematocrit, hemoglobin, plasma potassium, plasma magnesium, plasma sodium, and plasma glucose concentrations.

4. To determine the effect of sustained hypovolemic hypotension without pretreatment and following pretreatment with phenoxybenzamine or propranolol in adult SCWL hens on arterial pH, arterial pCO_2, arterial pO_2, and the incidence of respiratory arrest.

MATERIALS AND METHODS

I. Experimental Stock -- Mature SCWL hens (1.5 to 2.2 kg.), after a year of egg production at the Poultry Science Teaching and Research Facility, were housed in laying batteries at a constant environmental temperature for at least five days prior to use. Not all birds were producing eggs. Water and cage layer ration were supplied ad libitum. Feed was withdrawn twelve hours prior to each experiment. The birds were on a 14 hour light cycle. The research was conducted in the spring and summer. Thirty hens were divided into five equal groups.

II. Anesthesia -- Each animal was lightly restrained in a supine position on a small animal board. The legs were tied down and a wire was inserted through the nares to restrict movement of the head. The hens were anesthetized with sodium phenobarbital, 100 mg/kg, via the brachial vein. This plane of anesthesia is considered "light anesthesia." Toe pinch would produce slight withdrawal and comb pinch would produce vigorous head shaking. This anesthetic was chosen for its long action and margin of safety. Each bird then received an intracutaneous injection of about 0.5 ml of a two percent procaine solution at the surgical site on the ventral surface of the upper neck. After all surgical procedures, a piece of toweling was placed over the birds' head because this can produce a calming effect.

III. Surgical Procedure -- The carotid arteries and the jugular veins were exposed by making a three centimeter midline incision on the ventral surface of the neck in the upper cervical area. The right carotid

artery was then dissected free from the connective tissue and M. longus colli at about the level of the second cervical vertebra. A permanent ligature was placed around the cranial end of the exposed artery. A nick was made in the artery and a fluid filled polyethylene cannula (Intramedic Clay-Adams, New York, N.Y., PE-90: I.D. = .034", O.D. = .050") containing sodium heparin (0.25 mg/ml) was inserted about five centimeters toward the heart.

The right jugular vein was teased from all connective tissue. The ramifications of the vagus and glossopharyngeal nerves were carefully teased from a two centimeter segment of the vessel. The vessel was then looped with suture material for ease of identification for the subsequent dye injections.

Low-neck tracheotomy was performed to prevent the congestion in the larynx region often seen in avian species and for ease of artificial ventilation should the need arise. The tracheal tube was suctioned periodically with a suction catheter.

The animals were heparinized with 2.5 mg/kg sodium heparin via the carotid cannula upon completion of the surgical procedures. Heparin was readministered at two hour intervals.

IV. Drug Administration -- The treatment groups were set up in the following manner:

GRP #	TREATMENT	DOSE	n
1	sham operated, sample replacement	----	6
2	hemorrhaged	----	6
3	hemorrhage and PBZ	5 mg/kg	6
4	hemorrhage and PROP	.25 mg/kg + 5 ug/kg/min	6
5	sham operated, no sample replacement	----	6

Group #1 sampling blood losses were immediately compensated for by the infusion of equal volumes of donor blood.

All drugs were administered via the brachial vein. Phenoxybenzamine was injected 30 minutes prior to hemorrhaging. Birds receiving PROP underwent left brachial vein cannulation (Intramedic, PE-90) in the direction of the heart. A continuous infusion was necessary due to the shortness of action of this drug. The brachial cannula was attached by way of a three-way stopcock to a five ml glass syringe fitted into an infusion/withdrawal pump (Model 940, Harvard Apparatus Co., Millis, Mass.). The infusion was started 30 minutes prior to hemorrhaging.

V. Shock Protocol -- The experiment lasted 225 minutes. Every 45 minutes, beginning at time zero (T=0), blood samples were obtained and arterial blood pressure and EKG were recorded.

Starting at T=0, blood was withdrawn from the bird through the carotid cannula. This blood was shunted through the pressure transducer, through a dye tracer cuvette, and then into a lubricated glass 10 ml syringe fitted into a second infusion withdrawn pump (Model 950, Harvard Apparatus Co., Millis, Mass.). Bleeding rate was about one ml/kg/minute. Blood was quickly pumped from the infusion/withdrawal pump in ten ml intervals into a graduated cylinder sitting in a constant temperature water bath (Buchler Instruments, Fort Lee, N. J.). Reserve blood was agitated by a stream of room air.

Blood was removed until the mean arterial blood pressure (MABP), as measured periodically from the carotid cannula, reached 50 mm Hg. The amount of blood removed to reach this level of hypotension was designated the Initial Bleeding Volume (IBV). The IBV was generally obtained within

the first 15 minutes of the experiment. Subsequent small amounts of blood had to be removed to maintain the MABP at 50 mm Hg. This volume was designated the Secondary Bleeding Volume (SBV). The total volume of blood removed from the bird prior to death, or upon surviving 225 minutes of hypotension, was designated the Maximal Bleeding Volume (MBV).

Birds entering respiratory arrest were artificially ventilated (Harvard Small Animal Respirator, Harvard Apparatus Co., Millis, Mass.) for no longer than ten minutes, after which, the respirator was discontinued and the bird observed.

When a bird entered cardiovascular (CVS) collapse, as indicated by a rapid fall in the MABP, the right jugular vein was cannulated (Intramedic, PE-160, I.D. = .045", O.D. = .062") and the reserve blood reinfused at a rate not exceeding 2 ml/kg/minute. This would generally re-establish CVS status provided the fall in MABP was not of sufficient magnitude to prevent reversal.

VI. Blood Pressure Measurement -- The blood pressure was monitored via the carotid cannula by a Statham physiological pressure transducer (PA-23AC) connected to a Grass 7A polygraph. The MABP was obtained by electronically dampening the pressure oscillations. This was later determined more accurately by the following formula:

MABP = diastolic pressure + 3/8 pulse pressure (Sturkie, 1967).

Calibration of the recorder was accomplished by using either a pocket anaeroid barometer connected directly to the transducer or by the internal calibration mechanisms contained within the polygraph.

VII. Heart Rate Determination -- The HR was obtained from the BP tracings. Measurements were made over a six or ten second interval and

multiplied by ten or six,respectively. A three lead EKG was taken every 45 minutes to check for arrhythmias.

A chart speed of 10 or 25 mm/minute was used throughout the experiment, but every 45 minutes the chart speed was accelerated to 25 mm/second to obtain accurate BP and HR measurements.

VIII. Blood Chemistry Analysis -- Every 45 minutes, beginning at T=0, a 1.5 ml arterial sample was drawn through the carotid cannula after clearing the cannula dead space. Twenty microliters of this whole blood was used to determine the hemoglobin (Hb) concentration by the cyanomethemoglobin method (Lynch et al., 1969). The remaining blood was separated by centrifugation at 1000g for 15 minutes at 10°C (Sorvall RC-5 Superspeed Automatic Regrigerated Centrifuge). The plasma thus obtained was placed into small, capped glass vials that had been previously rinsed with triple distilled deionized water. These plasma samples were then frozen for subsequent analysis of magnesium, potassium, sodium, and glucose concentrations.

Every 45 minutes, beginning at T=0, three to five tenths of a ml of arterial blood was carefully withdrawn from the carotid cannula into a one ml syringe. This syringe was quickly capped and placed into an ice bath in a freezer for subsequent blood gas and pH analysis.

Synchronous with the arterial samplings, blood was withdrawn by venipuncture of a small shank or wing vein into a heparinized Micro-Capillary tube. This was centrifuged for ten minutes using an International Micro-Capillary Centrifuge (International Equipment Co., Boston, Mass.). Hematocrit values (HCT) were then determined on an International Micro-Capillary Reader.

Plasma sodium and potassium concentrations (mEq/L) were determined

in duplicate on a Beckman Flame Photometer (Model 105) using a 900 part per million lithium internal standard and appropriate plasma dilutions (Appendix A). Plasma magnesium was determined on a Perkin Elmer Atomic Absorption Spectrophotometer (Model 290B BMS3 Mark-2) using a lanthanum oxide diluent (Appendix A). Plasma glucose was determined by a glucose oxidase/peroxidase enzyme method (Sigma Chemical Co. as per Technical Bulletin #510). Blood gas and pH determinations were done on a direct-reading radiometer (BMS-3, Mark-2 Blood Micro System with a PHM Mark-2 Digital Acid Base Analyzer, Copenhagen, Dk.).

IX. Cardiac Output Determinations -- Cardiac output was measured by a dye dilution technique (Hamilton et al., 1932) at T=0, T=90, and T=180. The two groups receiving drug treatment had cardiac output (CO) determinations at T=-30 and T=0 only, because preliminary experiments showed the procedure was often fatal at a later time. During the CO procedure about four to five ml of blood were withdrawn from the carotid cannula through a dye tracer cuvette (General Medical Electronics, Middleton, Wisc.) at a rate of 10.3 ml/minute into a lubricated glass syringe fitted into an infusion/withdrawal pump (Model 950, previously mentioned).

The output signal of the GME dye tracer cuvette control unit was amplified by a Grass Model 7PL low level DC preamplifier and ultimately was recorded on an Esterline Angus single input analog recorder (Model E1101E with a twelve inch scale) at a chart speed of twelve inches per minute. This apparatus was calibrated by passing in vitro blood samples from each bird containing zero or ten mg of dye per liter of blood through the dye cuvette at the end of each experiment.

The baseline was set as blood was drawn through the cuvette. Two to three tenths of a mg of the concentrated cardio-green dye (Hyson,

Westcot, and Dunning, Inc., Baltimore, Md.) was injected rapidly into the exposed right jugular vein. When the recirculation effect was evident in the dye concentration curve, the pump was reversed and the blood re-infused.

The area under the curve was estimated with a compensating polar planimeter (Model 4236, Keuffel and Esser Co., Hoboken, N.J.) following extrapolation of the declining limb of the curve onto semilogarithmic paper and replotting the limb to within one percent of the baseline to eliminate recirculation effects (Appendix B). The CO and cardiac index (CI) were then calculated by the following formulae:

$$\text{Cardiac Output (CO)} = \frac{\text{mg dye injected}}{\frac{\text{area under curve}}{\text{mg min/liter}}} = \text{L/mihute}$$

$$\text{Cardiac Index (CI)} = \frac{\text{CO in ml/minute}}{(\text{Body weight in kg})^{.734}} = \text{L/min./kg}^{.734}$$

(Speckman and Ringer, 1963)

Stroke volume was estimated by dividing the CI by the HR. Stroke work was determined by multiplying the stroke volume by the MABP. Total peripheral resistance (TPR) was determined by dividing the MABP by the CI.

All birds, those that died during the hypotensive period and those that survived, were necropsied to determine the placement of cannulae, egg laying status, and the occurrence, if any, of subendocardial, hepatic or gastrointestinal hemorrhagic lesions.

X. Statistical Analysis -- One-way analysis of variance (ANOVA), using an f test, was utilized to determine if a significant difference existed between two means.

Comparison of the percent change from the initial value for the final three samples prior to death within and between treatments was done

by split-plot repeated measure ANOVA. Dunnett's test determined if significant differences existed between the hemorrhage group and each of the other three groups. This was followed by linear and quadratic orthogonal polynomial contrasting within each treatment for the final three sampling times to determine if average and/or curvilinear trends existed (Appendix C1).

A similar two-way block-design repeat measure ANOVA was used to analyze variation from the initial CI values, and the variance of parameters derived from the CI, e.g. TPR, stroke volume, and stroke work (Appendix C2).

RESULTS

EXPERIMENT I: A total of twelve hens were utilized in this experiment. All were anesthetized and cannulated as described previously. They were allowed to stabilize for ten minutes before initial measurements of HR, MABP, and CO. Immediately after these initial measurements, at T=-30 minutes, the appropriate drug treatment was administered. One group received PROP (0.25 mg/kg followed by a 5 ug/kg/minute infusion i.v.), the other PBZ (5 mg/kg i.v.). Thirty minutes later, at T=0, these parameters were remeasured.

A. Effect of PROP: One-way ANOVA within threatments showed PROP caused significant reductions in HR ($P<0.01$), CO ($P<0.5$), CI ($P<0.05$), MABP ($P<0.01$, and stroke work ($P<0.05$). The only parameters not affected were stroke volume and TPR ($P<0.05$), although these did not have a nonsignificant tendency to be reduced. ANOVA between treatments showed that PROP caused a significantly greater fall in HR ($P<0.01$) and CI ($P<0.05$) than did PBZ. There was also a tendency, although not significant, for the MABP of the PROP-treated animals to be lower than the PBZ-treated animals. The TPR did not differ between the two drug treatments, although the PBZ-treated animals have a nonsignificant tendency to have a lower TPR (Tables 1A and 1B and Figure 1).

B. Effect of PBZ: One-way ANOVA showed that PBZ did not significantly change any of the measured parameters except for a reduction in the MABP ($P<0.05$). There was a tendency for stroke work and TPR to be reduced. The HCT was significantly reduced ($P<0.05$) by 8.7 percent of the initial value (Table 5). The Hb value was not reduced.

TABLE 1A. The means ± S.E. of changes in cardiac index, mean arterial blood pressure, heart rate, and total peripheral resistance in adult SCWL hens thirty minutes after treatment with propranolol or phenoxybenzamine.

	Propranolol (n-6)[1]			Phenoxybenzamine (n=6)[2]		
	before treatment	30 min. after trt.	percent change	before treatment	30 min. after trt.	percent change
Cardiac Index ($ml/min/kg^{.734}$)	209.5 ±17.1	152.1 ±14.9	-27.5[3]	204.6 ±16.6	196.0 ±11.2	-4.20
Mean Arterial Blood Pressure (mm Hg.)	129.0 ±7.2	83.4 ±7.5	-35.3[4]	119.0 ±3.7	95.9 ±7.1	-19.4[3]
Heart Rate (b/min.)	266.6 ±9.2	204.2 ±9.1	-23.4[4]	334.2 ±9.0	322.5 ±19.0	3.50
Total Peripheral Resistance (PRU X 10)	6.316 ±.51	5.616 ±.51	-11.1	5.916 ±.50	4.938 ±.50	-16.5

[1]0.25 mg/kg bolus i.v. followed by 5 ug/kg/min. infusion
[2]5 mg/kg i.v.
[3]Significant change ($P<0.05$).
[4]Significant change ($P<0.01$.

FIGURE 1: The effect of a thirty minute pretreatment with propranolol or phenoxybenzamine on the cardiac index, mean arterial blood pressure, heart rate, and total peripheral resistance of adult SCWL hens.

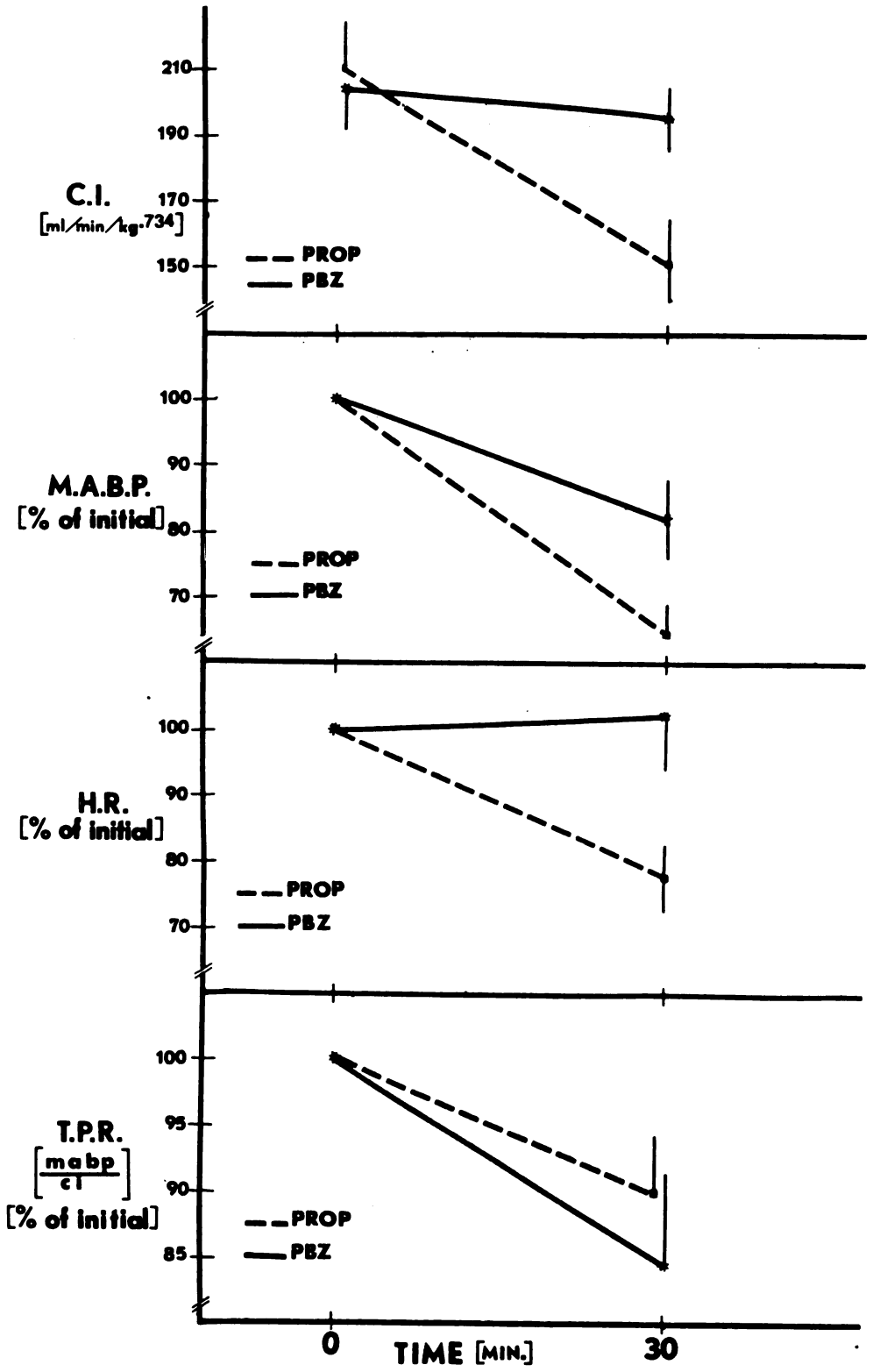
C.I.
[ml/min/kg.734]
210
190
170
150
PROP
PBZ
M.A.B.P.
[% of initial]
100
90
80
70
PROP
PBZ
H.R.
[% of initial]
100
90
80
70
PROP
PBZ
T.P.R.
[mabp/ci]
[% of initial]
100
95
90
85
PROP
PBZ
0
TIME [MIN.]
30

TABLE 1B. Mean ±S.E.of changes in cardiac output, stroke volume, stroke work, and hematocrit in adult SCWL hens thirty minutes after treatment with propranolol or phenoxybenzamine.

	Propranolol[1] (n=5)			Phenoxybenzamine[2] (n=5)		
	before treatment	30 min. after trt.	percent change	before treatment	30 min. after trt.	percent change
Cardiac Output (ml/min)	299.0 ±21.0	215.5 ±15.4	-27.93[3]	312.8 ±24.0	299.4 ±17.0	-4.28
Stroke Volume ($ml/b/kg^{.734}$)	.788 ±.058	.752 ±.076	- 4.57	.615 ±.046	.618 ±.043	+0.49
Stroke Work ($ml/b/kg^{.734}$)X (MABP)	101.6 ±9.0	64.2 ±10.1	-36.81[3]	72.2 ±5.8	58.6 ±4.6	-18.84
Hemato-crit (%)	---	---	---	25.92 ±0.45	23.67 ±0.49	-8.68

[1] 0.25 mg/kg bolus i.v. followed by 5 ug/kg/min. infusion.

[2] 5 mg/kg i.v.

[3] significant change at P<.05

EXPERIMENT II:

A. Hemodynamic Results: This experiment utilized 24 hens divided into four equal groups designated; control, hemorrhage, PROP + hemorrhage, and PBZ + hemorrhage. Inasmuch as some of the hens did not survive the entire 225 minutes of the experiment, the final three determinations of several parameters prior to death were recorded. These were labeled "sample 1" (90 minutes prior to the final sample), "sample 2" (45 minutes prior until the final sample), and "final sample" (prior to death). The actual times to the final sample are listed in Table 7. This temporal form of sampling synchronized the terminal changes in the measured parameters for each animal regardless of survival time. All electrolyte, HCT, Hb, glucose, and HR results were tabulated in this manner. The three samples were then compared to the initial pre-hemorrhage values to obtain accurate terminal percent changes. These percent values were then analyzed statistically as shown in Appendix C.

1. Heart Rate -- The hemorrhage group had a significantly higher ($P<0.05$) HR than the control group. One-way ANOVA of the absolute final HR value yielded the same level of significance. Dunnett's test indicated that the PROP + hemorrhage group had a significantly lower ($P<0.01$) HR than the hemorrhage group. The PBZ + hemorrhage group HR values did not differ from the hemorrhage group. These data are tabulated in Table 3 and are shown graphically in Figures 2A and 2B.

Neither average trend nor curvilinearity existed between sampling times. This means that, once the initial change in HR occurred, there would be no further change over time.

2. Cardiac Index -- The cardiac index was obtained at 90 minute intervals in the control and hemorrhage groups. The drug treatment groups

TABLE 2. Mean ±S.E. of changes in heart rate at the time of the final three samples, obtained at forty-five minute intervals, in adult SCWL hens subjected to sustained hemorrhagic hypotension without pretreatment and following pretreatment with propranolol or phenoxybenzamine.

No. of hens	Treatment	Initial Heart Rate (b/min.) T=-30	Initial Heart Rate (b/min.) T=0	Percent change from initial heart rate Sample 1	Sample 2	Final Sample
6	Control		303.3 ±13.1	-2.8 ±3.5	+0.8 ±4.5	+1.4 ±4.8
6	Hemorrhage		304.2 ±18.6	+15.7 ±4.8	+20.4 ±5.3	+21.9 ±5.2
6	Hemorrhage + Propranolol[1]	266.6 ±9.2		-18.0 ±3.9	-13.6 ±6.6	-27.0 ±4.7
6	Hemorrhage + Phenoxybenzamine[2]	334.2 ±9.0		+7.5 ±8.3	+10.3 ±9.4	+12.1 ±7.3

[1] .25 mg/kg bolus i.v. followed by 5 ug/kg/min. infusion.

[2] 5 mg/kg. i.v.

FIGURE 2A: The change in heart rate at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension.

FIGURE 2B: The change in heart rate at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension and pretreated with propranolol or phenoxybenzamine.

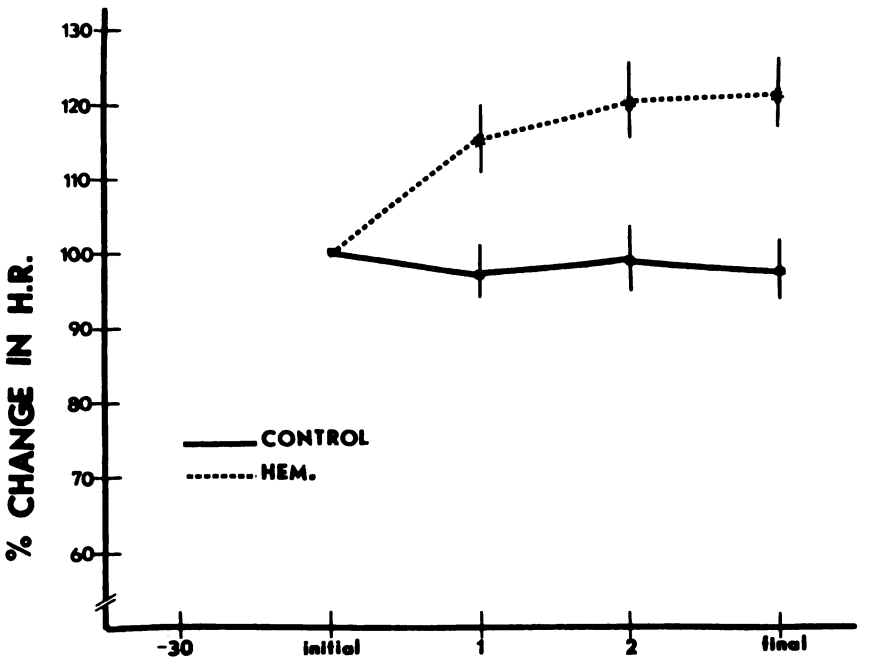

FIGURE 2A

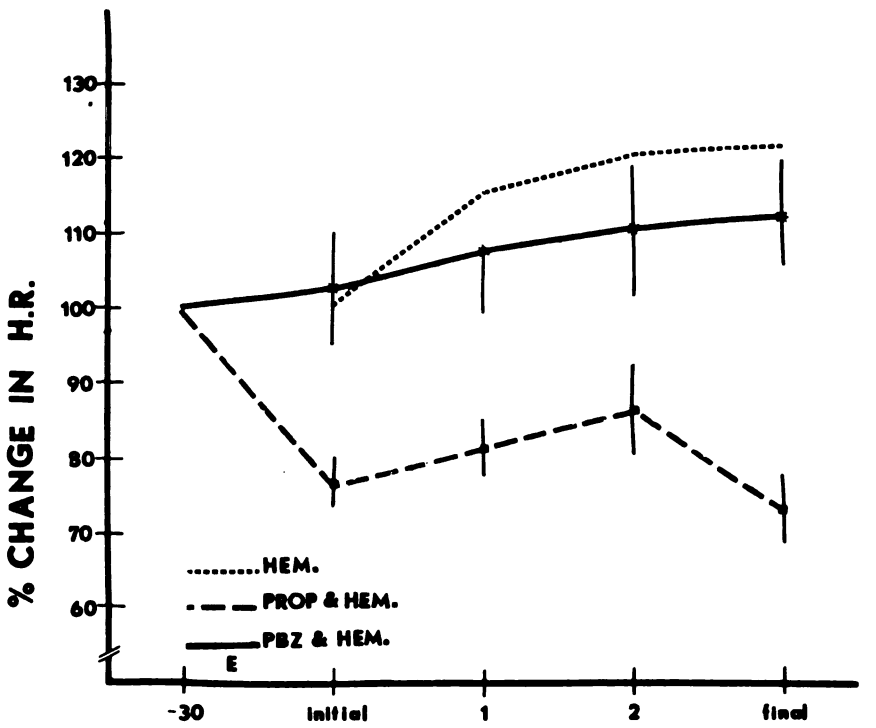

FIGURE 2B

had CI determinations only twice, i.e. at T=-30 and T=0 (immediately prior to hemorrhage), as previously described in Experiment I. Cardiac output values not corrected for differences in body weight are meaningless, therefore only weight-standardized CI values will be considered in this thesis.

A two-way block-design repeated measures ANOVA (Appendix C2) was used to compare the second and third CI determinations to the first within a treatment. The control group showed no significant difference ($P>0.05$) between CIs. The hemorrhage group did show a significant ($P<0.05$) decrease in the last two CI determinations below the initial CI value. These data are tabulated in Table 3 and are expressed graphically in Figure 3A.

3. Stroke Volume -- This parameter was analyzed in the same manner as the CI determinations. The T=90 and T=180 determinations did not differ significantly from the initial value in the control group, but did become significantly lower ($P<0.01$)for the latter two sampling times in the hemorrhage group (Table 3 and Figure 3B).

4. Stroke Work -- This parameter was analyzed in the same manner as the CI determinations. No change occurred in the control group, but a highly significant reduction ($P<0.01$) did occur in the hemorrhage group in the latter two sampling times (Table 3).

5. Total Peripheral Resistance -- The TPR of the hemorrhage group decreased significantly ($P<0.05$) over the first ninety minutes, whereas the control group did not change significantly. However, the magnitude of the change in the first 90 minutes in the hemorrhaged group, when compared to the change occurring in the control group, was not significant (Table 4).

6. Bleeding Volumes -- No significant difference existed in the IBV, SBV, or the MBV between the three hemorrhaged groups. The two drug treated groups did show a nonsignificant tendency to have lower IBVs and higher SBVs than the hemorrhage group. This is especially evident in the PROP + hemorrhage group (Table 5). Though no significant differences existed in the SBV/IBV ratios between treatments, there was a tendency for the ratio to be higher in the PROP + hemorrhage group (Table 4).

7. Survival Times and Mortalities -- No significant difference ($P > 0.05$) existed between survival times among the three hemorrhaged groups. There was, however, a nonsignificant tendency for the PROP + hemorrhage group to have a lower survival time than either of the other hemorrhaged groups (Table 4). Four out of six birds survived the hemorrhage procedure in both the hemorrhage and the PBZ + hemorrhage groups. Only two birds in the PROP + hemorrhage group survived. Mortalities for the hemorrhage, PBZ + hemorrhage, and PROP + hemorrhage groups were 33, 33, and 67 percent, respectively (Table 4).

8. Lesions and Arrhythmias -- Petechial hemorrhages were observed in the endocardium of two of the PBZ + hemorrhage birds, however, both of these birds lived the entire duration of the experiment. No hepatic or gastrointestinal lesions were observed in any of the other birds.

The EKG had arrhythmias in only one bird. This was in the PROP + hemorrhage group and this bird died early.

Traube-Herring waves were often markedly exaggerated after the primary fall in blood pressure. These were most prevalent in the hemorrhage group (occurring in three birds) but also occurred in each of the other hemorrhaged groups. They had a frequency of about one per minute. Small

TABLE 3. Means ±S.E. of changes in cardiac index, stroke volume, and stroke work in adult SCWL hens prior to, 90 minutes after, and 180 minutes after the onset of sustained hypovolemic hypotension (n=5 in both groups).

Time (min.)	Cardiac Index ($ml/min/kg^{.734}$)		Stroke Volume ($ml/beat/kg^{.734}$)		Stroke Work (MABP)X($ml/beat/kg^{.734}$)	
	Control	Hemorrhage	Control	Hemorrhage	Control	Hemorrhage
T = 0	211.7 ±11.7	209.4 ±7.8	.678 ±.045	.710 ±.011	89.2 ±4.2	93.5 ±5.6
T = 90	228.4 ±24.8	150.3[1] ±15.6	.723 ±.082	.446[2] ±.055	89.6 ±7.8	28.4[2] ±4.9
T = 180	239.3 ±29.3	140.7*[1] ±11.0	.772 ±.085	.451*[2] ±.031	91.3 ±8.1	28.3*[2] ±3.5

[1] Significant difference from T = 0 ($P<0.05$)

[2] Significant difference from T = 0 ($P<0.01$)

* n = 3

FIGURE 3A: The change in cardiac index in adult SCWL hens subjected to sustained hypovolemic hypotension.

FIGURE 3B: The change in stroke volume in adult SCWL hens subjected to sustained hypovolemic hypotension.

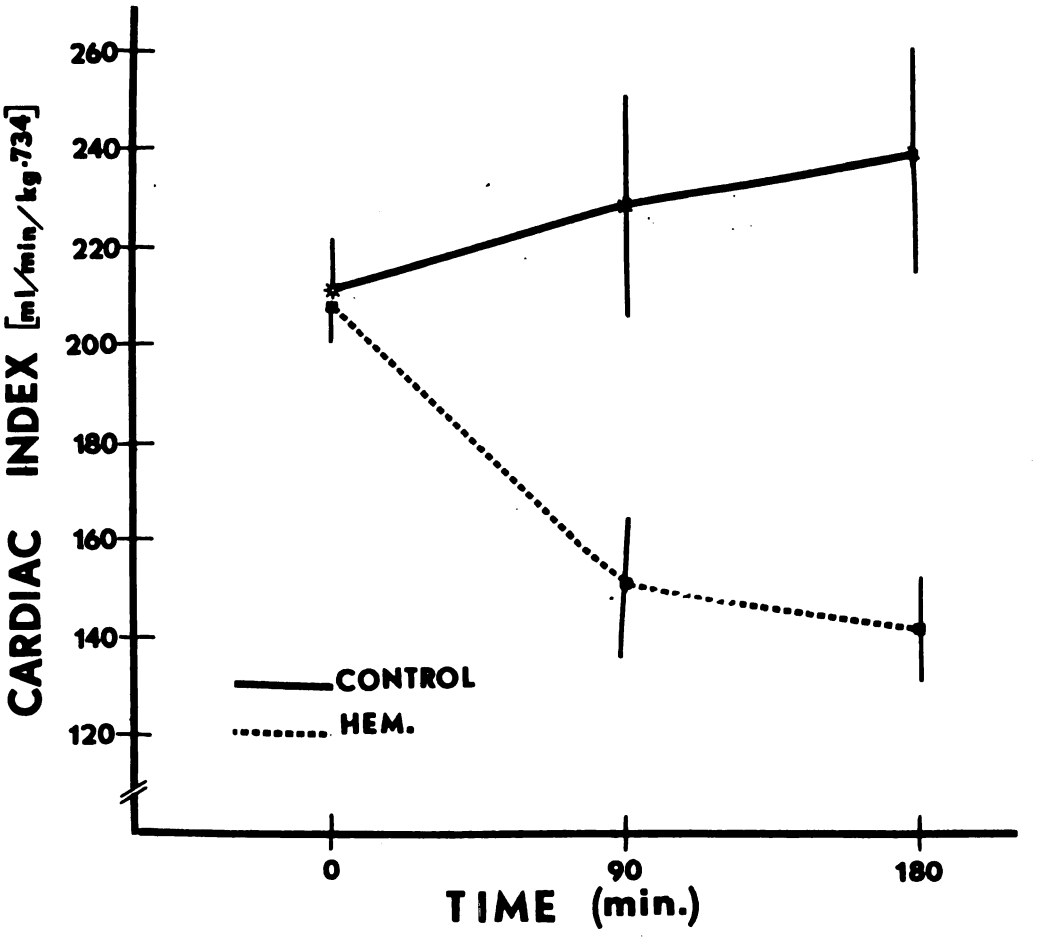

FIGURE 3A

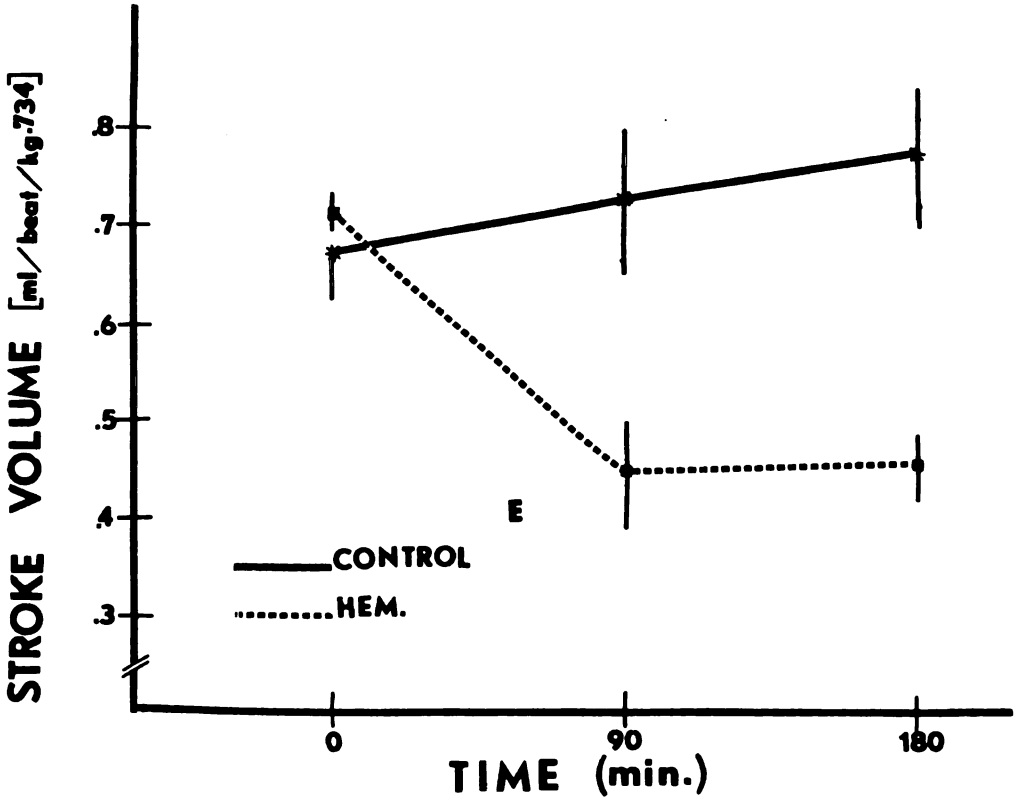

FIGURE 3B

TABLE 4. The mean ±S.E. for the total peripheral resistance values in adult SCWL hens prior to, 90 minutes after, and 180 minutes after the onset of sustained hypovolemic hypotension (n = 5 in both groups).

TREATMENT	TIME (min.)		
	0	90	180
Control	.634 ±.057	.590 ±.077	.526 ±.083
Hemorrhage	.634 ±.049	.430 ±.043	.360 ±.038

blood losses would cause these waveforms to become greatly exaggerated.

B. Hematological Results: Blood chemistry data were analyzed and recorded as described previously in Experiment IIA (Appendix C1).

1. Hematocrit -- The control group had a significantly greater ($P<0.01$) average HCT than any of the hemorrhaged groups. No significant difference ($P<0.05$) existed between any of the hemorrhaged groups. Analysis at individual times indicated that "sample 1" (the sample 90 minutes prior to the final sample before death) of the control group did not differ significantly from the hemorrhaged groups at that time. This means that the truly significant alterations in HCT occurred primarily in the hour preceding death (Table 6 and Figure 4A and 4B).

Only the hemorrhaged groups showed a significant ($P<0.01$) linear trend. This was in a curvilinear manner ($P<0.01$) for the PROP + hemorrhaged group.

2. Hemoglobin -- The average Hb for each of the hemorrhaged groups was significantly ($P<0.01$) lower than the control group. However, comparison within each of the hemorrhaged groups indicated that only the final two sampling times were significantly different from the control group. "Sample 1" for each of the hemorrhaged groups showed a non-significant ($P>0.05$) tendency to be lower than the control group, "sample 2" was lower ($P<0.05$), as was the "final sample" ($P<0.01$), (Table 7 and Figures 5A and 5B). No difference existed between the hemorrhaged groups.

No linear trend existed in the control group. The hemorrhage group showed a signigicant ($P<0.05$) linear trend, but in a curvilinear manner ($P<0.05$), with most of the change occurring one to two hours prior to death. Both drug treatment groups showed a significant ($P<0.01$) linear trend without curvilinearity.

TABLE 5. The mean ±S.E. of changes in initial bleeding volume (IBV), secondary bleeding volume (SBV), maximal bleeding volume (MBV), SBV/IBV ratio, survival times, and mortality percents in adult SCWL hens subjected to sustained hemorrhagic hypotension without pretreatment and following pretreatment with propranolol or phenoxybenzamine.

	Hemorrhage (n=6)	Hemorrhage+ Propranolol[1] (n=6)	Hemorrhage + Phenoxybenzamine[2] (n=6)
IBV (ml/kg)	11.45 ±1.88*	9.68 ±0.91*	10.20 ±1.03*
SBV (ml/kg)	18.69 ±3.69*	23.05 ±5.06*	20.69 ±2.31*
MBV (ml/kg)	30.14 ±2.71*	32.73 ±5.24*	30.89 ±3.08*
SBV/IBV	2.21 ±0.61*	2.49 ±0.63*	2.11 ±0.17*
Survival time[3] (minutes)	221.7 ±19.6*	169.2 ±27.4*	220.0 ±20.7*
Mortality	33	67	33

[1]0.25 mg/kg followed by 5 ug/kg/min. infusion i.v.
[2]5 mg/kg i.v.
[3]If animal survived 225 minutes + 30 minute post reinfusion observation period, the survival time was recorded as 255 minutes.
*values with this subscript show no significant treatment difference.

TABLE 6. Mean ±S.E.of the initial hematocrit, and the percent change from the initial value at the time of the final three samples, taken at 45 minute intervals, prior to death in adult SCWL hens subjected to sustained hemorrhagic hypotension without pretreatment and following pretreatment with propranolol or phenoxybenzamine.

No. of hens	Treatment	Initial Hematocrit (%) T=-30	Initial Hematocrit (%) T=0	Percent change from initial hematocrit Sample 1	Sample 2	Final Sample
6	Control		29.25 ±1.69	-14.48 ±3.25	-11.92 ±2.63*	-13.90 ±2.29*
6	Hemorrhage		28.17 ±1.48	-27.20 ±4.60	-41.88 ±3.00	-47.72 ±3.70
6	Hemorrhage+ Propranolol[1]	---	31.33 ±0.30	-22.28 ±2.58	-41.48 ±2.95	-50.85 ±4.49
6	Hemorrhage+ Phenozybenzamine[2]	25.92 ±0.45	23.67 ±0.49	-32.43 ±4.58	-39.89 ±2.37	-53.48 ±6.39

[1]0.25 mg/kg bolus followed by 5 ug/kg/min. infusion i.v.

[2]5 mg/kg i.v.

*this subscript means a significant ($P<0.01$) difference existed between this sample and the hemorrhage group.

FIGURE 4A: The change in hematocrit at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension.

FIGURE 4B: The change in hematocrit at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension and pretreated with propranolol or phenoxybenzamine.

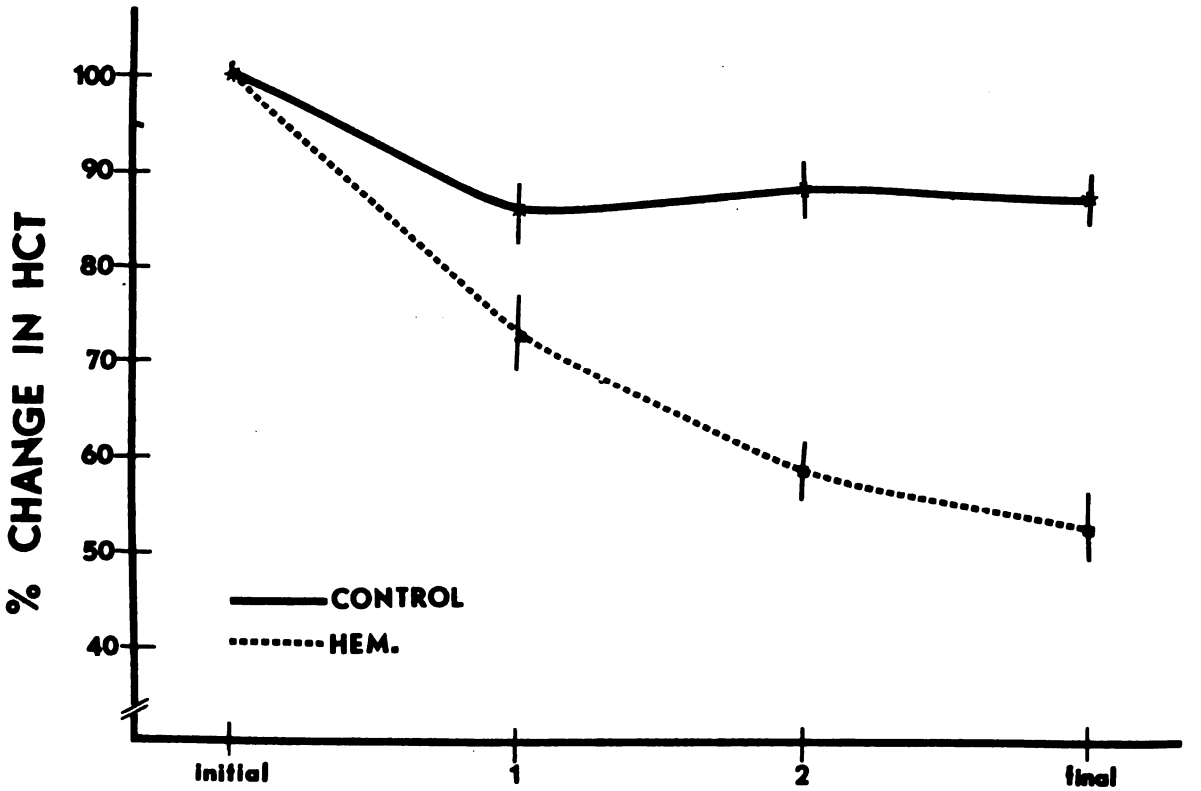

FIGURE 4A

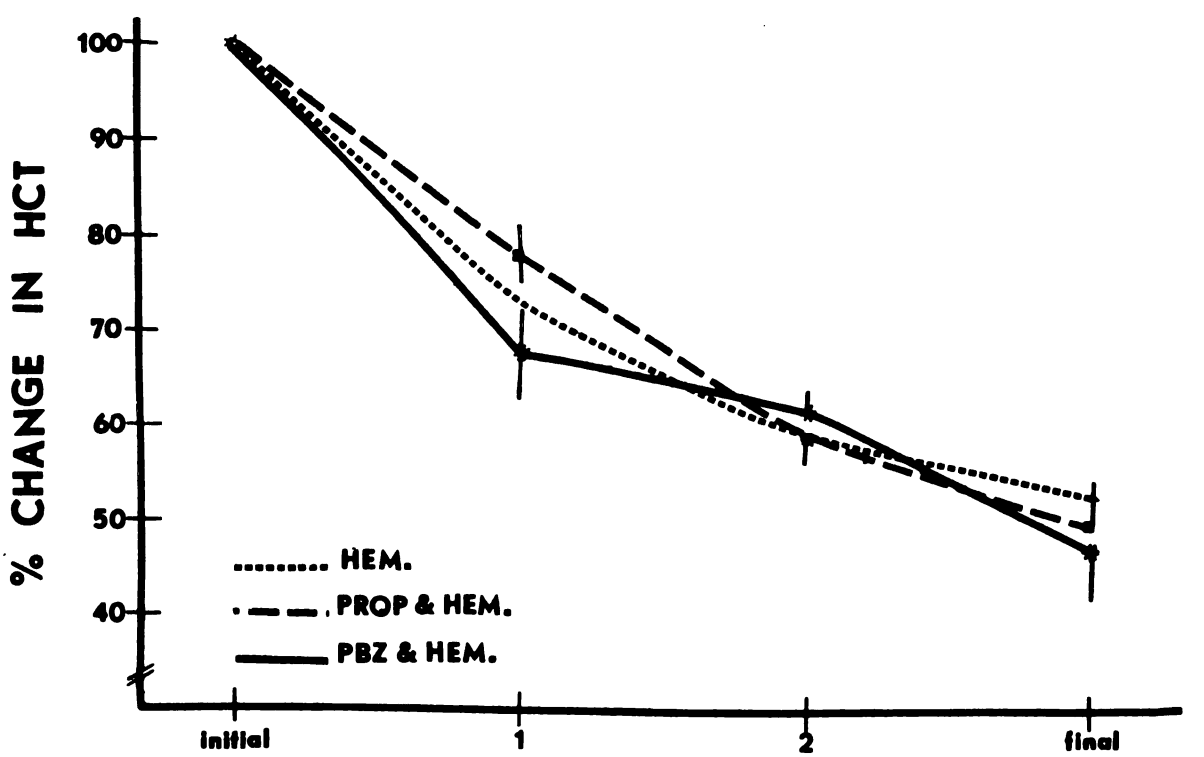

FIGURE 4B

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TABLE 7. The mean ±S.E.of the initial hemoglobin and the percent change from the initial value at the time of final three samples, taken at 45 minute intervals, in adult SCWL hens subjected to sustained hemorrhagic hypotension without pretreatment and following pretreatment with propranolol or phenoxybenzamine.

No. of hens	Treatment	Initial Hemoglobin (gm%)	Percent change from initial hemoglobin		
			Sample 1	Sample 2	Final Sample
6	Control	10.51 ±0.43	-10.74 ±2.96	-13.51 ±3.27	-12.47 ±3.51
6	Hemorrhage	9.90 ±0.61	-30.45 ±5.05	-51.46[3] ±10.04	-50.14[4] ±5.24
6	Propranolol[1]+ Hemorrhage	10.02 ±0.28	-25.06 ±4.37	-39.65[3] ±5.23	-57.35[4] ±4.54
6	Phenoxybenzamine[2] + Hemorrhage	9.43 ±0.18	-34.09 ±5.03	-48.95[3] ±3.16	-55.45[4] ±1.44

[1]0.25 mg/kg followed by 5 ug/kg/min. infusion i.v.

[2]5 mg/kg/ i.v.

[3]significantly different from the control group at that time ($P<0.05$).

[4]significantly different from the control group at that time ($P<0.01$).

FIGURE 5A: The change in hemoglobin at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension.

FIGURE 5B: The change in hemoglobin at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension and pretreated with propranolol or phenoxybenzamine.

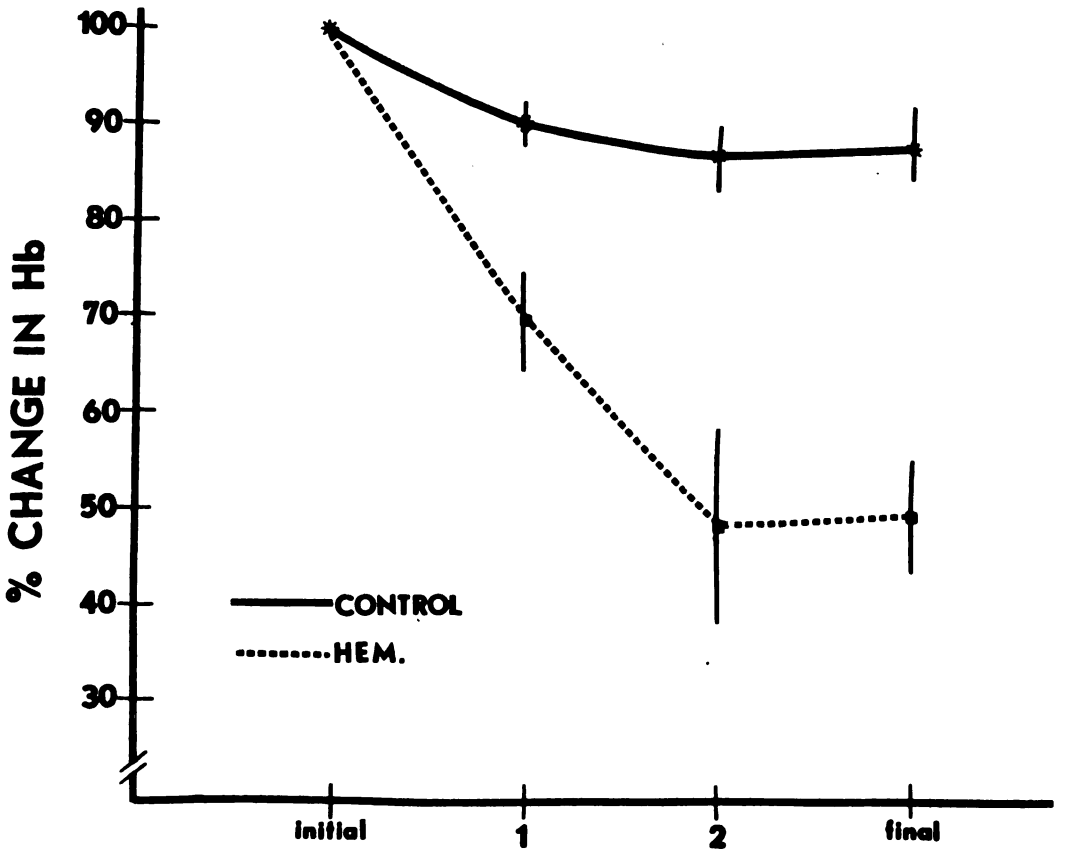

FIGURE 5A

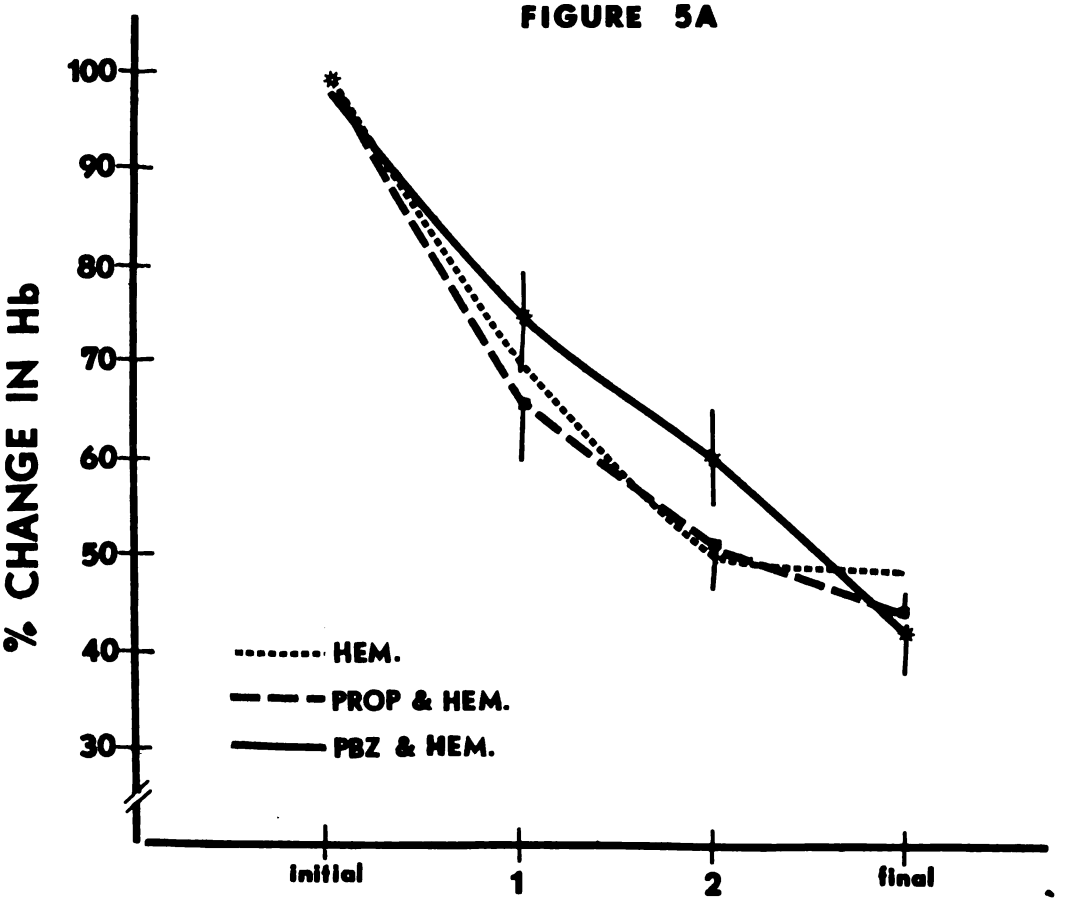

FIGURE 5B

3. Plasma Potassium -- The control group plasma potassium level was lower than the hemorrhage group ($P<0.05$) when averaged over the three sampling times. Likewise, the PBZ + hemorrhage group had a lower potassium level than the hemorrhage group, though this trend was not significant ($P<0.05$) (Figure 6A).

One-way ANOVA of the differences in the initial and final samples between treatments indicated that the hemorrhage and PROP + hemorrhage groups showed significantly greater differences than the control ($P<0.05$) or the PBZ + hemorrhage ($P<0.05$) groups. The PBZ + hemorrhage group had a significantly lower ($P<0.05$) average potassium level than the PROP + hemorrhage group (Figure 6B and Table 8) and a significantly ($P<0.05$) higher potassium level than the control group.

One-way ANOVA of the initial versus final potassium levels within each treatment indicated that the concentration increased over time in the control group ($P<0.01$), the PBZ + hemorrhage group ($P<0.01$), and in the PROP + hemorrhage group ($P<0.05$) (Table 8 and Figure 6B).

Hence, potassium was elevated in all of the groups. The magnitude of the increase in the hemorrhage and PROP + hemorrhage groups was significantly greater than the remaining two groups.

Only the hemorrhage and PROP + hemorrhage groups showed a linear trend ($P<0.01$) over time. None of the groups varied in a curvilinear manner.

4. Plasma Magnesium -- The plasma magnesium concentration did not vary significantly over time or treatments. One-way ANOVA comparing the initial and final samples within each treatment also showed no significant change (Table 8).

Trend analysis at individual sampling times indicated that the

FIGURE 6A: The change in plasma potassium concentration at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension.

FIGURE 6B: The change in plasma potassium concentration at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension and pretreated with propranolol or phenoxybenzamine.

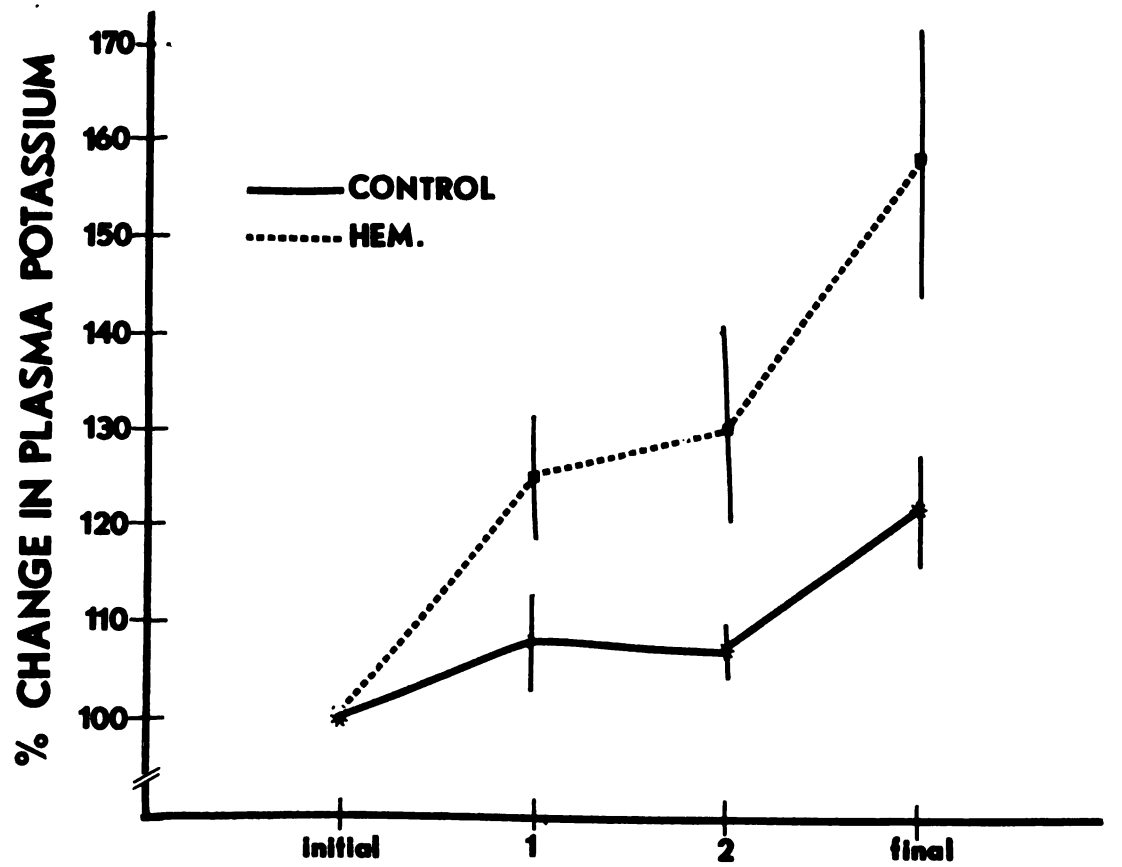

FIGURE 6A

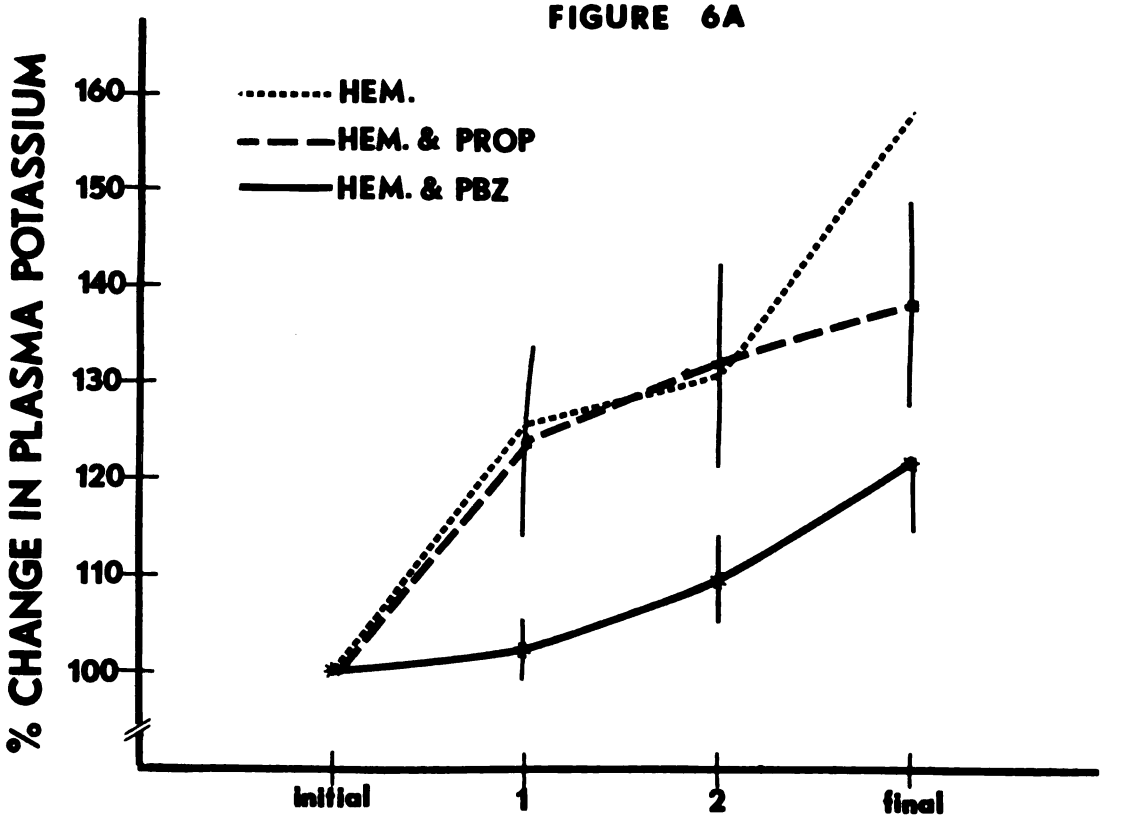

FIGURE 6B

TABLE 8. The initial and final mean ±S.E. the percent changes therein, and the mean time ±S.E to the final sample, for the levels of plasma potassium, magnesium, sodium, and glucose in adult SCWL hens subjected to sustained hemorrhagic hypotension without pretreatment and following pretreatment with propranolol[1] or phenoxybenzamine.[2]

Parameter	Treatment	Initial Sample	Final Sample	Percent Change	Mean time to final sample (min.)
Potassium (mEq/l) n=6	Control	2.41 ±.10[3]	2.91 ±.06	+20.7[4]	225 ±00
	Hemorrhage	2.92 ±.49	4.54 ±.70	+55.5	195 ±19
	PROP + hem.	2.88 ±.21	3.83 ±.21	+33.0[3]	157 ±25
	PBZ + hem.	2.48 ±.07	3.02 ±.13	+21.8[4]	195 ±19
Magnesium (mEq/L) n=6	Control	2.31 ±.15	2.42 ±.15	+ 4.8	225 ±00
	Hemorrhage	2.25 ±.11	2.35 ±.19	+ 4.4	195 ±19
	PROP + hem.	1.80 ±.08	2.02 ±.18	+12.2	157 ±25
	PBZ + hem.	2.67 ±.07	3.01 ±.22	+12.7	195 ±19
Sodium (mEq/L) n=6	Control	132.9 ±1.3	135.2 ±1.5	+ 1.7	225 ±00
	Hemorrhage	136.6 ±1.4	138.2 ±3.6	+ 1.2	195 ±19
	PROP + hem.	137.0 ±1.6	136.7 ±1.0	+ 0.2	157 ±25
	PBZ + hem.	135.2 ±1.0	144.2 ±2.1	+ 6.7[3]	195 ±19
Glucose (mg%) n=6	Control	216.8 ±19.2	221.6 ±11.9	+ 2.2	225 ±00
	Hemorrhage	189.9 ± 7.2	238.3 ± 9.8	+25.5[4]	195 ±19
	PROP + hem.	184.0 ± 3.8	213.3 ±23.8	+15.9	157 ±25
	PBZ + hem.	195.2 ± 5.4	218.6 ± 8.1	+12.0[3]	195 ±19

[1] 0.25 mg/kg bolus followed by 5 ug/kg/min. infusion i.v.

[2] 5 mg/kg i.v.

[3] Significant change by one way ANOVA f-testing ($P < 0.05$).

[4] Significant change by one way ANOVA f-testing ($P < 0.01$).

hemorrhage group had a nonsignificant tendency ($P>0.05$) to have a linear trend. The PROP + hemorrhage group did show a significant ($P<0.01$) linear trend, but in a curvilinear manner ($P<0.01$) with most of the change occurring two to three hours prior to death. The other groups showed no trends.

The initial versus final plasma magnesium concentrations are tabulated in Table 8.

5. Plasma Sodium -- Dunnett's test and one-way ANOVA failed to show any significant change in plasma sodium concentrations between or within treatments, with the exception of the PBZ + hemorrhage group, where one-way analysis did show that a significant increase ($P<0.05$) occurred between the initial and final samples (Table 8).

No linear or curvilinear trends occurred in plasma sodium concentration over time.

6. Plasma Glucose: Dunnett's test showed all of the hemorrhaged groups showed a nonsignificant tendency for plasma glucose to be higher than the control group (Figures 7A and 7B). The hemorrhage group had the largest tendency to increase ($P>0.05$). One-way ANOVA indicated that the change between initial and final samples was significant in the hemorrhage ($P<0.01$) and the PBZ + hemorrhage ($P<0.05$) groups (Table 8). The PROP + hemorrhage group tended to increase ($P<0.05$).

No linear or curvilinear trends existed over the final three sampling times. This indicated that the change which occurred in plasma glucose concentration must have occurred early in the experiment.

C. Respiration Data:

1. Arterial pH -- One-way ANOVA between initial and final samples

FIGURE 7A: The change in plasma glucose concentration at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension.

FIGURE 7B: The change in plasma glucose concentration at the time of the final three samples in adult SCWL hens subjected to sustained hypovolemic hypotension and pretreated with propranolol or phenoxybenzamine.

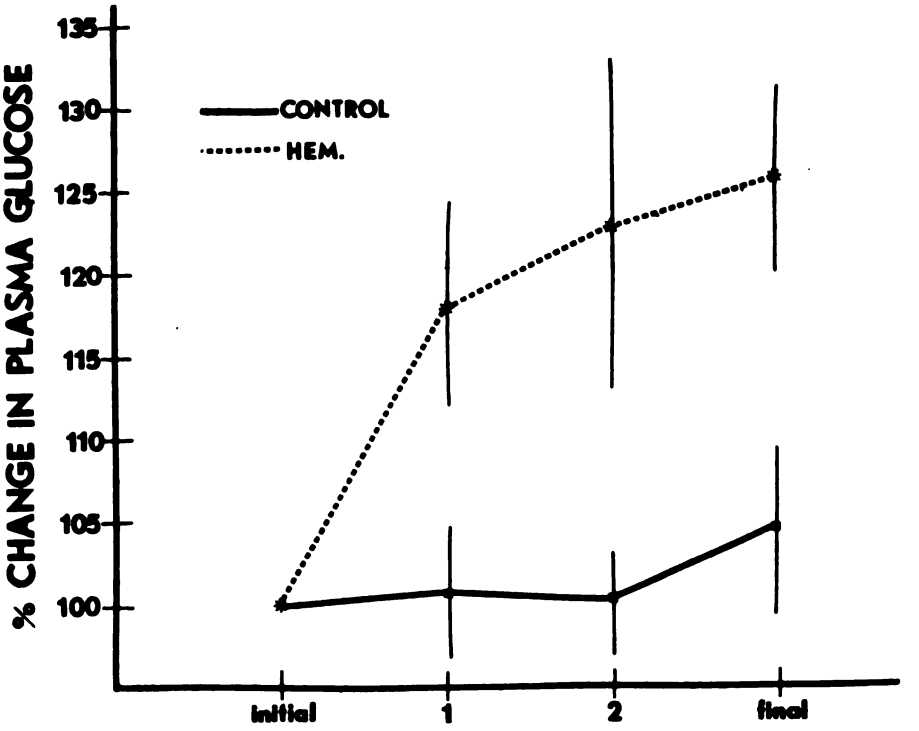

FIGURE 7A

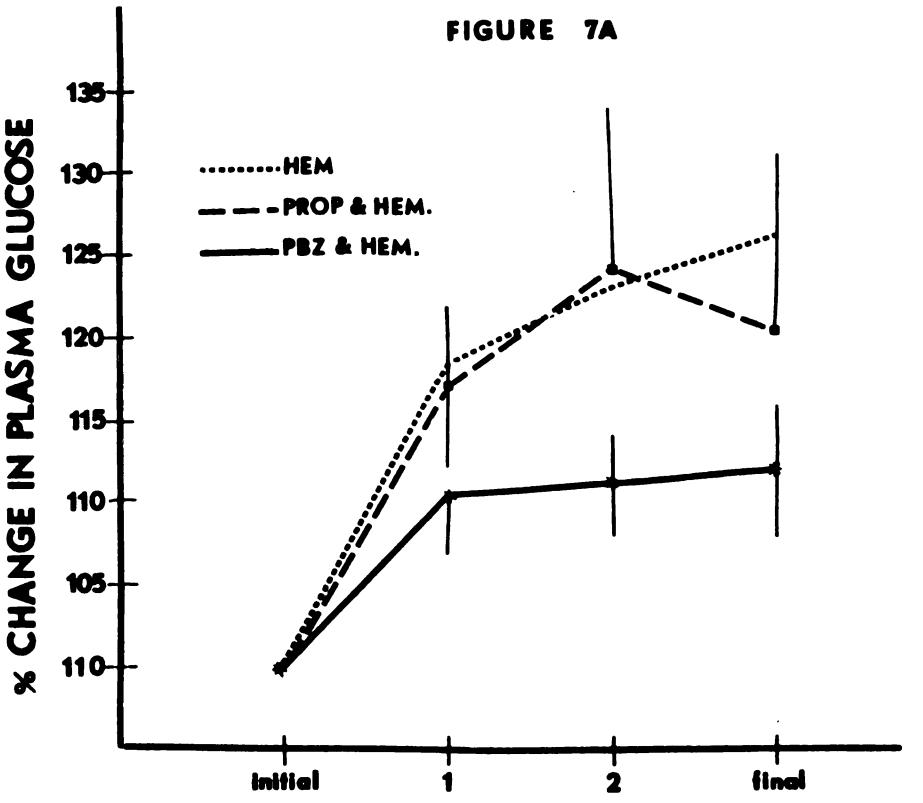

FIGURE 7B

within a treatment indicated that only the PROP + hemorrhage group showed a significant ($P<0.05$) increase in arterial pH over time. All of the other groups had tended to increase over the course of the experiment, with the hemorrhage group having the greatest trend of the three (Table 9 and Figure 8). Similar testing indicated that no difference existed between any of the initial or final pH values across the four treatments.

2. Arterial pCO_2 -- The pCO_2 was reduced in all of the groups, but only in the hemorrhage group was it significant ($P<0.01$). The PBZ + hemorrhage group showed a strong nonsignificant tendency to decrease ($P>0.05$) (Figure 8 and Table 9).

3. Arterial pO_2 -- No significant difference existed between or within treatments over time (Figure 8 and Table 9).

4. Respiratory Arrest -- The PROP + hemorrhage group had less of a tendency to become apneic. None of the PROP + hemorrhage birds died as a result of respiratory failure. The four deaths in this group were a result of cardiac failure as evidenced by a rapidly falling MABP and HR. Reinfusion of the shed blood reinstated normal, albeit hypotensive, CVS status in only one of these birds. As previously discussed in Experiment II6, two birds died in both the hemorrhage and the PBZ + hemorrhage groups. In each case, one death resulted from respiratory problems and the other from cardiac problems. The birds in the PBZ + hemorrhage group appeared to have more respiratory problems than the other hemorrhaged groups, as evidenced by the fact that three of these birds required some respiratory assistance during the experiment. This artificial ventilation never exceeded ten minutes in duration, but in two of the three instances this was sufficient to restore normal respiration. Though respiratory rates were not recorded, there was a general

TABLE 9. The mean ±S.E.of the initial and final values for arterial pH, pO_2, and pCO_2 in adult SCWL hens subjected to sustained hemorrhagic hypotension without pretreatment and following pretreatment with propranolol[1] or phenoxybenzamine.[2]

	Control (n=6)		Hemorrhage (n=6)		Propranolol+ Hemorrhage (n=6)		Phenoxybenzamine+ Hemorrhage (n=6)	
	initial	final	initial	final	initial	final	initial	final
pH (Temp. corrected)	7.530 ±.041	7.566 ±.027	7.467 ±.024	7.553 ±.031	7.480 ±.020	7.545[3] ±.008	7.531 ±.030	7.556 ±.026
pO_2 (mm Hg.)	81.02 ±3.70	81.80 ±6.44	80.07 ±4.64	88.00 ±3.25	94.03 ±3.61	93.28 ±7.10	93.15 ±3.00	94.17 ±4.00
pCO_2 (mm Hg.)	28.25 ±1.24	25.40 ±1.42	28.57 ±2.70	18.10[4] ±0.61	25.03 ±2.25	22.80 ±2.14	28.55 ±2.25	20.97 ±2.66

[1]0.25 mg/kg bolus followed by 5 ug/kg/min. infusion i.v.

[2]5 mg/kg/ i.v.

[3]Significant change from initial value ($P<0.05$).

[4]Singificant change from initial value ($P<0.01$).

FIGURE 8: The changes in arterial pH, pO_2, and pCO_2 concentrations from the time of the initial to the final sample in adult SCWL hens subjected to sustained hypovolemic hypotension without pretreatment and following pretreatment with propranolol or phenoxybenzamine.

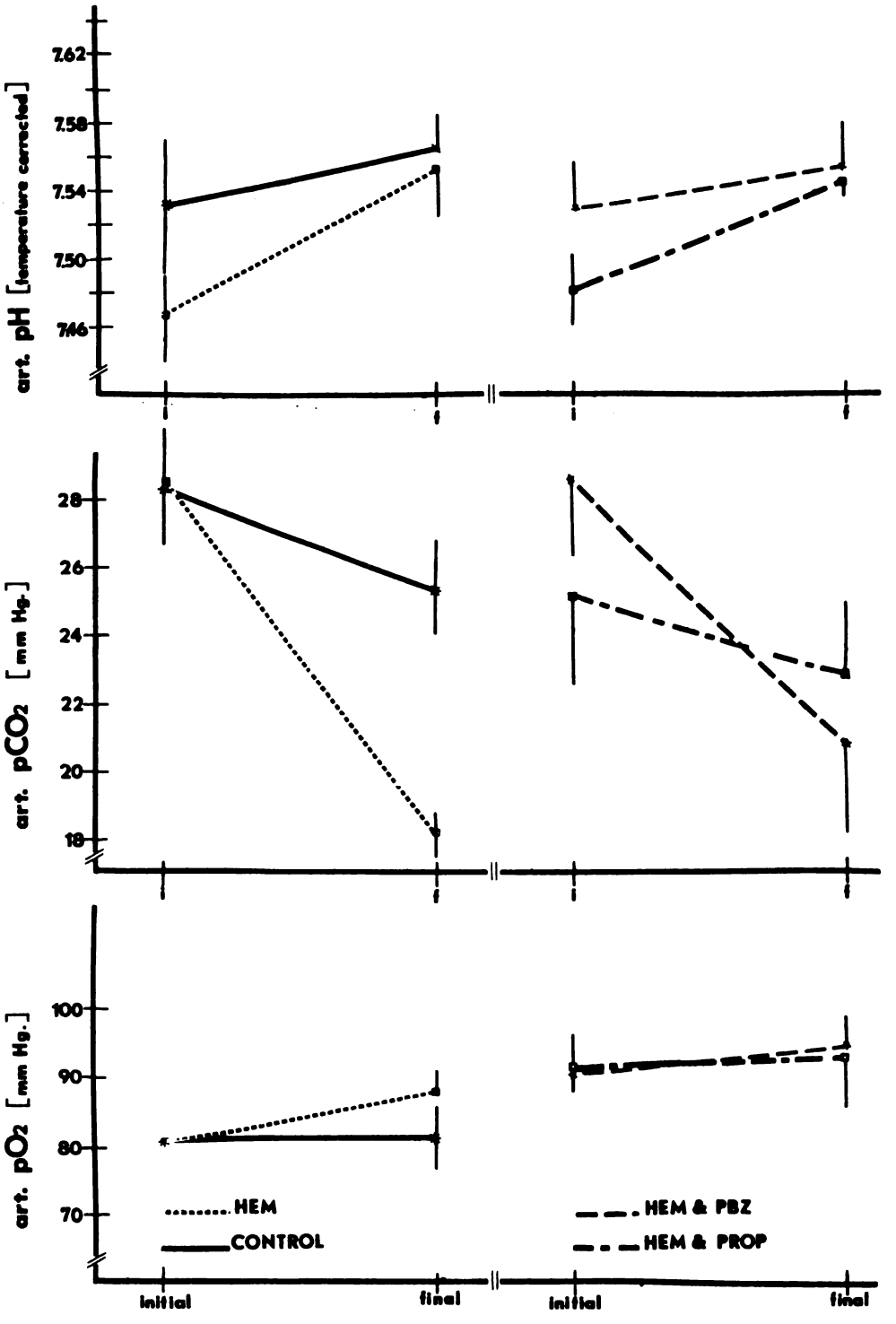
art. pH [temperature corrected]
7.62
7.58
7.54
7.50
7.46
art. pCO2 [mm Hg.]
28
26
24
22
20
18
art. pO2 [mm Hg.]
100
90
80
70
HEM
CONTROL
HEM & PBZ
HEM & PROP
initial
final
initial
final

FIGURE 8

tendency for the animals to become progressively tachypneic over time, especially in the hemorrhaged groups. The animals were hyperventilating by the end of the experiment.

D. Group #5 and Overall Grouped Data

1. Group #5 -- Group #5 was a control group, i.e. a nonhemorrhaged group, in which sampling losses were not replaced with donor blood. This group experienced a large fall in MABP and HCT, and an increase in HR when compared to the transfused control group (Table 10). The CI did not differ between the two control groups.

2. Overall Grouped Data -- The mean initial values for CI, HR, MABP, HCT, Hb, and plasma potassium, magnesium, sodium, and glucose concentrations for all the birds utilized in all of the experiments (n=30) are listed in Table 11.

TABLE 10. Means ±S.E. of changes in cardiac index, heart rate, mean arterial blood pressure, and hematocrit in adult SCWL hens after 225 minutes with and without replacement of sampling blood losses of 2.5 ml/hr.

Parameter	with replacement (n=6) initial	final	%change	without replacement (n=6) initial	final	%change
Cardiac Index ($ml/min/kg^{.734}$)	211.7 ±11.7	239.3 ±29.3	+13.0	224.2 ±26.0	257.2 ±45.4	+14.7
Heart Rate (beats/min)	303.3 ±13.1	299.0 ±34.4	+ 1.4	309.2 ±18.3	383.8 ± 8.0	+24.1
Mean Arterial Blood Pressure (mm Hg.)	131.7 ± 3.0	118.0 ± 4.0	-10.4	125.6 ±10.0	92.2 ±15.1	-26.6
Hematocrit (%)	29.2 ± 1.7	25.2 ± 1.1	-13.7	30.2 ± 1.5	22.1 ± 1.4	-26.8

TABLE 11. The mean values ±S.E. for cardiac index, heart rate, mean arterial blood pressure, hematocrit, hemoglobin, and plasma glucose, potassium, pH, magnesium, and sodium concentrations in thirty adult SCWL hens.

Parameter	mean initial value	
Cardiac index ($ml/min/kg^{.734}$)	211.5	±7.7
Heart rate (beats/min)	298.5	±6.7
MABP (mm Hg.)	127.3	±2.9
Hematocrit (%)	29.0	±0.6
Hemoglobin (gm%)	10.6	±0.7
Plasma potassium (mEq/L)	2.83	±0.13
Plasma Magnesium (mEq/L)	2.51	±0.12
Plasma Sodium (mEq/L)	136.4	±0.8
Plasma Glucose (mg%)	199.4	±5.2
Arterial pH (temp. corrected)	7.507	±0.013

DISCUSSION

I. EFFECTS OF PROP + PBZ ON HEMODYNAMIC PARAMETERS

It is well documented that PROP greatly reduced the inotropic and chronotropic state of the myocardium in mammals. In view of the large reductions in cardiac performance during beta-adrenergic blockade, myocardial beta-receptors also play an important role in avian species.

The hypotensive action of PROP in the hen was primarily mediated by its negative inotropic and chronotropic effects on the myocardium. The greatly reduced HR and stroke work resulted in the greatly reduced CI and MABP The peripheral vasculature was not altered by PROP inasmuch as the TPR did not change (Figure 1).

Conversely, the hypotensive effect of PBZ in the hen, as in the mammal, was mediated by peripheral vasodilation without significant positive inotropic effect on the myocardium. Though both drug treatments caused singificant reductions in the MABP, the PROP caused significantly greater reductions than the PBZ. This indicated that cardiac performance in the hen, not altered vascular tone, was the primary regulator of arterial blood pressure.

This latter supposition is logical since avian species have been shown to have a low arterial capacitance and low compliance in the abdominal aorta (Speckmann and Ringer, 1964). This low aortic compliance was reflected in pulse pressures often exceeding 100 mm Hg during the hypotensive phase in research for this thesis. The relatively stiff arterial system may represent a compromise situation related to the extremely high HR in the chicken, so the net result of smoothing flow in the periphery is still achieved. The low aortic compliance is likely due to the amount of collagen in the vessel walls and to the accumulation

of atherosclerotic plaque common in birds.

PBZ reduced the HCT by 8.7 percent of the initial value, indicating an increase in plasma volume. Avian extramuscular arteries are more numerous and more densely adrenergically innervated than in mammals (Folkow et al., 1967). Williams and Rodbard (1960) hypothesized, upon finding that PBZ caused a large increase in plasma volume in the hen, that the drug could conceivably release trapped plasma in extramuscular vessels by blocking the effectors of postganglionic neurons. Therefore, changes in nerve density or impulse frequency could effectively change the pressure-volume relationship in these vessels which could, in turn, regulate the effective circulating plasma volume. Though Williams and Rodbard (1960) did not find hypotension after PBZ treatment, reasearch for this thesis found hypotension, and numerous other researchers have found likewise (Harvey et al., 1954, Peterson and Ringer, 1968).

The fact that PBZ caused plasma volume expansion in the chicken and not in the dog (Stekiel et al., 1967) could be due to a larger capillary surface area in the bird. Folkow et al. (1967) estimated that the duck has three times the capillary surface area of the cat. Hence, if the fowl is similar to the duck in this regard, the fowl could be expected to experience large changes in transcapillary fluid movement following small changes in TPR.

II. EFFECT OF HEMORRHAGE IN THE FOWL WITH AND WITHOUT PRETREATMENT WITH PROP OR PBZ.

A. Hemodynamic Parameters -- The birds used in this research were normal and apparently in good health as evidenced by the overall mean initial hematological values listed in Table 11.

Whereas flying and diving avian species showed intense vasoconstriction

and maintained the MABP during large hemorrhages (Djojosugito et al., 1968), the chicken showed large and rapid falls in MABP with only minimal blood losses. The hen showed better survival after blood loss than the mammal, yet fell considerably short of the phenomenal tolerance of the duck or pigeon (Kovách et al., 1969). The sensitivity of the chicken is exemplified in Table 10, where a ten ml blood loss over a 225 minute duration caused large changes in HCT, Hb, and MABP. This confirmed the findings of Kutola et al. (1967) who found an eleven ml blood loss over two hours reduced the venous HCT by 18 percent. Though the hen cannot maintain the MABP after blood loss, she does have the ability to endure long periods of hemorrhagic hypotension without entering vascular decompensation.

The onset of decompensation in the mammal may result from a failure of the intense neurogenic vasoconstriction. This dilatation may be due to failure of the CNS-mediated neural activity, or is possibly the result of the accumulation of metabolic vasodilator substances, which may include MDF, CO_2, lactate, potassium ions, hydrogen ions, adenosine, histamine, bradykinin, or prostaglandins. Neurogenic vasoconstriction after initial blood loss is apparently not very intense in the hen since PBZ did not reduce the IBV with near the magnitude it has in the dog. The possibility then exists that vascular decompensation, if indeed it even does occur in the hen, would have a much less dramatic effect on the MABP than it does in the dog.

The hen does show a degree of vasomotor tone, as evidenced by the small fall in TPR upon alpha-adrenergic blockade (Figure 1). Neurogenic constriction in the duck is intense, inhibited by alpha-adrenergic blockade, and cannot be overcome by the accumulation of metabolic

vasodilator metabolites (Folkow et al., 1967). Sciatic vascular resistance increased eightfold during submersion in the duck (Butler and Jones, 1971). Isolated hind-limb perfusion studies have not been reported in the hen. Such studies in the turkey have shown that a skeletal muscle hyperemia does occur during the vasoconstriction caused by breathing a 20 percent CO_2 gas mixture. Similar studies in the duck have failed to demonstrate such a hypermia. Hence, large variations exist among bird species, with the most efficient MABP homeostasis being achieved by flying and diving birds.

The IBV is an indicator of an animal's ability to maintain the MABP. The chicken IBV is one half that of a dog when compared on a percent of initial blood volume basis (Table 12). This again indicates that vasoconstriction in the chicken is not as important as it is in the mammal with regard to blood pressure homeostasis.

Kovách and Szász (1968) reported that a loss of 54 percent of the initial blood volume in the pigeon resulted in only a 30 mm Hg drop in the MABP. Roughly 22 percent of the initial blood volume of the dog had to be removed to lower the MABP by 20 mm Hg (Hollenberg et al., 1970), however, after PBZ treatment only five percent had to be removed to obtain the same effect. Research for this thesis indicated that a blood loss of less than 10 percent was all that was necessary to reduce the MABP by 20 mm Hg in the hen, and PBZ did not change this value.

The SBV represents the movement of fluid into the vascular space in avian and mammalian classes. The magnitude of the SBV in a bird varies with the duration of the experiment. The experimental duration, i.e. the survival time, depends upon the level of hypotension and the bleeding rate. The bleeding rate in this research was twice that of Wyse and Nickerson

TABLE 12. The IBV, SBV, MBV, and initial blood volume data from research in the literature on the dog during hemorrhagic shock and on the chicken during sustained hypovolemic hypotention.

Species	n	IBV ml/kg	SBV ml/kg	MBV ml/kg	Initial Blood Volume ml/kg	Reference
Dog	6	35	17	52	105	Abel et al., 1967.
Dog	7	42	13	55	---[1]	Rothe and Selkurt, 1964
Dog	8	36	20	56	---	Grega et al., 1967
Dog	24	39	07	46	83	Stekiel et al., 1967.
Dog	11	45	07	52	86	Hollandberg et al., 1970
MEAN VALUES =		39.4(43)[2]	12.8(14)	52.2(57)	91.3	
SBV/IBV ratio = .32						
Chicken	13	12.6	24.8	37.4	57.2	Wyse and Nickerson, 1971
Chicken	6	11.4	18.7	30.1	---	This Thesis, 1979.
MEAN VALUES =		12.0(21)	21.7(38)	33.8(59)	57.2	
SBV/IBV ratio = 1.80						

[1]Value not given.

[2]Parenthetical values represent percent of the initial blood volume.

(1971) and the resultant bleeding volumes were somewhat smaller. The canine SBV is limited by the onset of decompensation. The SBV in the chicken is limited by a rapid CVS collapse culminating in the death unless the shed blood is quickly reinfused. The mean canine SBV from the five research articles cited in Table 12 is approximately 14 percent of the initial blood volume, as compared to 38 percent in the chicken.

The hemorrhaged chicken has been reported to mobilize 40 to 52 percent of the initial blood volume before dying (Wyse and Nickerson, 1971), whereas the dog mobilized only 10 percent. Wyse and Nickerson (1971) reported the plasma mobilization rates for the dog and chicken for the first 90 minutes of hypotension to be 6.7 and 15 percent of the initial blood volume per hour, respectively. The volume of fluid mobilized in the research for this thesis, as indicated by the fall in the HCT, was roughly 35 percent of the initial blood volume over a three hour duration, or about 11.7 percent per hour. Hence, the rate of transcapillary fluid influx is higher in avian species than in mammals and continues until death.

The duck has been shown to almost entirely replace a 40 ml blood loss (14 percent of the initial blood volume) in 20 to 25 minutes (Djojosugito et al., 1968). This high rate of transcapillary fluid influx in the hemorrhaged duck did subside over time despite a continued increase in flow resistance. This reduction could be a consequence of the reduced plasma oncotic pressure and increased tissue oncotic pressure. The duck, like the chickens in this research, may experience hyperglycemia which would increase the plasma osmolarity. This, coupled with a reduced Pc, could shift the Starling equilibrium, favoring fluid absorption despite the large fall in plasma oncotic pressure. Eventually the

forces could become balanced and a fluid efflux could occur in the terminal stages of hypovolemic hypotension (Wyse and Nickerson, 1971), though this was not observed in research for this thesis.

The TPR apparently falls, or at least does not increase, in the hemorrhaged chicken. How, then, does the chicken utilize the Starling forces to absorb fluid faster than the hemorrhaged dog? The low arterial pressure may cause some precapillary vessels to collapse, facilitating fluid absorption. The oncotic forces also favor fluid absorption. The respiratory alkalosis and the associated hypocapnia, may cause vasodilation(unlike the mammal), shifting the Starling equilibrium favoring fluid absorption.

Irreversible shock in mammals is thought to be partially the result of low tissue flow due to a low MABP and high TPR. The latter did not occur in the chicken and this may offer this species some protection.

Phenoxybenzamine treatment reduced the rate of transcapillary fluid influx in the hemorrhaged duck. PBZ also reduced the rate of fluid influx and then efflux across the capillary wall in the hemorrhaged dog (Stekiel et al., 1967). Hence, PBZ reduced the IBV and increased the SBV in dogs. The drug did not significantly change the IBV or SBV in the chickens in this research, though small trends did exist in the same direction as those observed in the dog (Table 5). Therefore, alpha-adrenergic receptors play a role in all three species, but to a much lesser extent in the chicken.

Beta-adrenergic blockade with PROP prior to hemorrhage greatly reduced the MABP, which could directly reduce the IBV as seen in Table 5. The mean SBV of this group of birds was the highest of all three hemorrhaged groups, even though their survival time was 50 minutes less than either

of the other hemorrhaged groups. Inasmuch as the rate of hemodilution in this group, as evidenced by the uniformity of the slopes for HCT and Hb (Figures 4B and 5B) after temporal adjustment for differences in survival times, is identical with the other hemorrhaged groups, one can assume that death resulted from similar vascular responses. The decreased survival time in this group appears to have been due to the negative chronotropic effects of the drug on the myocardium.

The question immediately arises as to whether or not the PROP + hemorrhage birds were stressed more than the other groups. However, the more relevant question might be why was it necessary to remove blood much more rapidly in these birds in order to maintain the MABP constant.

The ratio of SBV/survival time, which is an indicator of the volume of blood removed per kilogram per minute to maintain the MABP at 50 mm Hg for the PROP + hemorrhage group and the hemorrhage group was .136 and .084, respectively. If the SBV reflects the transcapillary fluid influx, PROP enhanced the rate of fluid mobilation almost twofold. This could have been due to alterations in either cardiac performance or peripheral vascular tone. The cardio-inhibitory effects of PROP alone could reduce the Pc facilitating plasma volume expansion. Beta-adrenergic blockade has been shown to produce a degree of vasoconstriction in mammals.

The SBV/IBV ratio was reduced in the dog pretreated with PBZ, indicating that alpha-adrenergic receptors are involved with MABP regulation. PBZ pretreatment did not change the SBV/IBV ratio in hens. Hence, the mechanisms involved with blood pressure regulation in the chicken depend less upon alpha-adrenergic innervation than on local control mechanisms and/or differences in anatomical design of the peripheral vasculature.

It should be noted here that the SBV/IBV ratios for the dog and chicken are .32 and 1.80 (Table 12), respectively. This again indicates the intrinsic differences in the control mechanisms between the two species; the dog acting primarily to maintain MABP and the chicken acting to maintain the intravascular volume.

The MBV's for avian and mammalian classes are remarkably similar. The MBV is obtained at a time when the critical events leading to death occur in both classes. Immediately following the MBV in the dog hemoconcentration begins to occur, and mortality increases as hemoconcentration progresses. The fowl, on the other hand, enters sudden death at the time the MBV is obtained.

Irreversible hemorrhagic shock in mammals is due either to cardiac failure or failure of the peripheral resistance. Inasmuch as the chicken apparently neither vasoconstricts nor enters vascular decompensation it appears that mammalian irreversible shock may result from failure of the peripheral resistance.

B. Hematological Parameters -- The hematological events which occur in the hemorrhaged dog up to the onset of decompensation are very similar to the events occurring in fowl. The primary differences in the two species are the rapid rate of fluid mobilization and long duration to the MBV in the fowl. The HCT, Hb, and plasma protein concentrations fall, while plasma potassium and glucose increase and plasma sodium and magnesium remain quite constant in both species during the interval of time leading to the MBV.

These trends all continue in fowl up to the time of death. The dog, however, undergoes a reversal of events: the HCT increases by 30 percent of the initial value, the Hb and plasma protein levels rise, the

glucose suddenly falls to severe hypoglycemia, but the plasma potassium continues to rise.

The failure of avian species to decompensate or enter shock irreversible to transfusion raises questions concerning the present understanding of the shock phenomenon, particularly in regard to the development of hyperkalemia and hypoglycemia in late shock. Before these questions can be addressed, a review of some of the biological differences between dogs, ducks, and chickens is necessary.

The skeletal muscle response for vasoconstriction during a reduction of the MABP in the dog is overcome, and a constant blood flow is maintained, by intrinsic neurologic responses, i.e. Baylis response, and by the accumulation of vasodilator metabolites. Mellander (1963) and others have hypothesized that after continued hypotension in the mammal, precapillary sphincter (if indeed such a sphincter exists outside of the bat wing) reactivity began to fall, while at the same time the reactivity of the postcapillary resistance vessels and capacitance vessels remained high. Eventually capacitance vessel reactivity fell, causing venopooling, decreased venous return, decreased CO, and a further fall in reactivity completing a vicious circle. It should be mentioned here that many subsequent researchers have failed to demonstrate changes in the pre/post capillary resistance ratio during shock in the mammal.

As mentioned previously, the duck is thought not to show an active hyperemia, or "neurogenic breakthrough," since no ascending vasodilation was seen in constant-flow hind-limb perfusion studies during neurogenic vasoconstriction. The chicken may behave more like mammalian species than the duck, though this has not been confirmed by experimental evidence.

The slopes of the HCT and Hb curves and the magnitude of the SBV

(both indicators of the rate of fluid transfer into the vascular system) were not affected by PBZ pretreatment in hemorrhaged chickens. Phenoxybenzamine reduced the rate of post-hemorrhage fluid influx in the duck. It is tempting to speculate that the difference between the chicken and duck in the rate of fluid mobilization under the influence of PBZ is somehow related to the ability of the duck to tolerate a more severe hemorrhage. Before such speculations may be made, it must be realized that many intrinsic physiological differences exist between the duck and chicken beside the vascular dissimilarities.

The duck has a much higher HCT and Hb, and an oxygen dissociation curve shifted considerably to the left of that of the chicken. The duck also has a higher degree of oxygen saturation in the arterial blood than the chicken, which has a high degree of unsaturation. The adult duck also has a higher level of adenosine triphosphate (ATP) in its erythrocytes than the chicken, about 12 versus 3 umoles ATP/ml of erythrocytes, respectively. The duck has all red muscle and a high myoglobin concentration. Vagotomy produced a 65 to 200 percent increase in HR in the duck, and only a 20 to 40 percent increase in the chicken, indicating that parasympathetic tone is more intense in the duck. Intracardiac glucagon (mammalian) caused an intermediate to severe hyperglycemia in the mammal or bird. However, the concomitant increase in plasma insulin seen in man, dog, and duck did not occur in the chicken.

The submerged duck maintained some degree of skeletal muscle blood flow, though primarily through non-nutritional vessels. This enhanced the utilization of venous oxygen stores, and the cortical concentration of reduced nicotinamide-adenine dinucleotide (NADH) increased only slightly after a minute dive (Jones and West, 1978). The chicken

tolerated a minute of submergence poorly by showing CVS difficulties (Bond, 1961) and greatly increased cortical NADH concentrations. If such large differences exist in the diving responses of these two species, it is not unreasonable to suspect large differences may also exist in their response to hemorrhage.

The increased potassium in hemorrhaged mammals is likely the result of a passive movement of potassium ions out of the skeletal muscle cells with the bicarbonate anions (HCO_3^-) in response to the intracellular accumulation of hydrogen ions. This hyperkalemia may be one of the primary initiating factors in reactive hyperemia in the mammal. A hyperkalemia-induced vasodilation in mammals may be involved in vascular decompensation in shock.

The hyperkalemia seen in the hens in this research was of a magnitude similar to that of mammals late in decompensation. Bond et al. (1977) reported plasma potassium in the hemorrhaged dog rose from 4 to 6 mEq/L (a 50 percent increase). The potassium in hens increased 20 to 55 percent (Table 8 and Figures 6A and 6B) during the duration of the experiment. Potassium levels of a 6 mEq/L in the mammal (but not exceeding 12 mEq/L) are vasodilator (Haddy and Scott, 1975), but will not cause nearly the vasodilation seen during exercise hyperemia. Therefore, the hyperkalemia probably plays only a small role in initiating vascular decompensation in late shock in the mammal. Nothing is known about the effect of eletrolyte alterations in the chicken during exercise, or active, hyperemia, or even if such forms of hyperemia occur.

The hydrogen ion concentration decreased while the extracellular potassium ion concentration increased in these hens. This is contrary to the popular opinion that the hyperkalemia is the result of the passive

movement of potassium ions out of the cell in exchange for accumulating hydrogen ions. It may be that the hen possesses a more dynamic buffering system than the mammal, even though Morgan and Chichester (1935) concluded that the buffering capacity of avian blood was about the same as that of man. Inhibition of the Na-K electrogenic pump could be another explanation for the hyperkalemia. The high heart rate also may have contributed to the hyperkalemia.

The production of carbonate for the eggshell results in an excess of hydrogen ions and reduces the blood pH. Since shell formation takes 20 out of the total 26 hours required for egg formation as a whole, and an adult hen will lay 250 eggs in a year, it can be appreciated that for half of its life the hen is faced with an incipient acidemia. Renal and pulmonary mechanisms play a major role in the preservation of the neutrality of the blood. Urinary excretion of photons in birds is by way of dihydrogen phosphate (as in mammals) and by the production of ammonia. Both of these mechanisms respond to experimentally-induced acidemia by hydrochloric acid infusion. Metabolic acidosis can also be compensated for by hyperventilation.

Alpha-adrenergic receptor stimulation inhibits insulin secretion (Akerblom et al., 1969). Insulin inhibits the efflux of potassium ions from both the liver (Mortimore, 1961) and the skeletal muscles (Zierler, 1959). Hence, PBZ may reduce plasma potassium levels. Alpha-adrenergic blockade can also increase pancreatic perfusion, which could lead to increased insulin release and plasma insulin concentrations, thereby decreasing the magnitude of a hyperkalemia. Alpha-adrenergic blockade can also decrease plasma corticosteroid concentrations (Edens, 1974) and, therefore, could decrease the extrusion of intracellular potassium

(Clauss and Ray, 1968). These may be the reasons why the increase in plasma potassium in the PBZ + hemorrhage group was of a lesser magnitude than seen in the other hemorrhaged groups (Figure 6B).

The hypoglycemia in mammals in late hemorrhagic and endotoxin shock is due to decreased glucose production by the liver and kidney, increased peripheral utilization of glucose, decreased pancreatic blood flow, and increased insensitivity to insulin. These all result in decreased intracellular glucose and cell death.

The injection of enough glucose to cause a significant hyperglycemia in the chicken doubled or tripled insulin levels within thirty minutes. The hyperglycemia from one catecholamine injection did not affect insulin release (Langslow and Hales, 1971). Catecholamine injections caused hyperglycemia in both the fowl and mammal, yet while the plasma FFA concentration fell in the mammal, it did not change in the chicken. There is generally an inverse relationship between glucose and FFA concentrations in the mammal, whereas the FFA concentration stays constant in the chicken. Massive doses of mammalian insulin did not affect plasma FFA concentrations, and resulted in only a slight hypoglycemia in the hen. This difference is not due to pancreatic glucogon since results following pancreatectomy are the same. The avian insensitivity to exogenous insulin is well documented.

The avian liver has a well developed insulin-destroying mechanism. Differences in mammalian and avian nervous systems may also contribute to the avian insensitivity to insulin. Some researchers have observed that avian hepatic and skeletal muscle glycogen stores are decreased by repeated administration of insulin. These results are the opposite of what occurs in mammals, and can also be produced in the fowl by the

continuous administration of catecholamines. Thus, insulin-induced hypoglycemia in the hen may encourage the adrenal-medullary release of glycogenolytic catecholamines, which may favor the release of glucose into the blood stream. Likewise, the massive adrenal-medullary discharge during hemorrhagic shock causes large and continous increases in plasma glucose (Figures 7A and 7B).

Beta-adrenergic blockade with PROP has been shown to depress the insulin increase in response to elevated glucose and to prevent the catecholamine-stimulated lipolysis and FFA release in vivo and in vitro (Akerblom et al., 1969). In glucose-loaded rats, the insulin/blood glucose ratio, an indicator of the response of the pancreatic islet cells to glucose stimulation, increased threefold in controls and did not change in PROP-treated rats. Therefore, beta-adrenergic receptors must be intact for complete insulin response to hyperglycemia to be elicited in the mammal. Though Figure 7B appears to show that the PROP + hemorrhage group had high glucose levels, one-way ANOVA showed that this was the only hemorrhaged group failing to show a significant hyperglycemia (Table 8). The two PROP + hemorrhage birds which survived the experiment had the largest increases in plasma glucose for that group. This is because the birds, dying early in the procedure due to the cardiodepressive effects of beta-adrenergic blockade, did not have time to become hyperglycemic.

It cannot be ascertained if the elevated glucose levels were the result of hypoinsulinemia or decreased reactivity to insulin. It is clear that no trend toward hypoglycemia existed in the hemorrhaged chickens as it did in the mammal (Strawitz et al., 1961).

It is tempting to speculate that hyperglycemia in the fowl raised the

plasma osmolarity enough to provide a considerable osmotic gradient for fluid influx from the interstitial and intracellular spaces. As mentioned previously, this gradient, coupled with a reduced Pc, could eventually reach an equilibrium with the decreased plasma oncotic pressure. The rate of fluid influx would then decrease, or even reverse. Wyse and Nickerson (1971) reported a nonsignificant tendency for the plasma protein and HCT to increase in the terminal stages of hemorrhagic hypotension in their hens, though this was not seen during research for this thesis.

The lower degree of hyperglycemia in the PBZ + hemorrhage group (Figure 7B) than the hemorrhage group could be the result of the drugs insulin-elevating effects, as discussed previously. This is supported by the fact that PBZ has been shown to increase the plasma FFA concentration during shock in mammals (Kashyap et al., 1975). Alpha-adrenergic blockade has also decreased the cortisone level in the chicken, which could also reduce the plasma glucose (Edens, 1974).

The normal value for the oxygen (O_2) combined with Hb for the chicken is about 12 ml O_2 per 100 ml of blood (or 12 vol%) at a Hb of 10 grams per 100 ml of blood (10 gm%), pCO_2 of 40 mm Hg, pO_2 of 95 mm Hg, if it is assumed that one gram of Hb will combine with 1.34 ml of O_2 (as in mammals). The O_2 combined with Hb value below which electrocortical alterations begin to occur in the hen is about 8.0 vol%. This value was obtained by using the lower critical pO_2 of 70 mm Hg (Richards and Sykes, 1967) and the O_2 dissociation curve of Choidi and Terrman (1965). If, as in this research, the Hb was reduced by 50 percent, the pCO_2 was 20 mm Hg due to hyperventilation (shifting the O_2 dissociation curve far left), and the pO_2 was 90 mm Hg, then the O_2 combined with Hb value would

be about 6 vol%. This is considerably below the lower limit for normal electrocortical behavior. In fact, a reduction of Hb by more than 30 percent, while the pCO_2 was below 20 mm Hg, could cause cortical dysfunction. If this is truly the case, the birds in this research began showing electrocortical alterations for the last one and one-half hours of the experiment (Table 7).

Although this cerebral ischemia may be a contributing factor in the CVS collapse (or possible peripheral vascular failure) in the fowl, it is not the primary cause of death. This was proven by Wyse and Nickerson (1971), who reinfused all the resuspended shed erythrocytes in their hemorrhaged hens after two hours of hypotension and found the survival times were not changed. Therefore, although hypoxia caused a large fall in TPR (Besche and Kadono, 1978) and the birds in this research were quite hypoxic, this does not appear to be the ultimate cause of death.

Recent research has indicated that, following hemorrhage in the hen the intraerythrocytic concentration of 1,3,4,5,6 -- myoinsitol pentophosphate increased, which shifted the O_2 dissociation curve far to the right and enhanced O_2 delivery to the tissues (Jones et al., 1978). This is different from mammals, where the main controller of O_2 transport is increased intracellular concentrations of 2,3 -- diphosphoglycerate. These researchers also found the rate of erythrogenesis following hemorrhage in the hen was comparable to that of the dog.

Many other factors could be involved, such as direct inhibition of the vascular smooth muscle by hypoxia, metabolic acidosis, and/or catecholamine depletion. There may be a decreasing adrenergic effect on resistance vessels. Plasma prostaglandin E_1, which increases between 90 and 180 minutes after shock in mammals, and can cause inhibition of

adrenergic constriction, may be involved. Cellular swelling and a catecholamine refractoriness due to low pH are other possibilities. MDF, histamine, and/or bradykinin may also be involved.

Further studies involving local blood flow regulation in the gut and skeletal muscles of the chicken should be conducted. Capillary filtration coefficients (CFC) and other perfusion studies should be performed on the hen to determine the effects of hypoxia, histamine, prostaglandin, adenosine, electrolytes, and bradykinin on local blood flow. The duration and magnitude of active and reactive hyperemias in the chicken also needs to be determined. Studies elucidating the process of local blood flow regulation in the chicken would not only benefit shock research, but expand current understanding of poultry physiology as well.

SUMMARY AND CONCLUSIONS

1. Propranolol significantly reduced heart rate, arterial blood pressure, cardiac index, and stroke work, thirty minutes after administration. There was a tendency for stroke volume and total peripheral resistance to be reduced. Phenoxybenzamine pretreatment did not change the heart rate, mean arterial blood pressure, cardiac index, stroke volume, stroke work, or hemoglobin. There was a tendency for total peripheral resistance to be reduced. Hematocrit was reduced by nine percent of the initial value by PBZ treatment.

2. Hemorrhage reduced cardiac index, stroke volume, stroke work, and total peripheral resistance. Heart rate increased in all the hemorrhaged groups except for the PROP + hemorrhage group, where heart rate remained subnormal throughout the experiment. Bleeding volumes were not affected by alpha- or beta-adrenergic blockade. Survival time was lower in the PROP + hemorrhage group and mortality was higher (67%). Mortality for both of the other hemorrhaged groups was 33%.

3. Hemorrhage caused the hematocrit and hemoglobin to fall progressively throughout the duration of the experiment. Mammals do the opposite in late shock. Plasma potassium and plasma glucose concentrations increased progressively throughout the duration of the experiment in the hemorrhaged groups. Small increases also occurred in the control group. Mammals demonstrate severe hypoglycemia after hemorrhage. Plasma magnesium and plasma sodium concentrations did not change in the hemorrhaged groups.

4. Hemorrhage caused progressive reductions in the arterial pCO_2, and a concomitant increase in the arterial pH. The arterial pO_2 was not changed by hemorrhage. One death occurred in each of the hemorrhaged groups due to respiratory arrest, except for the PROP + hemorrhage group where all deaths appeared to be of cardiovascular causes. The birds in this latter group experienced no respiratory difficulties, whereas each of the other hemorrhaged groups required artificial ventilation in at least one instance to keep a bird alive.

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APPENDIX A

PROCEDURE FOR PLASMA ELECTROLYTE DETERMINATIONS

Sodium Concentration: Fifty ul of plasma was added to 10 ml of a 90 ppm lithium internal standard diluent. Analysis by automatic feed flame photometer.

Potassium Concentration: Fifty ul of plasma was added to 10 ml of a 90 ppm lithium internal standard diluent. Analysis by automatic flame photometer.

Magnesium Concentration: 0.2 ml of plasma was added to 3.8 ml of lathanum oxide diluent. Analysis by automatic feed atomic absorption spectrophotometer.

APPENDIX B

SAMPLE CALCULATION OF CARDIAC OUTPUT, CARDIAC INDEX, AND TOTAL PERIPHERAL RESISTANCE

Body weight = 1.7 kg
Dye Injected = 0.2 mg
Area under curve = 6.5 in^2
Calibration = 10 mg/l. = 6 in.defl.
Chart speed = 12 in./minute
MABP = 100 mm Hg

Cardiac Output =

$$\frac{.2 \text{ mg}}{6.5 \text{ in}^2\left(\frac{10 \text{mg/l./12 in./min.}}{6 \text{ in.}}\right)}$$

$$= \frac{0.2 \text{ mg}}{.903 \text{ mg min/l}}$$

$$= 0.222 \text{ l./min.} = 222 \text{ ml/min}$$

Cardiac Index =

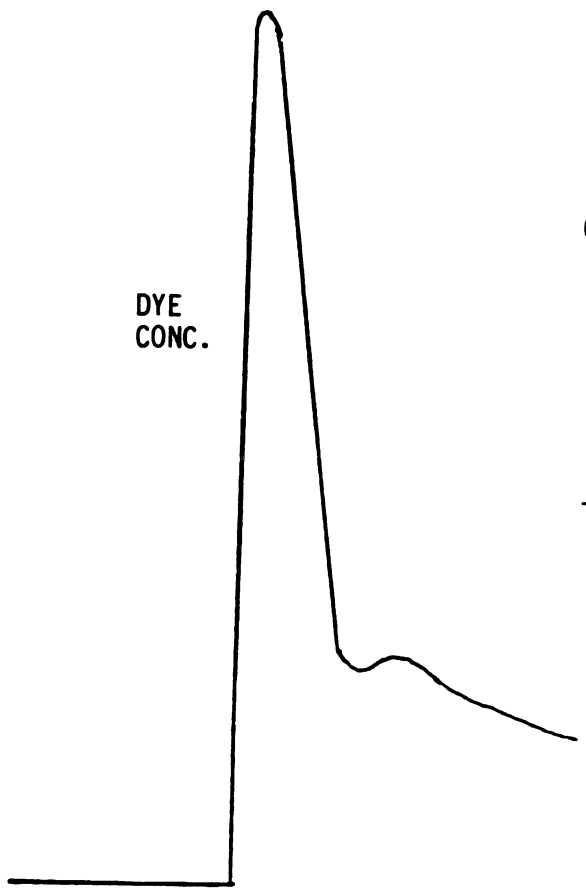
$$\frac{222 \text{ ml/min.}}{1.7^{.734} \text{kg}}$$

$$= 150.5 \text{ ml/min./kg}^{.734}$$

Total Peripheral Resistance =

$$\frac{100 \text{ mm Hg}}{150.4 \text{ ml/min./kg}^{.734}}$$

$$= 0.66 \text{ arbitrary units}$$



APPENDIX C

STATISTICAL ANALYSIS':

1. Split-plot repeated-measure ANOVA

	Source of Variation	d.f.	SS	MS
i =	Treatments	3	SS_t	MS_t
E1 = i/j =	Birds/treatment	20	SS_{E1}	MS_{E1}
k =	Sampling times	2	SS_s	MS_s
	Treatment X Sample	6	SS_{ts}	MS_{ts}
E_2 =	Birds X Sample	40	SS_{E2}	MS_{E2}
	Total		SS_y	

F-testing:

MS_t/MS_{E1} vs. $f\alpha,3,20$ ---------- treatments

MS_s/MS_{E2} vs. $f\alpha,2,40$ ---------- samples

MS_{ts}/MS_{E2} vs. $f\alpha,6,40$ ---------- treatment-sample interaction

$SS_y = \Sigma\Sigma\Sigma y^2ijk - \underbrace{(\Sigma\Sigma\Sigma yijk)^2/72}_{C.F.}$

$SS_t = \Sigma y^2_i../18 - C.F.$

$SS_{E1} = \Sigma\Sigma y^2ij./3 - \Sigma y_i{}^2../18$

$SS_s = \Sigma y^2.._k/24 - C.F.$

$SS_{ts} = \Sigma\Sigma y^2_{i\cdot k}/6 - (C.F. + SS_t + SS_s)$

$SS_{E2} = SS_y - (SS_t + SS_{E1} + SS_s + SS_{ts})$

C.F. = Correction Factor

$y_i..$ = trt. total of 18 observations

$y_{ij}.$ = bird total of 3 observations

$y.._k$ = sampling time total of 24 observations

$y_{i\cdot k}$ = trt. total at one time (6 obs.)

Treatment comparisons: (Averaged over sampling times) using Dunnett's test with group #2 as the 'control.'

$t_1 = \bar{y}_1 - \{\bar{y}_2 / \sqrt{2MS_{E1}/18}\}$

$t_3 = \bar{y}_3 - \{\bar{y}_2 / \sqrt{2MS_{E1}/18}\}$ all vs. $t_d,\alpha,3,20$

$t_4 = \bar{y}_4 - \{\bar{y}_2 / \sqrt{2MS_{E1}/18}\}$

APPENDIX C (continued)

Sampling time comparisons: (averaged over treatment) using orthogonal polynomials (L, Q).

sample times

1
2 }45 minutes
3 }45 minutes
4 death

$q_L = \bar{y}_3 - \bar{y}_1$

$q_Q = \bar{y}_3 + \bar{y}_1 - 2\bar{y}_2$

$F_L = q_L^2/2MS_{E2}/24$ vs. $f\alpha,1,40$ ---------- average trend

$F_Q = q_Q^2/6MS_{E2}/6$ vs. $f\alpha,1,40$ ---------- curvilinear

2. Block design repeated measure 2-way ANOVA

Sources of variation	d.f.	SS	MS
Individuals	4	SS_i	
Sampling times	2	SS_t	
Error	8	SS_E	MS_E

$SS_y = \Sigma\Sigma y^2_{ij} - \underbrace{(\Sigma\Sigma yij)^2/18}_{\text{C.F.}}$

$SS_i = \Sigma y_i^2./3 - C.F.$

$SS_t = \Sigma y^2_{.j}/6 - C.F.$

$SS_E = SS_y - SS_i - SS_t$

$y_i.$ = total on 1 indiv. (3 observations)

$y_{.j}$ = total at 1 time (6 observations)

Dunnetts test: 0 vs. 90: $t_D = \bar{y}_{.1} - \bar{y}_{.2}/\ \sqrt{2MS_E/6}$

0 vs. 180: $t_D = \bar{y}_{.1} - \bar{y}_{.2}/\ \sqrt{2MS_E/6}$

both vs. $t_{D,\alpha/2,2,10}$