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THE ROLE OF CHEMORECEPTORS IN ERYTHROPOIESIS AND THE CARDIOVASCULAR RESPONSES TO ANEMIA

by

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THE ROLE OF CHEMORECEPTORS IN ERYTHROPOIESIS AND THE CARDIOVASCULAR RESPONSES TO ANEMIA

David Black Gillis

Abstract

We have studied several aspects of the role of chemoreceptors in anemia and erythropoiesis in cats.

It has been reported that removal of carotid bodies in cats results in a brief, profuse reticulocytosis which is followed by a progressively severe anemia. We excised carotid bodies bilaterally in cats and followed the course of erythropoiesis with serial bone marrow aspirations, hemoatocrit determinations, and reticulocyte determinations. $T_{1/2}$ for plasma Fe^{59} removal, erythrocyte Fe^{59} incorporation, and Cr^{51} RBC lifespan determinations were performed on five operated and three control cats. We found no significant differences between the groups. We concluded that the carotid body has no direct effect on erythropoiesis although other evidence suggests it influences the erythropoietic response to hypoxia by reducing hypoxemia through hyperventilation. We suggest the anemia reported in a prior study was secondary to sepsis resulting from an indwelling femoral vein catheter. Our data also suggests that erythrocyte incorporation of Fe^{59} in cats averages 95%, in contrast to other reports of 20% incorporation.

We have studied the effect of acute, isovolemic anemia induced by Dextran-for-blood exchange transfusion on the chemoreceptor hypoxic

response curve. Carotid chemoreceptors demonstrated variable changes in the hypoxic sensitivity as manifested by changes in sinus nerve chemoreceptor fiber firing rates after induction of anemia with constant Pa_{O_2}. Aortic chemoreceptor fibers, however, consistently manifested an increased firing rate with induction of anemia. Partial correction of the anemia resulted in a return of the hypoxic sensitivity towards the control value in aortic chemoreceptors but responses to re-infusion of red cells had variable effects on carotid chemoreceptors. We concluded that aortic chemoreceptors manifest significantly increased hypoxic response during anemia, carotid chemoreceptors respond inconsistently. We suggest that aortic chemoreceptors may be the afferent sensor mechanism for initiating sympathetic cardiovascular responses to anemia in the first several hours after induction of acute anemia.

A model for studying the effects of chemoreceptor denervation on organ blood flows and on the cardiac output was developed and validated. Pilot studies suggested the model is suitable for accurate measurement of blood flows and cardiac output in normal, anesthetized cats, and during hypoxia and severe anemia. Our values for organ blood flows in the intact anesthetized cat agree well with those found by other authors in other animals.

While the number of cats studied were small, the results suggest that aortic chemoreceptors play a significant role in the elevation of cardiac output in the first hour after induction of acute, isovolemic anemia. After 1-2 hours, cardiac output returned towards its predenervation levels. Cardiac output responses to hypoxia were also

reduced by aortic chemoreceptor denervation. Organ blood flows underwent major changes in some vascular beds with denervation of the aortic chemoreceptors in the presence of anemia or hypoxia.

CONTENTS

NOTE

All statistical data are presented as mean $\pm$ standard deviation unless otherwise indicated. Probability values were determined by Student's 't' test.

GENERAL INTRODUCTION

It was the purpose of this study to investigate a possible role of the arterial chemoreceptors in hematologic and circulatory responses to anemia, to develop a model for study of chemoreceptor influence on cardiac output and organ blood flow in anemia and hypoxia, and develop preliminary data based on this model. These endeavors can be grouped in three categories: 1) erythropoietic consequences of carotid body excision, 2) the influence of acute normovolemic anemia on the hypoxic response of aortic and carotid body nerve fibers, and 3) validation of the radioactive microsphere method for study of changes in organ blood flow in anemia and hypoxia with preliminary studies of the effects of aortic body denervation on the circulatory response to hypoxia and acute anemia. All original studies reported here were conducted in the cat.

The carotid body is a minute organ weighing about 2 mg in the cat, and located immediately posterior to the ascending pharyngeal and occipital arteries at the carotid bifurcation bilaterally. It is innervated by efferent sympathetic fibers from the superior cervical ganglion via the ganglio-glomerular nerve and pre-ganglionic parasympathetic fibers via the carotid sinus nerve. Afferent innervation is via the carotid sinus nerve, a branch of the glossopharyngeal nerve. Its major blood supply is via branches of the carotid artery and the major venous drainage is via small branches into the superior vena cava (Chungcharoen, et al., 1952). The carotid body receives a blood flow of about 2 liters per 100 gm. per minute, about five times the specific blood flow of the kidney (Daly, et al., 1954). Both preganglionic and postganglionic fibers (from the superior cervical ganglion) influence its blood flow, the predominant effect being a tonic vasocon-

striction mediated by the sympathetics (Purves, 1970). Fay (1970) has reported oxygen consumption of 1.5 mg 0_2/min/100 gm, less than 20% of the value reported by Daly (1954), who estimated 9 ml 0_2/min/100 grams.

The predominant functional cell type of the carotid body is the Type I (glomus or chemoreceptor) cell, characterized by its irregular shape, multiple cellular processes, a round or ovoid nucleus, and a cytoplasm filled with dark-staining granules containing a high concentration of dopamine and lesser quantities of epinephrine and norepinephrine (Zapata, et al., 1969; Chiocchio, et al., 1966; Chen and Yates, 1969).

Type I cells frequently have a prominent Golgi apparatus, apparently associated with the formation of cytoplasmic granules which can be seen in some micrographs to appear to bud from the Golgi saccules in much the same manner as vesicles in pancreatic cells. The vesicles are characterized by dense staining circular outlines and a halo of lightly staining material adjacent to the enclosing membrane. They appear in diameters of 350 to 2300 Å, suggesting a true diameter of 2000-2300 Å. Areas of rough endoplasmic reticulum occur and, rarely, a cilia is found with a 9 + 0 filamentous structure. Occasional junctional complexes are seen between adjacent Type I cells.

Enclosing the Type I cell almost completely is the Type II cell which has classically been assigned a supportive role because of its resemblance to satellite cells and sustentacular cells in other locations in the body. Very little surface area of the Type I cell is left uncovered by the Type II cell. Type II cells contain cilia and Golgi bodies but vesicles are neither so prominent nor numerous.

Nerve fibers are encased by the type II cells in much the same fashion as by Schwann cells.

The fine structure of the carotid body has been described in a number of species. There is moderate variation in the glomus cell to nerve relationship and evidence for several major types of glomus cell (Grimley and Glenner, 1968; Biscoe and Stebhens, 1966; Al-Lami and Murray, 1968; Al-Lami and Murray, 1968b; Morita et al., 1969; McDonald, 1974; de Castro, 1926; de Castro, 1928; Chen et al., 1969; Ross, 1959; Kobayashi, 1968; Lever et al., 1959; Morita et al., 1970).

The structure of the Type I and II cells together with their nerve fibers has been described as forming cords or glomeruli. These nests of cells are surrounded but not penetrated by a network of capillaries. Usually the Type I cell is separated from the capillary by one or more layers of the enclosing Type II cell, by the basement membrane, and by the capillary endothelium. The latter is fenestrated in the manner of some endocrine organ capillaries. The average distance of the Type I cell from the capillary lumen appears to be 8-30 micra. In the cat the widened vessels are called sinusoids whereas in the monkey and man typical capillaries are found. The arterioles supplying these capillaries are presumably innervated by sympathetic and parasympathetic efferents.

Several types of nerve endings have been reported in the carotid body. One type, which appears to be sympathetic efferents, has compact bulbous endings with numerous synaptic vesicles of the S-type, 300-400 Å in diameter. This type of ending is frequently seen adjacent to the Type I cell and is suggestive of a typical efferent synapse. It is reported that 95% of the nerve endings on Type I cells are affer-

ent and frequently form reciprocal synapses with the Type I cells (McDonald, 1974). There is controversy over the existence and number of sub-types of Type I cells and the effect on the electron-dense granule content during stimulation. The fine structure of the innervation is also controversial, the original concept of simple afferent innervation by the glossopharyngeal nerve introduced by De Castro (1926, 1928) having been supplanted by several theories. The Type I cell has been considered a transducer sensing Pa_{O_2}, Pa_{CO_2}, and pHa in the arterial blood perfusing it and stimulating the afferent endings of the glossopharyngeal nerve. This concept requires a neurotransmitter, acetylcholine and various catecholamines have been considered.

The identification of the postulated transmitter substance in the carotid body continues to be controversial. Although there is much evidence to suggest acetylcholine is the transmitter substance, the presence of large amounts of catecholamines in the carotid body has suggested that catecholamines might be the transmitter substance.

Chen and Yates (1969) used autoradiographic and cytochemical techniques to localize catecholamines and indolamines in the Type I cell of the carotid body in hamsters. Over 50% of administered radioactive catecholamines and indolamine precursors localized to granules, over 90% to Type I cells. Reserpine depleted the granules and they were restored in 3-5 days. Chen and Yates concluded Type I granules synthesize catecholamines which then become particle bound. Most of the incorporated substance appeared to be catecholamine rather than indolamine.

In a related study Chen, Yates and Duncan (1969) studied the effect of hypoxia and reserpine on the appearance and number of granules in

the carotid body Type I cell and found no changes in granule density or number. They suggested reserpine releases the catecholamine intracellularly rather than extracellularly, for although the number of granules does not decrease and part of their contents remain, their catecholamine content is decreased as evidenced by the bichromate reaction. These studies also suggested the Type I cell does not release significant amounts of catecholamines in response to hypoxia.

Zapata, Hess, Bliss, and Eyzaguirre (1969) found 24 ng of adrenaline, 98 ng of noradrenaline and 207 ng of dopamine in the average cat carotid body. Previous sympathectomy did not alter these catecholamine levels, indicating the principle concentrations were not confined to sympathetic nerve endings. In carotid bodies exposed to hypoxia in situ and in vitro the catecholamine levels were essentially unchanged but reserpine administered four hours before excision of the organ resulted in marked reduction of the amine concentration without alteration in the normal response to natural stimuli or to acetylcholine. Adrenalectromy, sympathectomy, section of the sinus nerve, and hypoxia failed to cause microscopic changes in the granules. Neither catecholamines nor nialamide, a monoamide oxidase inhibitor, increased chemoreceptor discharge in vitro. The preparations did respond briskly to acetylcholine and catecholamines did not inhibit this response.

A series of papers by Eyzaguirre and colleagues has provided considerable experimental evidence of a possible role for acetylcholine (ACh) as the neurotransmitter for chemoreceptor impulses. Using in vitro carotid bodies to avoid the effect of circulatory changes, they noted a marked increase in discharge rates when ACh was introduced into

the superfusion fluid. The effect of ACh was potentiated by CO_2 and eserine. Cholinergic blocking agents such as tetraethylammonium and hexamethonium caused stimulation in small dosages but depression at higher dosages. Their blockade of ACh effects was antagonized by strong natural stimuli.

Eyzaguirre et al. (1965) found 20-30 micrograms ACh per gram of cat carotid body. Using two carotid bodies in tanden in the bathing medium they demonstrated that anoxic or electrical stimulation of the upstream body resulted in stimulation of the downstream carotid body. They suggested the chemical released might be ACh. In 1968 Eyzaguirre and Zapata (1968) showed the stimulation of the downstream organ was markedly augmented by addition of eserine to the bathing media whereas hexamethonium inhibited the effect of either 1 microgram of ACh or anoxic stimulus on the upstream carotid body. Acetylcholinesterase blocked the Loewi effect in this experiment. Denervated carotid bodies with degenerated efferent nerve endings gave qualitatively similar results, suggesting little of the Loewi effect originated from the ACh of the nerves, but rather arose primarily from the release of ACh from the carotid body cells.

Metz (1969) has also reported that in eserinized dogs ventilated with an hypoxic gas mixture there was a 42% increase in the ACh in carotid body effluent blood when the ventilating gas is made hypercapnic as well as hypoxic. Addition of hemicholinium to the perfusing blood of the carotid body uniformly reduced the ACh in the venous effluent.

There has been little experimental evidence presented that catecholamines function as the neurotransmitter in chemoreceptors although

some investigators have demonstrated them to be pharmacologically active in the carotid body. Jacobs et al. (1968) showed dopamine to be a potent stimulant of carotid chemoreceptors in the dog but it does not stimulate the cat. Eyzaguirre and Koyano (1965), like Jacobs, could not stimulate the cat carotid body with dopamine nor with epinephrine, tyramine, or norepinephrine. Biscoe (1965) and Joels and White (1968) have reported increased impulse activity after catecholamine infusions. The reduction in carotid body blood flow by vascular constriction and its contribution to this effect has not been elucidated.

Biscoe and Stehbens suggested in 1966, and Biscoe in 1971, that most of the nerve synapses on the Type I cell were efferent rather than afferent. In 1969 Biscoe, Lall, and Sampson (1970), reporting denervation studies, suggested most nerve endings on Type I cells were efferent and hypothesized they functioned to modulate the Type I cell sensitivity to physiologic stimuli. This concept has been challenged by Hess and Zapata (1972) and more recently by McDonald and Mitchell (1975), who found no degeneration of the synaptic endings on the Type I cell to suggest an efferent nature. They identified reciprocal synapses and accumulations of dense-cored vesicles at the afferent synaptic sites and postulated that it was afferent nerve endings, not the Type I cells, which are chemosensitive and that the Type I cells function to modulate the chemosensitivity of afferent nerve endings, presumably by the release of dopamine.

Although the identity of the neurotransmitter in the carotid body has not been established, it has been generally accepted that the carotid body responds to acidemia, hypoxemia, and increasing levels

of arterial carbon dioxide. Since de Castro's suggestion, based on anatomic evidence, many authors have confirmed such functions. Heymans and Heymans in 1927 and Heymans, Bouchaert and Dautrebande in 1930 demonstrated sensitivity of the carotid bodies to hypoxia and hypercarbia. Hornbein, Griffo and Roos (1961) used integrated chemoreceptor nerve discharges to show increasing discharge rates with hypoxemia and potentiation of the hypoxic response by hypercarbia. Biscoe and Sampson (1967) showed in the single nerve preparation that the chemoreceptor response to hypoxemia could be represented as a rectangular hyperbola while the response to increasing hypercarbia was nearly linear. Joels and Neil (1960) showed the carotid body responded to changes in pHa without changes in the Pa_{CO_2}. Hornbein and Roos (1963) and Gray (1968) demonstrated that the stimulant effects of carbon dioxide are effected through changes in pH in the carotid body.

Carotid body nerve impulses are conducted via the carotid sinus nerve to the glossopharyngeal nerve and hence to the brain stem, terminating in the nucleus tractus solitarius (Cottle, 1964) and perhaps other areas. Heymans and Heymans (1927) demonstrated trunkal chemosensitivity in the dog resulting in reflex increase in ventilation. Heymans et al., (1930) and Comroe and Schmidt (1938) then described the carotid chemoreceptors as a source of respiratory stimulation in response to hypoxia. Stimulation of the carotid bodies has also been shown to have marked effects on cardiovascular function, as will be discussed in more detail below. In summary, however, carotid body stimulation results in a decrease in heart rate and contractility, an increase in pulmonary vascular resistance, vasoconstriction of certain

vascular beds, splenic contraction, and increases in adrenal medullary and adrenal cortical secretions (Marotta, 1972).

THE AORTIC BODIES:

Although extra-carotid chemoreceptor tissue had been functionally demonstrated by Heymans and Heymans (1927) and anatomically by several workers, it was Comroe (1939) who first localized the extra-carotid chemoreceptor functions to the anatomic aortic chemoreceptor tissue and characterized its blood supply as systemic. The light microscopic characteristics of the aortic body are similar to those of the carotid body (Nonidez, 1937; Nonidez, 1935-36; Becker, 1967; Knoche, 1963; Coleridge, 1967; and Coleridge, 1970), as is the electron microscopic picture (Knoche, 1963).

As described by Howe (1956), the aortic bodies are found in four general locations: 1) root of the right subclavian artery, 2) root of the left subclavian artery, 3) on the ventral surface of the aortic arch, and 4) between the ascending aorta and the pulmonary trunk. The afferent impulses travel via a branch of the vagus nerve, joining the superior laryngeal nerve immediately before it enters the vagus (Coleridge, 1967; Coleridge, 1970; and Hammond, 1941). Becker (1967) has shown the histochemical composition of the aortic and carotid bodies to be similar. Paintal and Riley (1966) and Paintal (1967) have shown the aortic bodies are stimulated by pharmacological agents which stimulate the carotid bodies.

Similarities in response to physiological stimuli have also been observed although these have not been extensively reported for the aortic body. Diamond and Howe (1956) reported increase in aortic

body impulse activity with hypoxia as did Lee (1964, Paintal and Riley (1966), and Paintall (1967). Sampson and Hainsworth (1972) have reported the aortic body responds to hypercapnia with increased discharge of impulses, but the impulse activity usually decreased with decreases in pHa.

The reflex responses to aortic body stimulation differ from those of the carotid body inasmuch as stimulation of ventilation is less, heart rate and contractility are increased, and there is an intense vasoconstriction of some vascular beds. These responses will be presented in more detail below.

PART I

INTRODUCTION

In 1937 it was generally believed that hypoxia acted directly on the bone marrow resulting in an increase in the circulating red cell mass. In that year Latner (1937) reported that removal of the carotid sinuses and denervation of the aortic arch in rabbits led to reticulocytosis, a transient anemia, a subsequent increase in red cell count, and stabilization at some level of hemoglobin above preoperative levels. His initial findings led him to more systematic studies with controls. In a second series Latner (1938) reported essentially the same results in operated animals. The anesthetic agent was not reported. In control animals under ether anesthesia no change in the blood picture was noted. The intense reticulocytosis (20%) disappeared by the third post-operative week and the initial anemia had reversed by the third post-operative week. Latner then postulated that the area of the carotid sinus in the normal animal reflexly influences the bone marrow.

The literature reflects little pursuit of these observations until 1951 when Grant, suspecting that carotid chemoreceptors had been damaged or destroyed in Latner's preparation, denervated the carotid and aortic areas in rabbits under pentobarbital anesthesia and exposed the animals to discontinuous hypoxia in an altitude chamber. Control animals had no significant response to the hypoxic exposure but denervated animals markedly increased their hematocrit and oxygen capacity. Both denervated and control animals had similar responses to hemorrhage and to Cobalt injection. Denervated animals were found to

have extremely low hemoglobin oxygen saturation values while breathing the hypoxic gas mixture, consistent with their lack of ventilatory response to hypoxia. Grant concluded chemoreceptor organs were not required for the erythropoietic response to hypoxia or other modes of erythropoietic stimulation.

In 1955 Terzioglu et al., performed similar aortic and carotid denervations in rabbits and found no consistent changes in the erythrocyte count or hemoglobin concentration. They did find 3-4 fold increases in the reticulocyte count. They found no change in the acid-base status of denervated animals compared to controls when breathing room air, making it unlikely that the usual chemical stimuli of the chemoreceptors played any role in hematopoietic changes secondary to the denervation. Upon exposing their denervated rabbits to hypoxia they too noted a more profound erythropoietic response than in control animals.

Gilfillan et al., in 1967 exposed dogs to altitude (3,800 meters) after section of the sinus nerves and denervation of the aortic arch area. They found a markedly increased hematopoietic response to altitude hypoxia in the operated dogs compared to intact animals.

The literature at this point suggested interruption of chemoreceptor function resulted in a profuse reticulocytosis in rabbits but no general impairment of erythropoietic activity. On the contrary, hypoxia resulted in augmented erythropoietic responses in the glomectomized animal, consistent with their reduced arterial oxygen saturation compared to intact animals. No reticulocytosis had been reported in the dog.

In 1971 Tramezzani et al., reported a series of studies in cats in which bilateral carotid body resection had been performed. This group reported that in normal cats hematocrit values decreased from 39% to 17.3% eighteen days after removal of the carotid bodies. There was a brief, profuse reticulocytosis (5-40%) which then declined to below normal values and continued at less than 0.1%. They reported the removal of carotid bodies in cats subjected to daily bleeding resulted in ablation of the reticulocyte response to that bleeding. Injection of one ml of carotid body extract into normal cats and in carotid body resected (CBR) cats resulted in an intense reticulocytosis ranging from 3.5% to 30% twenty-four hours later. Similar effects were produced with carotid body efferent blood (venous) injected into CBR cats. The mode of injection of the carotid body extract was not reported. The reticulocyte response to bleeding was reported ablated by carotid body resection and CBR cats experienced more rapid falls in hematocrit with daily bleeding than did normal cats.

In studies utilizing the polycythemic rat as an assay animal Tramezzani et al., found injection of a feline carotid body extract increased the Fe^{59} incorporated by the rat bone marrow. Carotid bodies in chronically bled cats were reported to be enlarged by a factor of 3. They concluded the carotid body secreted one or more hormones necessary for normal erythropoiesis in the cat.

Thus, after several decades in which only a profuse reticulocytosis had been attributed to removal of carotid bodies, Tramezzani reported the carotid body was essential for feline erythropoiesis.

This was not, however, the first suggestion that the carotid body might subserve some hormonal function. Heath et al., (1970) had reported in a series of forty human autopsy cases that the carotid body was uniformly enlarged in patients who had a clinical history suggestive of chronic hypoxia. He noted that Pearse (1969) classified carotid body Type I cells in the APUD series "for which a hormonal function may be predicted." Edwards et al., (1971) have reported enlarged carotid bodies in guinea-pigs, rabbits, and dogs from high altitudes.

We studied the effect of carotid body resection on feline erythropoiesis using peripheral blood indices, bone marrow examination, and radio-isotope tests of plasma iron turn-over, marrow uptake, and red-cell survival time.

METHODS

Eight adult male cats (3.8 - 5.2 kg) were isolated and administered routine feline immunizations. After 6 to 8 weeks all were free of symptomatic disease and considered to be in a stable, uniform metabolic state. We distributed cats into three groups: 1) operated cats (five animals) who underwent bilateral carotid body resection as described below, 2) a sham-operated control (one animal), and 3) unoperated controls (two animals).

SURGICAL PREPARATION AND HEMATOLOGIC STUDIES:

We resected carotid bodies under halothane in oxygen general anesthesia. This mode of anesthesia was chosen to avoid arterial hypoxia at the time of surgery and to minimize nonspecific stimuli to the bone marrow. Using sterile technique we opened the neck ventrally in the midline. Carotid bodies were identified and excised using a dissecting microscope. Surgical specimens were fixed in Boins solution, sectioned, and stained with iron hematoxylin analine blue for histological verification of carotid body removal. At the conclusion of the study we sacrificed this group and excised the carotid bifurcation bilaterally and sectioned it for histological study. We used iron hematoxylin analine blue stained sections to verify adequacy of carotid body excision.

The sham-operated control animal underwent similar anesthesia and surgical trauma except the carotid bodies were not removed. Instead, the superior laryngeal nerves were sectioned bilaterally. The unoperated control animals underwent no surgical procedures other than those described below in conjunction with the isotope studies.

We utilized a battery of hematological studies to establish baseline values for the erythropoietic status of each animal and to follow responses to the operative intervention. We used EDTA anticoagulated peripheral venous blood for peripheral blood studies (except for some hematocrit determinations which were performed on heparinized blood). All hematocrit determinations were in triplicate by the microhematocrit method. We counted erythrocytes and leukocytes using the Unopette System and hemocytometer as described by Platt (1969). Hemoglobin was determined in triplicate by the methemoglobin method. Slides for reticulocyte counting were stained with Breckers new methylene blue and counter-stained with Wright's stain. We used a Miller disc (Brecher and Schneiderman, 1950) for reticulocyte counts.

Serum iron and total iron binding capacity were determined in five randomly selected cats (Olson and Hanlin, 1969) 1 to 2 weeks prior to conduct of the isotope studies. The uncertainty this limited sampling introduced into iron turnover calculations was accepted in order to reduce to a minimum blood sampling on day 1 of isotope studies. It is believed the animals constituted a population of adequate uniformity to validate this assumption.

We examined bone marrow on each animal during the baseline period and repeatedly throughout the study. We aspirated marrow under light halothane in oxygen general anesthesia with 1% lidocaine HCl local skin infiltration. An 18 guage Rosenthal needle was inserted along the long axis of either femur, entering medial to the greater trochanter, and several ml of marrow aspirated into a heparinized syringe by gentle suction. Marrow smears were prepared on glass slides and

stained with Wright's-Giesma stain.

ISOTOPE STUDIES:

Ferrokinetic studies: Generally, ferrokinetic techniques used in humans were adapted for use in the cat. This involved modification of plasma sampling times to accommodate to the cat's usual plasma iron turnover, reduction of injected dosages of isotopes and reduction of sample volumes consistent with the body weight of the cat.

The animals were initially prepared for the isotope study by the surgical placement of a polyethylene catheter in either external jugular vein under halothane in oxygen anesthesia. We advanced the catheter to the superior vena cava and utilized it for withdrawal of venous blood samples and injection of isotope label over the following 24 hours. We then removed the catether and conducted further sampling by venepucture of the anterior forelimb vein.

Figure 1 illustrates the general procedure which will be described in detail. A 9 ml blood specimen was removed from the catheter and immediately divided into two aliquots, 3 ml for Cr^{51} studies and 6 ml for Fe^{59} studies. We anticoagulated the 6 ml aliquot with heparin, centrifuged it, and removed 3 ml of the resulting plasma. We added 1-2 microcuries per kilogram of body weight of the cat of Fe^{59} as iron citrate to the plasma sample and incubated it at 37°C for 30 minutes. A 25 microliter aliquot was pipetted off and diluted to 25 ml with 0.9% saline to yield a 1:1,000 dilution plasma standard. The remaining plasma was transferred to a syringe, weighed to the

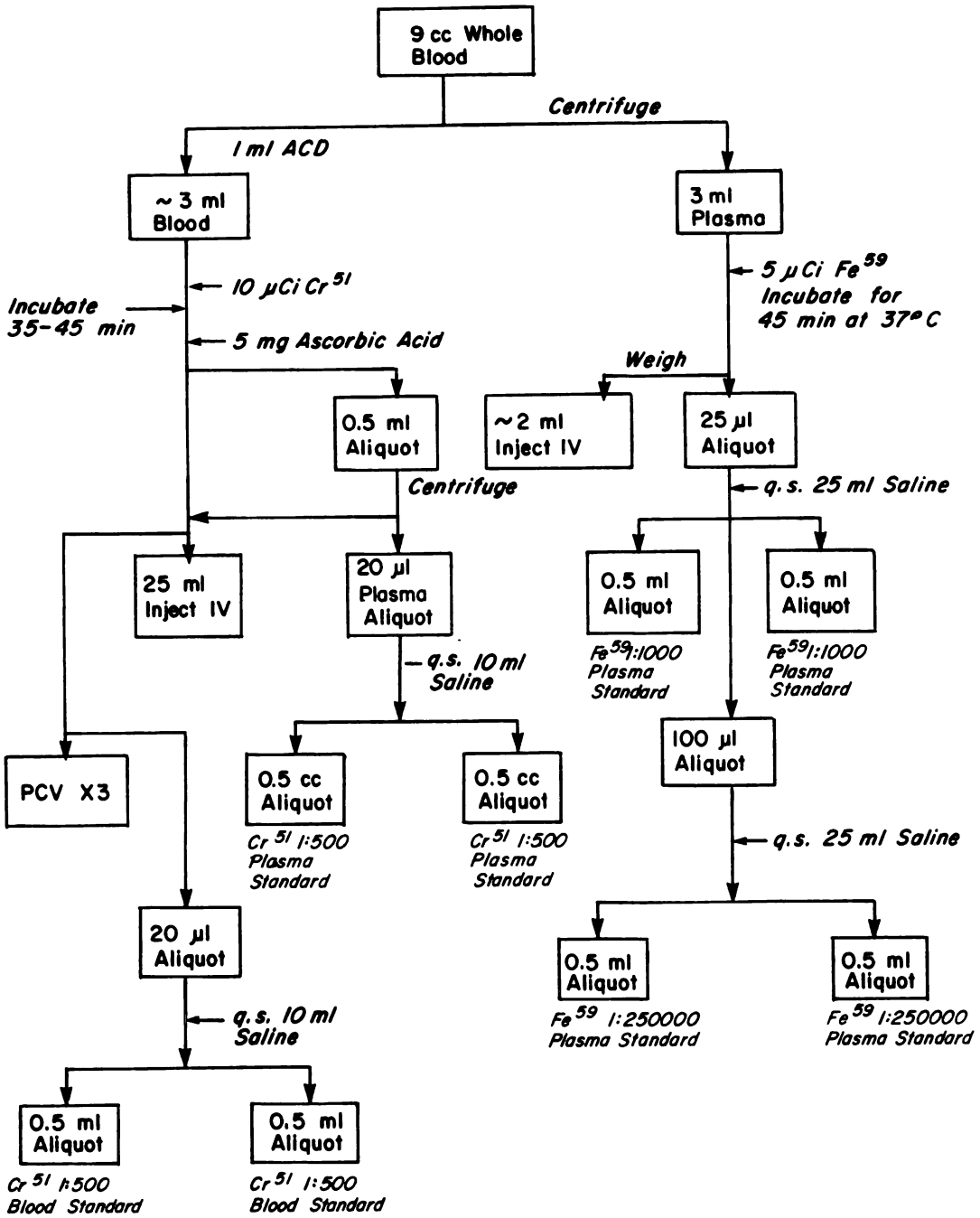


Figure 1

Preparation of Isotope Standards for Fe^{59} and Cr^{51}

nearest 0.1 mg and injected via the jugular catheter at the time chromium-labeled red cells were injected. We flushed the catheter with heparinized saline. The empty syringe was reweighed and the injected volume calculated from the injected weight.

After injecting the Fe^{59} label, we prepared eight plasma samples from blood obtained from the jugular vein catheter over the following 24 hours, and used these to determine plasma iron clearance. Four samples were taken within 4 hours of injection of the label. We re-infused red cells remaining after plasma was removed from samples to minimize blood loss to the animal. We obtained whole blood samples and hematocrit determinations 24 hours later and periodically for the following 34 days for determination of erythrocyte iron incorporation. The plasma and whole blood specimens so obtained were of 500 microliter volume. Red cells in whole blood specimens were hemolyzed by freezing, and all standards were batch counted in a two-channel gamma spectrometer as described below.

Specific activity of the Fe^{59} used in this study was carefully monitored, and the injected isotope dose was calculated to saturate not more than 50% of the latent iron binding capacity estimated by the average latent iron binding capacity of the group. Analysis of the ferrokinetic data will be described below in conjunction with analysis of the Chromium-51 data.

A 3 ml sample of venous blood was anticoagulated with 1 ml of ACD and 10-25 microcuries of Cr^{51} as sodium chromate was added and the mixture incubated at room temperature for 30-45 minutes with intermittent swirling. Following incubation 15 mg of ascorbic acid was added to stop Cr^{51} labeling. We prepared 20 microliters samples

of plasma and whole blood from this mixture and diluted them to a 10 ml volume with 0.9% saline, establishing 1:250 dilution plasma and whole blood standards. We did Microhematocrit determination in triplicate, transferred the blood to a syringe, weighed it to the nearest 0.1 mg, and injected it via the jugular catheter, which was then flushed with heparinized 0.9% saline. The empty syringe was re-weighed and the injected weight calculated and converted to injected volume using the appropriate density factor. We prepared 500 microliter samples of plasma and whole blood from venous blood at 10 and 30 minutes following injection of the label and obtained additional whole blood specimens from the anterior forelimb periodically for 34 days for use in determination of erythrocyte lifespan. The Cr^{51} and Fe^{59} specimens were counted with appropriate standards in a two-channel gamma spectrometer with an energy window of 280-360 Kev for Cr^{51} and 1.0 - 1.5 Mev window for Fe^{59}. All specimens were batch counted to avoid need for isotope decay correction.

DATA ANALYSIS:

We calculated plasma iron clearance, plasma iron turnover, and red cell iron incorporation from the Fe^{59} samples. Blood volume, red cell volume, and red cell lifespan were calculated from the Cr^{51} samples. We utilized a PDP-12 computer and two programs developed in FOCAL-12 language, FEDATA and FEUPTK for these calculations. Logic and accuracy of the two computer programs developed were cross checked by hand calculated values on two animals with good correlation. Raw counts of both the plasma iron and whole blood sample radioactivity

accepted by FEDATA were corrected for background and the Fe^{59} counts were extracted from the Cr^{51} window counts, as determined by appropriate standards. The program then calculated the activity of raw plasma and blood standards for both Cr^{51} and Fe^{59}. Using the plasma Fe^{59} standard and the injected volume of Fe^{59} labeled plasma, the total injected Fe^{59} dose was calculated. The plasma iron samples were identified and their activity expressed as a percent of the injected dose. The program then selected the samples occuring within 2 hours of injection of the label and performed a least squares exponential fit to these samples. This best fit line was extrapolated to injection time of the label and the volume of dilution (plasma volume) calculated. Utilizing the packed cell volume, blood volume was estimated. Time for removal of 50% of the labeled iron from plasma ($T_{1/2}$) was calculated from the fitted curve.

The slope and intercept of the best fit line, the variation in the slope and intercept, and the correlation coefficient were calculated. Using the animal's hematocrit an estimate of the blood volume and red cell volumes were calculated. We calculated activity of all plasma sample specimens as a percent of injected dose of Fe^{59}.

The program then utilized the injected volume of Cr^{51} labeled red cells, the packed cell volume of the injectate, and the activity of the Cr^{51} blood and plasma standards to calculate the acitivty of labeled erythrocytes in the injected Cr^{51} dose. We used whole blood samples obtained at 15 and 30 minutes after injection of the Cr^{51} label to calculate volume of dilution of the label (blood volume). Again utilizing the animal's hematocrit we made estimates of the red

cell volume and the plasma volume for the 15 and 30 minute sample times.

A second program, FEUPTK, calculated the red cell iron incorporation of the Fe^{59} label and prepared the whole blood Cr^{51} activities for determination of the red cell life span. Raw counts were corrected for background and overlap of the Fe^{59} into the Cr^{51} window as described in FEDATA. The injected dose of Fe^{59} was used, in conjunction with the blood volume as determined by Cr^{51} at 30 minutes in FEDATA, to express the activity as percent of the injected Fe^{59} dose of Fe^{59} in each sample taken from day 1 through day 34. Each blood sample was corrected for its hematocrit on the assumption that the red cell mass remained constant. A printout of sample activity expressed as percent of the injected Fe^{59} dose versus sample time was obtained (red cell iron incorporation). A printout of Cr^{51} sample activity versus sample time was also obtained.

The Cr^{51} sample activity was converted to percent of sample activity of day 1 and we graphed the time required for loss of one-half the label, determined by inspection (red cell half life).

Isotope studies were conducted once on each cat. Cats with carotid body resection underwent these studies at one week (1 cat), 2 weeks (2 cats), 6 weeks (1 cat) and 8 weeks (1 cat) after removal of the carotid bodies.

We studied a sham-operated animal one week post-operatively. The unoperated controls were studied upon completion of the baseline studies.

We sacrificed all operated cats at conclusion of the study, re-

moved the area of the carotid bifurcation, prepared serial sections of the specimen at 200 micron intervals, and conducted a microscopic search for remnants of carotid body tissue.

RESULTS

We confirmed successful bilateral removal of the carotid bodies in four cats and unilateral removal in cat No. 2 by histological examination of surgical specimens. Histological examinations at conclusion of the study confirmed these findings.

Table 1 lists hematocrit variations during the course of study for all cats and includes those of Tramezzani et al. (1971) for comparison. The mean change in hematocrit in the glomectomized animals at the end of study was -3.4 ± 1.8 compared with an average change of -2.3 ± 3.5 for the unoperated control animals. This is not significantly different ($P > 0.5$). There were variations on a day-to-day basis in all animals, usually related to the stress of anesthesia and other actions which possibly induced splenic contraction. Abrupt transient decreases were noted at the time of jugular vein catherization and the extensive blood sampling in the following 24 hours. Baseline hematocrits averaged 42.5% for unoperated controls and 42% for carotid body resected cats. All cats, with the exception of the sham-operated control, had a fall in hematocrit in the course of the study but by the end of the study average hematocrits had returned to 38.5% in controls and 38.6% in carotid body resected cats.

At no time in any cat did bone marrow reveal decreased cellularity. On the contrary, any change was always of increasing cellularity. Reticulocyte counts were always below 0.5% of circulating red cells, but were always present. No increase was noted after carotid body removal although toxic stippling did occur frequently. A typical course of hematocrit values for a control and an operated cat is

Table 1

	Hematocrit Determination				End-of-Study		
	Baseline Period++	5-7 Day Post-Op	13-15 Day Post-Op	20-22 Day Post-Op	Day Post-Op	Hct	Hct Change from Baseline
Glomectomized							
1	43 (1)	46	37*	45	49	41	-2
2	39 (4)	36	36	37	90	34	-5
3	45 (2)	42	35*	39	40	44	-1
4	42 (2)	43	40	39	80	38	-4
5	41 (2)	38		40	72	36	-5
Sham-Operated Control+	42 (2)	43*	42	42	27	43	+1
Unoperated Control							
1	41 (3)	36**	38	39	25	39	-2
2	44 (2)	40**	38	38	32	38	-6
Tramezzani et. al.	39	34	21	17			

* 24cc blood removed for isotope studies, 3cc whole blood and 5cc packed cells returned to animal.

** Control cat hematocrit vealues are post-isotope studies, as no surgery was performed on controls.

\+ Sham-Operation consisted of 3 hours Halothane anesthesia and bilateral section of superior laryngeal nerves.

++ Hematocrits in base line period are average values. Number of determinations is given in parenthesis.

illustrated in Figure 2.

Because of technical difficulties a satisfactory plasma iron removal and plasma volume determinations in carotid body resected cat No. 3 was not obtained. Data for the initial 120 minutes (the single exponential portion of the curve) of the plasma iron removal curve is plotted in Figure 3.

The mean serum iron concentration for five randomly selected cats from the group was 92.4 ± 7.05 mg/100 ml blood. Mean total iron binding capacity was 233.6 ± 14.09 for this group. Half time for removal of Fe^{59} averaged 34.8 minutes in the 3 controls and 34.9 minutes in a 4 operated cats. Fractional iron transfer rate thus averaged 30.6 per day in controls and 30.2 per day in operated cats. These differences are not significant ($P \geq 0.50$).

Maximum erythrocyte Fe^{59} incorporation averaged 93% in controls and 92% in carotid body resected cats as shown in Figure 4. This again is not a significant difference ($P > 0.50$). Blood volume in the unoperated controls was 66 and 68 ml/kg of body weight and in operated cats was 64.8 ± 0.97 ml/kg. Red cell lifespan averaged 9.5 ± 1.25 days in carotid body resected cats and 9.88 ± 0.7 days in control cats, uncorrected for Cr^{51} elution, the rate of which has never been determined for cats. Some hemolysis of undetermined origin occurred in Cr^{51} specimens of two control cats and two operated cats, resulting in an early rapid loss of Cr^{51} label. After 2 to 3 days the rate of dissappearance of C^{51} was similar in all animals ($P > 0.50$).

With one exception all animals tolerated removal of carotid bodies well, maintaining weight and erythropoietic function. One

FIGURE 2

Typical response of hematocrit in one control cat and one glomectomized cat. Arrows indicate day of glomectomy and isotope study in upper curve and day of isotope study only in lower curve.

Figure 2

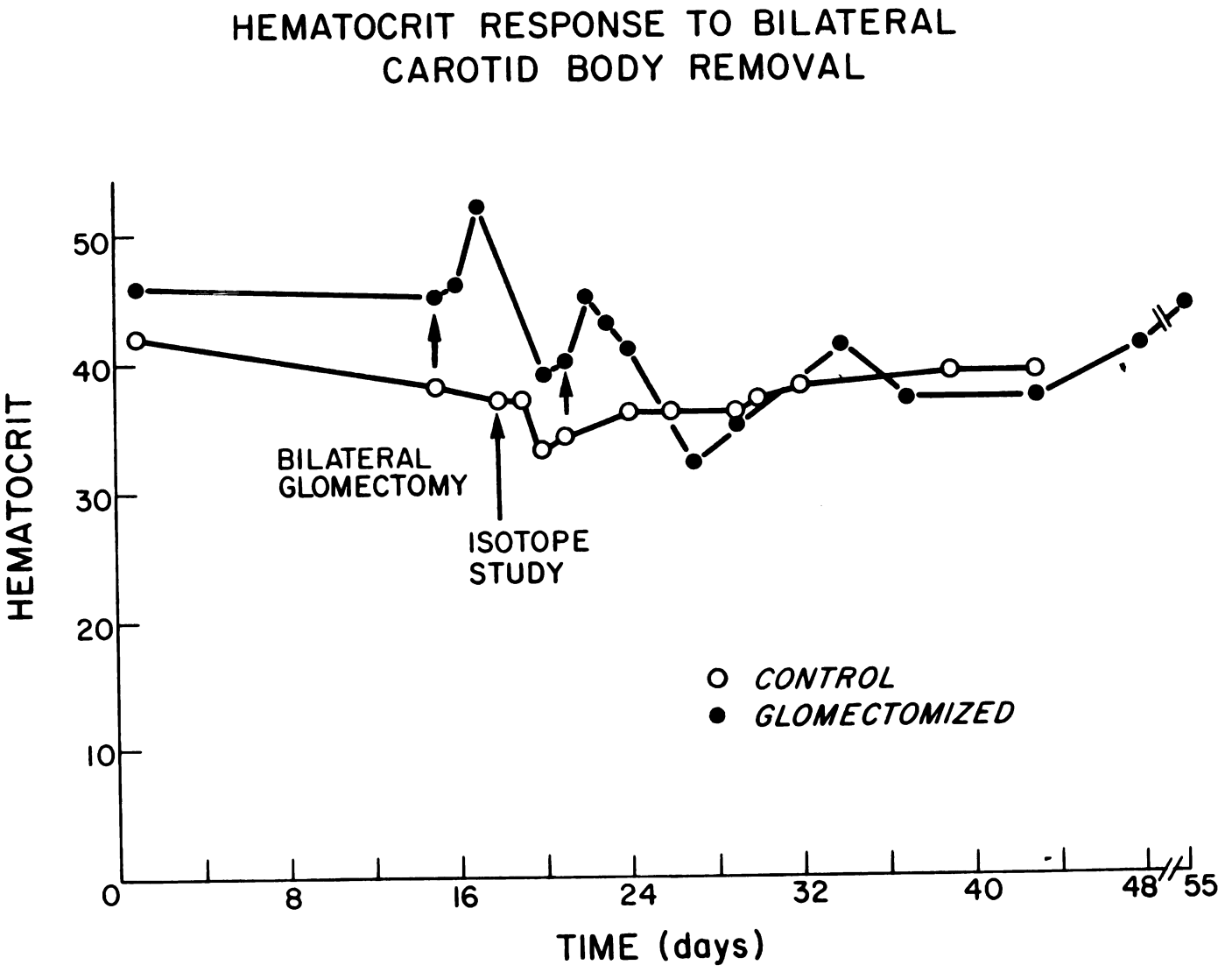

HEMATOCRIT RESPONSE TO BILATERAL CAROTID BODY REMOVAL
HEMATOCRIT
50
40
30
20
10
0
8
16
24
32
40
48
55
TIME (days)
BILATERAL GLOMECTOMY
ISOTOPE STUDY
CONTROL
GLOMECTOMIZED

FIGURE 3

Semi-logarithmic graph of plasma Iron removal data in control and operated cats.

Figure 3

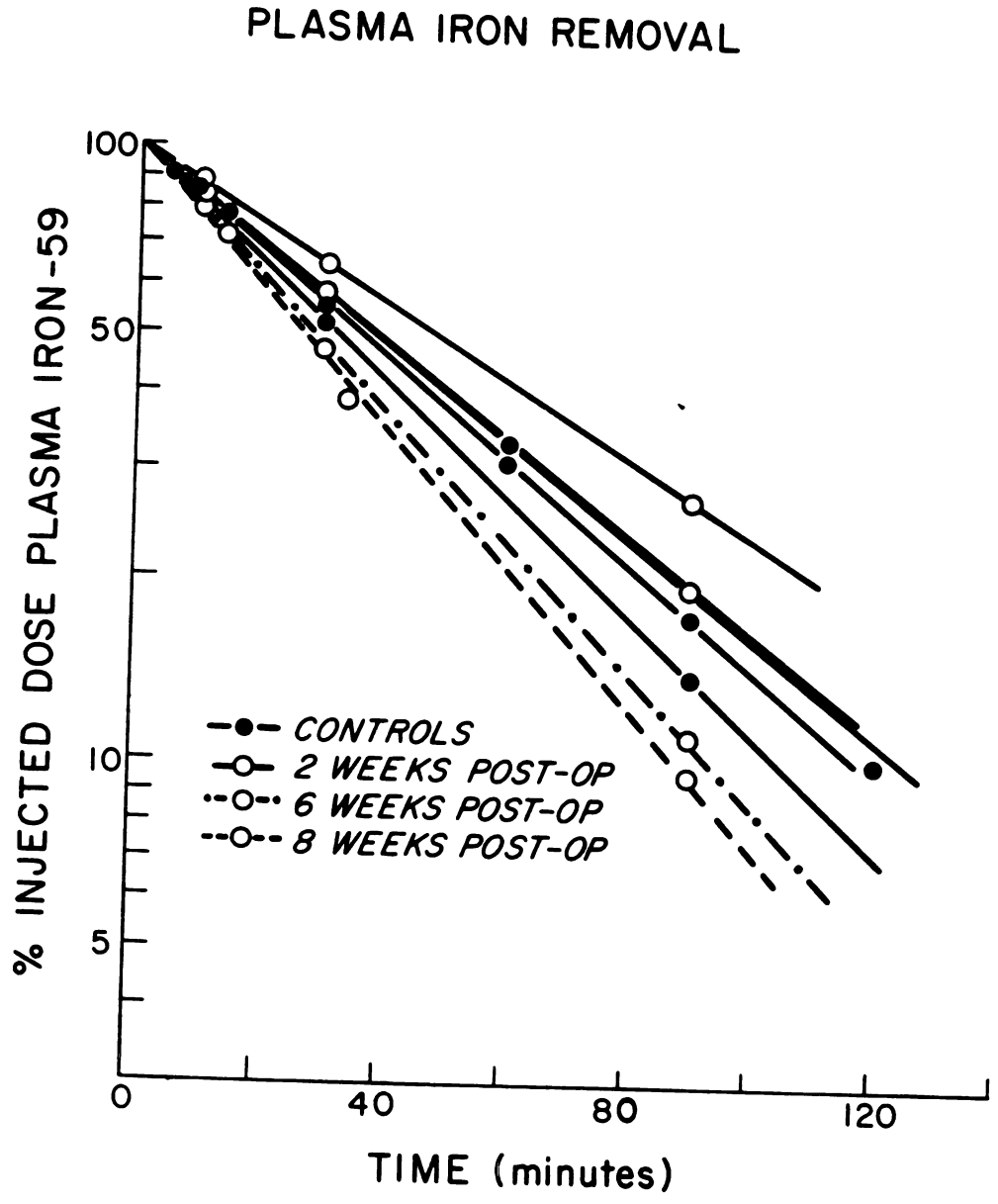
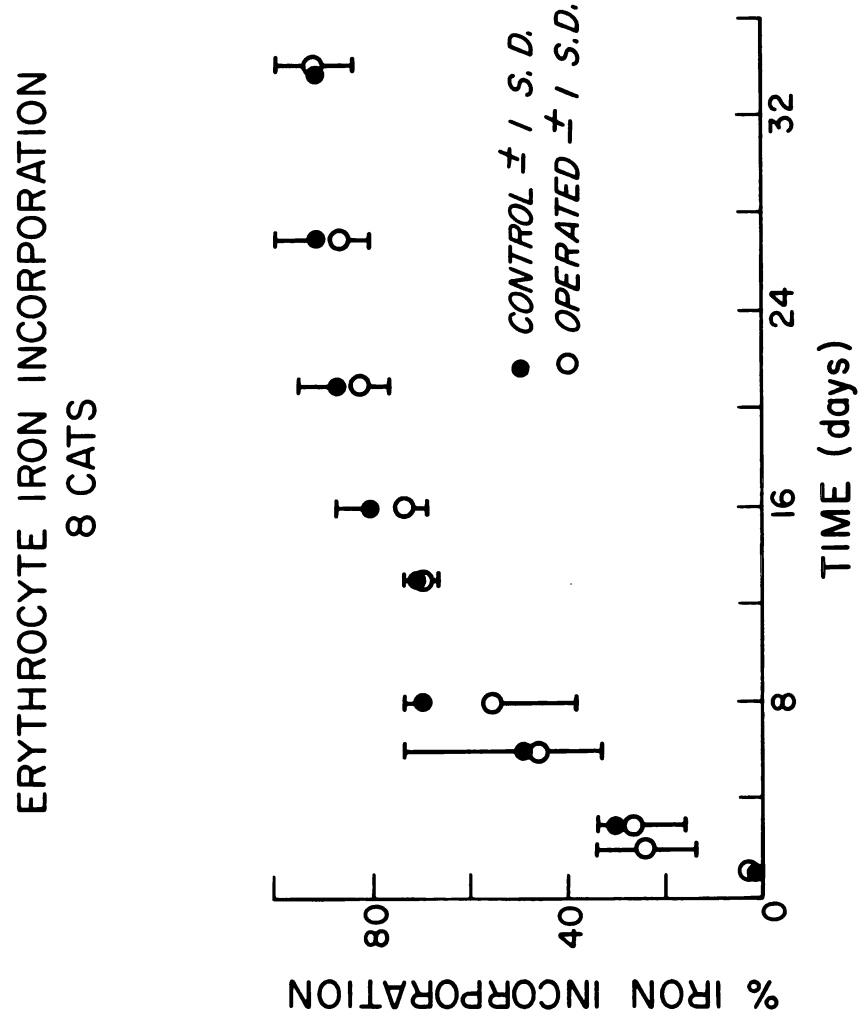

PLASMA IRON REMOVAL
% INJECTED DOSE PLASMA IRON-59
100
50
10
5
0
40
80
120
TIME (minutes)
CONTROLS
2 WEEKS POST-OP
6 WEEKS POST-OP
8 WEEKS POST-OP

FIGURE 4

Summary of erythrocyte labeled Iron incorporation data for all cats, control and glomectomized.

Figure 4


ERYTHROCYTE IRON INCORPORATION
8 CATS
% IRON INCORPORATION
0
40
80
8
16
24
32
TIME (days)
● CONTROL ± 1 S.D.
○ OPERATED ± 1 S.D.

cat, No. 5, experienced a severe post-operative infection with fever to 105°F and leukocytosis to 35,000 WBC/mm^3. With intensive fluid and antibiotic therapy he recovered fully. Cats number 1 and 2 died suddenly of urethral calculus and bladder obstruction at 102 and 144 days postoperatively, respectively. Such a demise is a frequent occurrence in male cats at this institution and is presumably related to diet.

DISCUSSION

Early investigators determined that resection of the area of the carotid sinus resulted in a brief profuse reticulocytosis and an increased hemoglobin concentration upon exposure to an hypoxic environment (Latner, 1938; Grant, 1951; Terzioglu, 1955; and Gilfillan, 1967). Tramezzani, however, reported a profuse reticulocytosis followed by a progressive, severe anemia (Tramezzani, 1971) in the cat. These cats usually died within three weeks of removal of their carotid bodies (Tramezzani, personal communication). Unfortunately the only hematopoietic entity studied by Tramezzani was the hematocrit and the reported fall after carotid body resection was of a rate suggesting an additional mechanism in addition to the proposed marrow arrest of red cell production. Additionally, no controls were included in their study. Our study was designed to test again the critical response of the hematopoietic system to removal of the carotid bodies while allowing determination of the specific mechanism responsible for any change observed. For these reasons we selected Fe^{59} clearance from the plasma and erythrocyte Fe^{59} incorporation, in addition to bone marrow cellularity and hematocrit, as indices of marrow activity, and erythrocyte survival time as an index of toxic effects on the red cell population.

Validity of erythrocyte iron incorporation as a test of marrow function is generally accepted in humans and in most animal species but its validity in the cat was considered questionable inasmuch as the only literature report of normal values was a maximal incorporation of 20% of injected iron dose. Accordingly, if only 20% of in-

jected Fe^{59} could be expected to be utilized by the marrow in normal cats this test would be an insensitive index of marrow activity in the cat. We hypothesized the reported values were in error and further hypothesized that the error lay in use of Fe^{59} of such low specific activity by other investigators that the plasma iron binding capacity was exceeded by the total injected iron, resulting in much of the label being lost as free iron via renal excretion. We were accordingly careful to use Fe^{59} of such specific activity that the necessary activity could be injected with full confidence the total injected iron was 30-50% of the available iron binding capacity. Indeed, erythrocyte Fe^{59} incorporation in our controls averaged 93%. Cats tested after carotid body resection averaged 92% erythrocyte Fe^{59} incorporation. There was no significant difference in plasma Fe^{59} removal, and no decrease in bone marrow erythroid element cellularity. We could thus find no evidence for bone marrow depression after removal of carotid bodies.

Although there were occassional large transient variations in hematocrit in all animals, these changes could usually be related to surgical or anesthetic stress and no sustained changes occurred during our study period. We could find no evidence for shortened red cell lifespan, control and carotid body resected cats having almost identical values. Blood volume remained similar in the two groups.

The failure to find any significant alterations in hematopoietic function in healthy cats after carotid body resection led us to speculate that perhaps the indwelling femoral vein catheter used by

Tramezzani et al., became a source for overwhelming sepsis with consequent anemia. This has subsequently been confirmed (Tramezzani, personal communication). In contrast, our cats, with one exception, remained healthy after removal of the carotid body. One cat became septic but recovered with intensive treatment. All cats tolerated the repeated venepunctures well and we were thus able to avoid the complications of long-term indwelling catheters.

Tramezzani et al., also reported preparation of carotid body extracts which, when injected into the polythemic rat, resulted in an increase in iron utilization. Similar effects from injection of carotid body effluent blood were reported. They hypothesized erythropoietin was responsible for this effect. We did not test this hypothesis directly, but Lugliani et al. (1971) could find no evidence for anemia in 57 human patients who had undergone bilateral carotid body resection for asthma. The reticulocyte response to bleeding of one liter of blood was normal in seven of this group. Lugliani et al., concluded the carotid body was not an essential source of erythropoietin in humans. Others have suggested the absence of anemia in humans after removal of the carotid bodies does not prove carotid bodies do not produce erythropoietin (Lancet, 1972).

More directly to the question was a study of Fogh et al. (1972), which found hypoxia resulted in serum erythropoietin concentrations greater in carotid body resected cats than in normal controls. There was no detectible erythropoietin by biological assay in homogenates of the carotid body prepared as described by Tramezzani et al. Similarly, Paulo, et al., (1972 and 1973) reported serum erythropoietin

levels were greater in carotid body-ablated cats and rabbits than in controls when exposed to hypoxia.

There seems to be little support for the thesis that the carotid body is a source of erythropoietin in cats or that it is essential to normal erythropoiesis in man or animals.

PART II

INTRODUCTION

Increased cardiac output is a well-established consequence of severe anemia in man and animals. The mechanism of this increase has continued to be a source of controversy. Some investigators have concluded cardiac output increases in response to humoral stimulation, others have demonstrated sympathetic nervous system influence. Confusion may have resulted from indiscriminate comparisons of man and animals; acute and chronic anemia; moderate and severe anemia; isolated anemia and anemia complicated by other pathologic processes. An ancillary problem is the identification of a mechanism or mechanisms by which the animal senses the anemic state, an afferent source of the 'anemic signal' which, when activated, might call to play sympathetic or humoral compensatory functions. We have tested the hypothesis that aortic and/or carotid chemoreceptors function as the anemic 'sensor', through the stimulatory effect of reduced oxygen content on chemoreceptor hypoxic sensitivity.

Perhaps the most prominent cardiovascular compensation to anemia is an increase in the cardiac output. This is a constant feature in man in chronic anemia (Sharpey-Schafter, 1944; Bradley and Bradley, 1947; Brannon, 1945). Hatcher, in 1954, noted increased cardiac outputs in dogs made anemic by chronic hemorrhage. Most subsequent experimental work in anemia has used a dog model. We are unaware of any reports of cardiovascular effects of acute anemia in the cat.

Two primary mechanisms for the increased cardiac output have been proposed. Some investigators feel there is little change in

the contractile state of the heart and attribute the rise in cardiac output to a decrease in peripheral vascular resistance. With decreasing hematocrit there is a fall in viscosity, resulting in lowered peripheral vascular resistance. Fowler and Holmes (1964) noted increased cardiac output in dogs after Dextran-for-blood exchange transfusion, and demonstrated lesser increases in dogs with Kreb's red cell suspension exchange transfusion. They attributed these differences to the effects of lowered viscosity in the Dextran-for-blood exchange. Escobar et. al., (1966), studying anemic dogs, could not account for all the rise in cardiac output on the basis of decreased viscosity. On the other hand, Murray and Escobar (1968) reduced oxyhemoglobin content with methemoglobin exchange transfusion and found a decrease in cardiac output. They found similar results with normal whole blood transfusion, but increased cardiac output after Dextran-for-blood exchange transfusion.

Although viscosity changes are important, most investigators accept an increased contractile state as the primary determinant of the increased cardiac output in anemia. The mechanism of this increase in contractility is still controversial.

An increase in initial fiber length (Frank-Starling mechanism), resulting from elevated right atrial pressure, has been considered a possible mechanism for increasing cardiac output in anemia. Increases in right atrial pressure were not found in the dog after Dextran-for-blood exchange transfusion (Sunahara and Beck, 1954; Fowler, Franch, and Bloom, 1956). Gold and Murray (1969) have reported that a one-for-one Dextran-for-blood exchange transfusion

in the dog results in a blood volume expansion of about 15-20%, presumably secondary to the oncotic effects of 6% Dextran solution. Sunahara and Beck (1954) have demonstrated, however, that right atrial pressure influences the anemic increase in cardiac output only in volume-depleted dogs. In short, there is little evidence that increased central venous pressure is responsible for increasing the cardiac output in acute anemia.

An increased contractile state of the heart could result from sympathetic stimulation secondary to anemia. Addressing this possibility, Lovegrove, Gowdey, and Stevenson (1957) found similar increases in cardiac output in response to anemia in intact dogs and after adrenalectomy or pharmacologic ganglionic blockade. But Hatcher, Sadik, and Baumber (1959) found acute T_{1-5} sympathectomy markedly reduced the cardiac output response to anemia while dogs chronically denervated (in a similar sympathetic distribution) had little impairment in their response to anemia.

Glick, Plauth, and Braunwald (1964) found significant differences in responses when comparing awake controls with awake dogs after chronic, complete cardiac denervation. The denervated group had less increase in cardiac output, and achieved this increase primarily by an increase in right atrial pressure and stroke volume with little increase in heart rate. The intact dogs had greater increases in cardiac output, which were achieved primarily by an increase in heart rate with little increase in stroke volume.

Escobar et. al., (1966) found the stroke volume and work indices increased for any given end-diastolic volume in dogs made anemic by

Dextran-for-blood exchange transfusion. They found propranolol blunted the increase in cardiac output, contrary to Gowdey (1962), who could find no effect of dichloroisoproterenol on cardiac output increases after Dextran exchange transfusion. Neil, Oxendine, and Moore (1969) could not blunt cardiac output increases after anemia in awake, trained dogs given propranolol. Contradictory results have followed, i.e., Fowler and Holmes (1971) made dogs anemic while controlling heart rate, left atrial pressure, and aortic pressure but still found increases in ventricular contractility. They were able to reduce the anemia-induced changes by administering propranolol. Chapler, Hatcher, and Jennings (1972) also found propranolol reduced cardiac output increases after Dextran-for-blood exchange transfusions, if given within the first hour.

It now appears that conflicting appraisals of the role of the sympathetic nervous system in the cardiac output elevations following anemia may arise when observations made within the first 1-2 hours are not differentiated from observations made latter in the anemic state. Justus, Cornett, and Hatcher (1957) have demonstrated that blood from a dog made anemic within the hour does not increase the cardiac output in a recipient dog, whereas blood withdrawn after three hours of anemia will increase the cardiac output of a recipient dog. This effect has been further studied by Hatcher et. al., (1963) and by Hatcher and Jennings (1966).

A reasonable model can be synthesized, with an early response of the sympathetic nervous system effecting primarily cardiac sympathetic stimulation resulting in an increase in cardiac output. This

increase is facilitated by a fall in peripheral vascular resistance secondary to reduced viscosity of blood in anemia. At 1-3 hours a humoral factor is present in sufficient quantity to maintain the elevated cardiac output without requiring enhanced sympathetic outflow.

If we accept this model, we must ask "What is the afferent mechanism?" Recent evidence suggests carotid and aortic chemoreceptors could be the sensor mechanism which stimulates the sympathetic nervous system. Several authors have reported increased stimulation of aortic and carotid chemoreceptors in cats inhaling carbon monoxide (Paintal, 1967; Mills and Edwards, 1968; Edwards and Mills, 1969). Carboxyhemoglobin formation reduces arterial oxygen content without a reduction in arterial oxygen partial pressure--a circumstance similar to the anemic state. We have attempted to determine the effect of acute, normovolemic anemia, induced by Dextran-for-blood exchange transfusion, on chemoreceptor neural activity in aortic and carotid bodies of the cat.

METHODS

We studied the effect of acute isovolemic anemia on hypoxic stimulation of aortic and carotid chemoreceptor fibers in cats weighing 3.2 to 6.5 kg. All experiments were conducted using intraperitoneal induction of anesthesia (chloralose, 40 mg/kg and urethane, 200 mg/kg) with supplemental intravenous administration as required. We paralyzed all animals with 20 mg gallamine triethodide intravenously and administered 10-20 mg per hour for maintenance of muscle relaxation.

SURGICAL PREPARATIONS

Bilateral femoral artery and unilateral femoral vein catheters were placed, and arterial blood pressure was recorded continuously with a Statham transducer and Grass Model 5 polygraph. We made a ventral mid-line incision in the neck to expose the trachea, which was ligated and transected. A tracheostomy canula was inserted into the distal tracheal segment and the animal ventilated with a Harvard small-animal respirator with minute ventilation adjusted to maintain a Pa_{CO_2} of approximately 40 torr. The esophagus was double ligated and transected. The upper esophageal segment was reflected superiorly to expose the tissue planes containing the aortic and carotid sinus nerves. Exposure of a unilateral aortic nerve and carotid sinus nerve was performed and both nerves were further dissected and prepared for recording.

For aortic nerve recordings we transected the aortic depressor nerve at its junction with the superior laryngeal nerve and freed

it for several millimeters peripherally. For carotid sinus nerve recordings we transected the glossopharyngeal nerve at its junction with the carotid sinus nerve and dissected the latter free as it coursed toward the carotid body. Such dissections were made on each nerve as required to obtain a satisfactory functioning chemoreceptor fiber or small group of fibers. While the nerve rested on a stainless steel platform submerged in warm liquid paraffin, it was desheathed with the use of a dissecting microscope and micro-surgical instruments. The nerve was progressively separated into smaller fasicles until a strand with only several active chemoreceptor fibers was identified. We identified chemoreceptor fibers by 1) a random pattern of impulses, and 2) the characteristic burst of impulse activity immediately following the intravenous injection of 0.2 ml of a 100 mcg/ml solution of sodium cyanide.

The nerve strand selected for recording was placed on bipolar platinum electrodes, the impulse activity pre-amplified with a Grass preamplifier and displayed on oscilloscopes for viewing and film recordings with a camera. The impulse spikes were gated and a corresponding standard pulse generated and counted with an electronic pulse counter. In initial experiments 1 minute counting intervals were determined with a stopwatch. In later experiments a digital clock automatically interrogated the digital counter at 1 minute intervals and recorded the counts on paper tape. A continuous paper write-out of integrated pulse activity was simultaneously recorded on a polygraph and correlated with the digital count rates by timing marks generated by the digital clock.

The standard pulse, corresponding to gated (counted) chemoreceptor nerve impulses, was recorded photographically to allow later resolution of any counting ambiguities. In some experiments arterial blood pressure was recorded photographically for heart rate determination during counting periods. Both arterial blood pressure and tracheal P_{CO_2} were recorded on the polygraph.

DEXTRAN-FOR-BLOOD EXCHANGE TRANSFUSION

All Dextran exchange transfusions were performed as follows. Exchange transfusion was performed by withdrawing blood in 10 ml increments from a femoral artery catheter and infusing Dextran 60 in corresponding volumes by the femoral vein catheter. The volume exchanged was sufficient to reduce the hematocrit to 15-20% of red cells and ranged between 60-120 ml. Exchange transfusion was completed in 10-15 minutes. Blood was withdrawn in plastic syringes and stored in plastic beakers (heparanized) at room temperature. For re-infusion studies plasma was removed and the remaining red cells re-infused.

DATA COLLECTION AND ANALYSIS

The effect of rapid, isovolemic anemia on the sensitivity of chemoreceptor fibers to hypoxia was studied utilizing two methods: 1) we performed a single-point analysis by ventilating the animal with a gas mixture of O_2 and N_2 approximating that of room air prior to and during the Dextran exchange tranfusion (DET). After a control period, the exchange was completed and ventilation continued

constant for an additional 30 minute period. Impulse activity was recorded during the control period and during the 30 minute post-exchange period. Arterial pH, P_{O_2}, P_{CO_2} and hematocrit were determined during each period.

2) We performed a multi-point analysis by determining an oxygen response curve for the chemoreceptor fiber(s). The curve was determined while ventilating the animal with mixtures of O_2 and N_2 designed to vary Pa_{O_2} between about 30 torr and 200 torr. During each gas mixture we allowed impulse activity to stabilize and counted nerve firing rate for three minutes. In the following minute we recorded nerve activity on film and withdrew arterial blood for gas analysis.

In constructing a given hypoxic response curve we used only those data in which the variation of Pa_{CO_2} and pHa among points did not exceed 6 torr and 0.1 units respectively, and in which mean arterial blood pressure was above 100 torr, to exclude effects of these variables on the nerve impulse response. Data excluded are depicted in parentheses in the appropriate tables and underlined in figures. Curves were hand-drawn to best fit by inspection. The impulse rates at Pa_{O_2} of 100, 75, and 35 torr were determined by inspection from each curve and the impulse rate tabulated as a percent change from the firing rate noted at a Pa_{O_2} of 100 torr in the appropriate control curve.

After determining an initial hypoxic response curve (control) we conducted the Dextran-for-blood exchange transfusion in conjunction with the single-point analysis. We then determined a second

hypoxic (anemic) response curve. In some animals red blood cells taken from the phlebotomized volume were re-infused and impulse activity recorded for 30 minutes. A third (re-infused) hypoxic response curve was then determined.

We analyzed hypoxic response curves statistically utilizing a non-linear least squares computer program, BMD07RT, modified January 1, 1973. This program was developed by the Health Sciences Computing Facility, University of California at Los Angeles and was run on an IBM 37145 system. Aortic and carotid hypoxic response curves were analyzed utilizing two models:

$$Y = A/x \quad \text{and} \quad Y = A/x^2$$

We calculated the value of 'A' and its standard deviation for each curve and mean square error of the fit. As all aortic data fit the first degree equation best, and most carotid data fit the first degree equation best, we performed Student's 't' test on A values from this model. The value of 't' was calculated as follows:

$$t = \frac{A_1 - A_2}{\sqrt{\frac{(n_1-1)(s_1^2) + (n_2-1)(s_1^2)}{n_1 + n_2 - 2}}}$$

where A is as defined above,

n is number of data points in a curve

s is the standard deviation of A

Degrees of freedom were determined as $n_1 + n_2 - 2$. Probabilities were determined from standard two-tailed 't' tables (Snedecor and Cochran, 1971).

The dependence of 'A' values on hematocrit was analyzed using least squares linear regression analysis. We analyzed only sets of data which included a re-infusion curve by this method. Significance of the slope, b, of this regression was determined by Student's 't' test, 't' value being determined by dividing the slope, b, by the sample standard deviation of the regression coefficient, s_b, (Snedecor and Cochram, 1971, Chapter 6.

RESULTS

Single-Point Analysis of Normovolemic Acute Anemia and Discharge Frequency of Chemoreceptor Fibers

Aortic Chemoreceptors

In nine cats in which satisfactory data was obtained the Dextran-for-blood exchange transfusion resulted in an increase in discharge activity in aortic chemoreceptor fibers as listed in Table 2. This elevated discharge rate was maintained for the entire thirty minute observation period in all cats except No. 5. In this animal the discharge rate had returned to control levels in 30 minutes. A 40 torr increase in arterial pressure accompanying the exchange transfusion in this cat may have contributed to the decrease in firing rate. In contrast with other animals in this series, cat No. 5 also exhibited marked variations in minute-to-minute impulse activity (Figure 5 B). Generally, the elevated impulse activity was relatively stable (Figure 5A, cat No. 2).

Carotid Chemoreceptors

In eight cats in which satisfactory data was obtained the Dextran exchange transfusion resulted in an increase in discharge in carotid chemoreceptor fibers in seven cats. There was no significant change in discharge activity in cat No. 17. Two other cats, No. 10 and No. 11, showed only temporary increases in discharge activity, the count rate returning towards control values by the end of the observation period. These data are presented in Table 3 and illustrated in Figure 6.

Table 2
Aortic Chemoreceptor Fibers-Single Point Analysis

Cat #	Min	Imp/Min	△%	Pa_{O_2} torr	△	Pa_{O_2} torr	△	pHa unit	△	Blood Pressure mmHg	△	Hematocrit % rbc	△
1	Con	19		87.4		36.0		7.279		180/110		41.0	
	10	35	+84										
	30	35	+84	89.5	+2.1	36.4	+0.4	7.333	+.054	185/105	+5/-5	13.5	-27.5
2	Con	100		90.2		27.8		7.381		160/85		44.5	
	10	267	+167										
	30	406	+306										
	40	491	+391	90.1	-0.1	33.7	+5.9	7.331	-.050	215/100	+55/+15	15.0	-29.5
3	Con	331		98.3		40.7		7.220		180/75		30.0	
	10	1632	+393										
	30	929	+180	95.7	-2.6	37.7	-2.6	7.265	+.045	160/100	-20/+20	10.0	-20.0
4	Con	57		68.6		44.3		7.204		160/90		45.0	
	10	137	+140										
	20	145	+154	67.7	-0.9	45.7	+2.4	7.225	+.021	180/85	+20/-5	20.0	-25.0
5	Con	32		86.2		33.9		7.319		140/105		41.0	
	10	78	+145										
	30	35	+9	80.6	-5.6	40.9	+6.1	7.250	-.069	180/110	+40/+5	18.0	-23.0
6	Con	226		55.6		48		7.113		190/130		37.0	
	10	160	+15										
	20	248	+10	56.0	+0.4	49.6	+0.9	7.210	+.097	170/105	-20/-25	15.5	-21.5
7	Con	38		63.5		38.3		7.241		185/130		40.0	
	10	54	+42										
	30	69	+82	72.5	+9.0	38.0	-0.3	7.264	+.023	160/105	-25/-25	*	*
8	Con	32		98.5		31.9		7.333		180/115		34.0	
	20	59	+69	103.4	+4.9	37.3	+5.4	7.378	+.045	180/105	0/-10	17.0	-17.0
9	Con	175		81.8		41.6		7.203		150/125		50.0	
	10	239	+37										
	30	215	+23										
	81	234	+34	81.3	-0.5	41.3	-0.3	7.290	+.087	170/115	+20/-10	17.0	-33.0

Figure 5

A.
Effect of Dextran-for-blood exchange transfusion on aortic chemoreceptor impulse activity, Cat No. 2, constant Pa_{O_2}.

B.
Marked variability in effect of Dextran-for-blood exchange transfusion on aortic chemoreceptor activity, Cat No. 5, constant Pa_{O_2}.

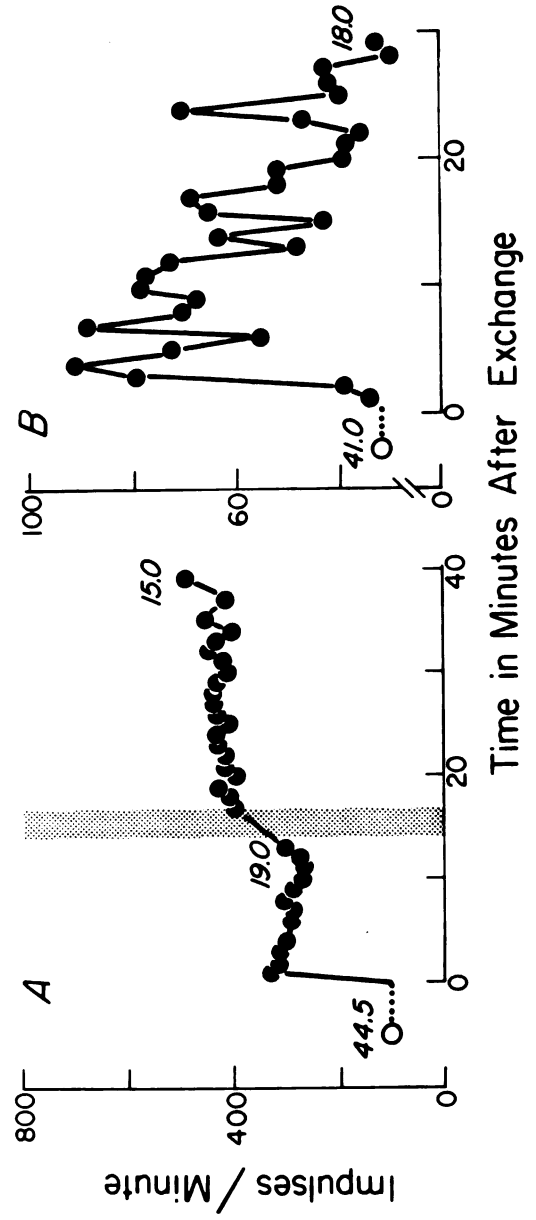

Impulses / Minute
800
400
0
A
44.5
19.0
15.0
0
20
40
100
60
0
B
41.0
18.0
0
20
Time in Minutes After Exchange

Figure 5

Table 3
Carotid Chemoreceptor Fibers-Single Point Analysis

Cat #	Min	Imp/Min	Δ%	Pa_{O_2} torr	Δ	Pa_{CO_2} torr	Δ	pHa unit	Δ	Blood Pressure mm Hg	Δ	Hematocrit % rbc	Δ
10	Con	118		107.1		25.1		7.425		185/115		46.5	
	10	264	+127	101.9		24.2		7.422					
	25	164	+37	100.1	-4.4	31.2	+6.5	7.398	-.025	165/105	-20/-10	13.0	-33.5
11	Con	402		108.7		29.0		7.384		190/120		32.5	
	10	828	+105										
	30	618	+54										
	45	570	+45	109.9	+1.2	30.7	+1.7	7.339	-.045	185/110	-5/-10	18	-14.5
12	Con	23		79.3		38.9		7.423		215/165		43.0	
	10	193	+739										
	30	190	+726	80.2	+0.9	43.5	+4.6	7.382	-.041	200/125	-15/-40	14.5	-18.5
13	Con	114		96.7		33.8		7.324		160/90		44.0	
	10	164	+61										
	30	174	+53	101.2	+4.2	34.1	+0.3	7.387	+.063	175/80	+15/-10	19.0	-25.0
14	Con	155		95.8		34.9		7.343		120/85		42.0	
	10	217	+40										
	30	221	+42										
	50	260	+68	97.7	+1.9	35.5	+0.6	7.358	+.015	135/80	+15/-5	16.5	-25.5
15	Con	200		91.5		34.8		7.309		170/115		39.5	
	10	239	+19										
	30	309	+53	88.0	-3.5	37.8	+3.0	7.290	-.010	185/115	+15/0	18.0	-21.0
16	Con	60		92.1		30.4		7.482		175/125		45.0	
	10	162	+170										
	30	157	+162	107.5	+15.4	30.8	+0.4	7.483	+.001	180/115	+5/-10	17.0	-28.0
17	Con	10		93.7		31.4		7.353		175/150		47.0	
	10	103	+1										
	30	98	-4	97.3	+3.6	32.4	+1.0	7.360	+.007	165/130	+10/-20	22.0	-25.0

Figure 6

A.
Effect of Dextran-for-blood exchange transfusion on carotid chemoreceptor impulse activity, Cat No. 13, constant Pa_{O_2}. Open circle is control count.

B.
Effect of Dextran-for-blood exchange transfusion on carotid chemoreceptor activity, Cat No. 11. Open circle is control count.

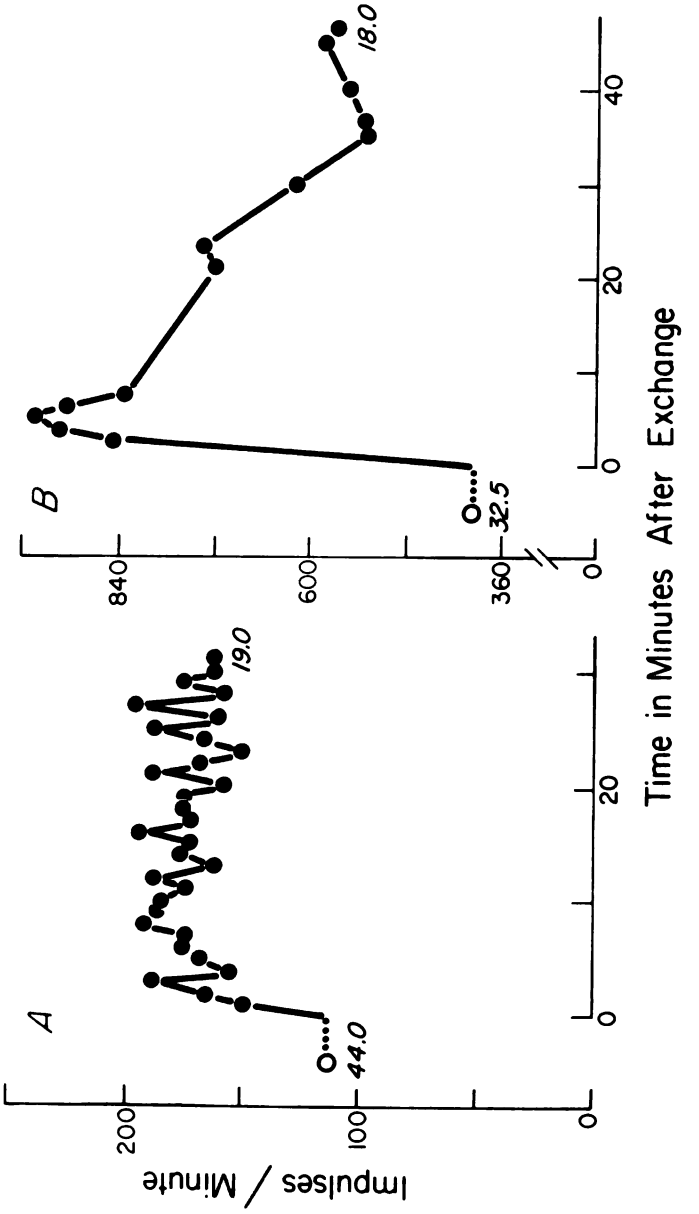

A
B
Impulses / Minute
200
100
0
840
600
360
0
O....
44.0
19.0
O....
32.5
18.0
0
20
40
Time in Minutes After Exchange

Figure 6

Multi-Point Analysis (Oxygen Response Curve) of Effect of Normovolemic Acute Anemia on Discharge Frequency of Chemoreceptor Fibers

Aortic Chemoreceptors

In the six cats in which satisfactory oxygen response curves were obtained, the response curve was shifted to the right and upwards after the Dextran-for-blood exchange. These data are summarized in Tables 4, 4A, and 4B and illustrated in Figure 7 (Cat No. 2). The increased discharge frequency was apparent at all oxygen levels in all cats except cat No. 5, in which increases were evident only for Pa_{O_2} of 70 torr or less.

Carotid Chemoreceptors

In the seven cats in which satisfactory oxygen response curves were obtained before and after the Dextran-for-blood exchange, the response curve after the exchange was variously shifted or not at all. These data are summarized in Table 5 and 5A. Figure 8 illustrates the response of cat No. 11, in which there was no shift in the oxygen response curve with exchange transfusion. In cats No. 13, 41, and 15, the response curves after exchange transfusion were shifted upwards and to the right at higher levels of Pa_{O_2}, but in lower levels was unshifted or shifted to the left of the control curve. This is illustrated in Figure 9, cat No. 14. In cats No. 12 and 15 the post-transfusion curve was shifted upwards and to the right in its entirety. These variations in response could not be related to changes in blood gas tensions or in arterial blood pressure.

Table 4

Aortic Chemoreceptor Fibers Oxygen Response Curves

Cat #	Hct %	Pa_{CO_2} torr	pHa	Blood Press	100 torr	Δ%	75 torr	Δ%	50 torr	Δ%	35 torr	Δ%
1	41.5	35.6-41.1 (44.5)	7.253-7.286 (7.232)	180-200 / 100-135	17		26	53	37	235	122	617
	13.5	36.4-40.6 (34.7)	7.252-7.33 (7.344)	160-195 / 90-125	30	76	60	253	130	664	285	1570
	25.5	27.8-40.8 (45.1)	7.265-7.284	180-215 / 110-140	22	30	40	135	95	460	215	1164
2	44.5	29.6-33.6 (27.8,37.3)	7.360-7.411 (7.318)	160-180 / 85-95	80		140	75	350	335	830	937
	14.5	32.8-35.3	7.331-7.375	170-215 / 60-105	400	400	700	775	1370	1612	2550	3087
	28.0	30.0-34.4	7.302-7.335	195-215 / 85-115	290	262	460	475	880	1000	1730	2062
3	30.0	38.7-43.7 (47.6)	7.218-7.234	170-195 / 75-115	240		380	58	740	208	1200	400
	9.5	33.3-37.7 (31.3)	7.265-7.306 (7.313)	150-165 / 75-105	770	221	950	296	1300	441	1750	629
4	45.0	44.3-46.7	7.193-7.217	150-175 / 75-90	45		60	33	100	122	200	344
	19.0	44.4-48.1	7.225-7.253	155-180 / 70-85	85	89	115	155	180	300	375	733
5	41.0	29.7-35.1	7.319-7.380	140-170 / 105-120	30		35	17	55	83	85	183
	18.0	40.0-46.1 (49.5, 51.5)	7.218-7.250 (7.148,7.170)	135-180 / 70-110	35	17	40	33	135	350	815	2616
	28.5	40.5-46.0	7.212-7.288	110-165 / 60-110	40	33	40	33	75	150	260	766
6	34.0	29.4-32.2	7.314-7.313	175-200 / 115-150	20		40	100	88	340	150	650
	17.0	29.9-35.4 (37.3)	7.361-7.399	150-185 / 85-110	53	165	70	250	132	560	192	860

Table 4A

Statistical Analysis of Aortic Chemoreceptor Hypoxic Response Curves for $Y = A/X^2$ Model

Cat	Hct	A ± S.D.	Mean Square Error	n	P
1	41.5	$1.67 \times 10^5 \pm 6.37 \times 10^3$	1.14×10^2	6	<.001
	13.5	$3.30 \times 10^5 \pm 2.84 \times 10^4$	6.54×10^2	6	<.059
	23.5	$2.68 \times 10^5 \pm 1.78 \times 10^4$	4.53×10^2	5	
2	44.5	$9.95 \times 10^5 \pm 2.92 \times 10^4$	1.59×10^3	5	<.001
	14.5	$3.05 \times 10^6 \pm 2.75 \times 10^5$	6.99×10^4	7	<.005
	28.0	$2.20 \times 10^6 \pm 1.01 \times 10^5$	1.44×10^4	7	
3	30.0	$1.57 \times 10^6 \pm 1.89 \times 10^5$	3.39×10^4	6	<.010
	19.5	$3.72 \times 10^6 \pm 8.76 \times 10^5$	1.69×10^5	6	
4	45.0	$1.92 \times 10^5 \pm 1.77 \times 10^4$	6.55×10^2	5	<.001
	19.0	$3.30 \times 10^5 \pm 3.74 \times 10^4$	1.98×10^3	6	
5	41.0	$2.41 \times 10^5 \pm 3.95 \times 10^4$	3.97×10^3	7	<.001
	18.0	$7.80 \times 10^5 \pm 1.70 \times 10^5$	2.67×10^4	5	<.010
	28.5	$4.06 \times 10^5 \pm 3.86 \times 10^4$	5.47×10^3	7	
6	34.0	$1.91 \times 10^5 \pm 9.44 \times 10^3$	7.38×10^1	7	<.025
	17.0	$2.68 \times 10^5 \pm 3.53 \times 10^4$	8.60×10^2	7	

Table 4B

Least Squares Regression Analysis of Hematocrit and 'A' Value

Cat	b^*	S_b^*	P Less than	Chemoreceptor
1	7.1974×10^3	1.44×10^2	0.025	Aortic
2	5.0482×10^4	2.7946×10^3	0.050	Aortic
3	1.2590×10^4	6.3982×10^3	0.40	Aortic
11	2.95889×10^5	6.6257×10^4	0.20	Carotid
12	2.2232×10^4	3.5790×10^3	0.10	Carotid
14	2.5308×10^4	1.3696×10^4	0.40	Carotid
15	3.8702×10^4	2.1312×10^4	0.40	Carotid

*b is slope of the best fit least squares regression of A value on hematocrit.

**S_b is the sample standard deviation of the regression coefficient.

Figure 7

Effect on hypoxic response curve of Dextran-for-blood exchange transfusion and partial correction of anemia by re-infusion of red cells, Cat No. 2, aortic chemoreceptors.

Figure 7

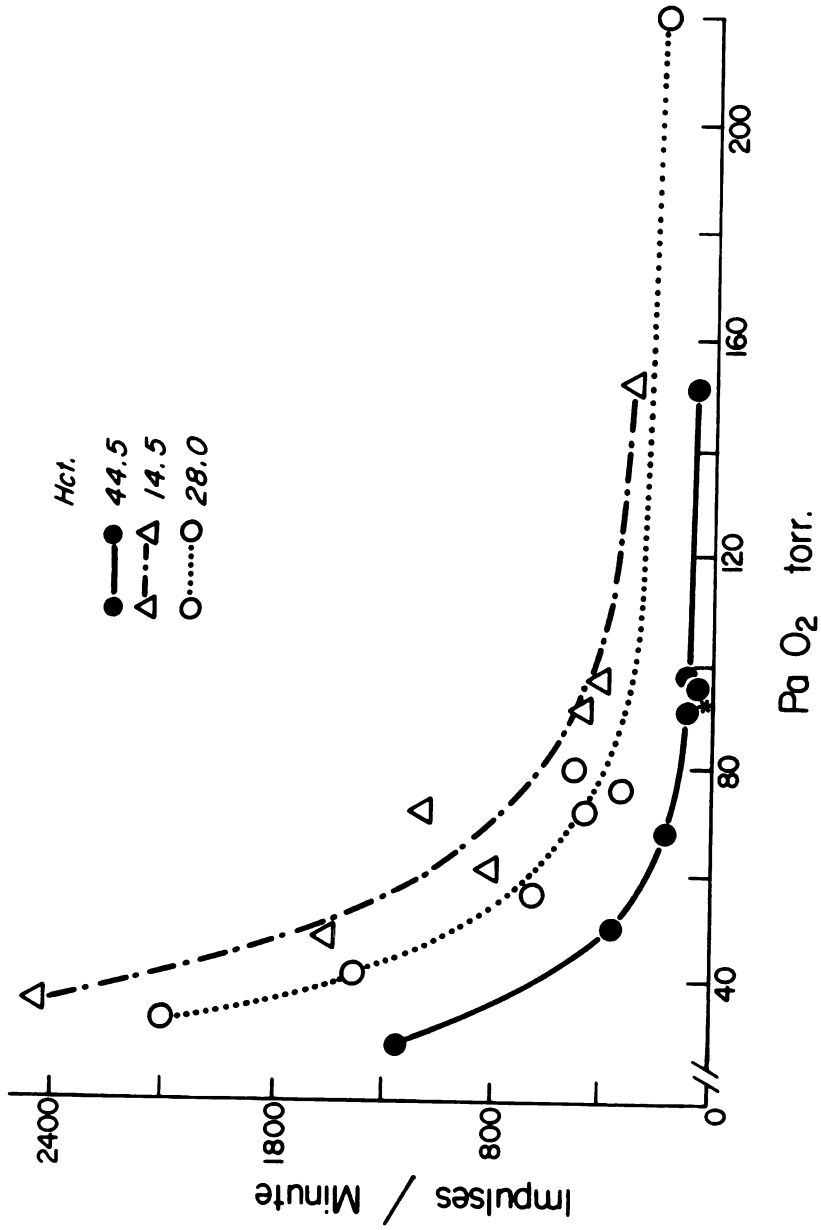

Hct.
44.5
14.5
28.0
2400
1800
800
0
40
80
120
160
200
Impulses / Minute
Pa O2 torr.

Table 5
Carotid Chemoreceptor Fibers
Oxygen Response Curves

Cat #	Hct	Pa_{CO_2} torr	pHa	Blood Press	100 torr	△%	75 torr	△%	50 torr	△%	35 torr	△%
11	32.5	29.0-32.5	7.354-7.384	190-205 / 120-130	600		1620	170	3120	420	4380	630
	18.0	29.0-30.7	7.394-7.415	165-185 / 95-110	600	0	1620	170	3120	420	4380	630
	24.5	29.0-32.9	7.373-7.404	185-190 / 115	600	0	1620	170	3120	420	4380	630
12	43.0	36.7-41.3	7.373-7.447	205-215 / 145-165	25		50	100	200	700	500	1900
	15.0	38.3-40.5 (43.5)	7.347-7.420 (7.382)	185-205 / 110-130	50	100	130	420	325	1200	625	2600
	24.0	37.3-41.9	7.385-7.413	205-215 / 125-140	80	260	225	800	430	1620	710	2740
13	44.0	31.6-33.8 (29.6)	7.324-7.353 (7.374)	160-205 / 80-120	125		225	80	450	260	640	412
	19.0	33.0-34.7	7.358-7.387	165-175 / 80-90	175	40	245	96	420	235	615	392
14	42.0	33.9-35.7 (30.5)	7.359-7.367 (7.406)	120-150 / 80-100	140		175	25	370	164	800	471
	16.5	33.6-35.5 (37.7)	7.354-7.372 (7.313)	135-145 / 80-90	210	50	300	114	450	221	565	303
	29.0	31.0-35.7 (36.9)	7.338-7.410 (7.312)	140-155 / 90-105	180	29	270	93	390	178	515	268

Table 5 (continued)

Cat #	Hct %	Pa_{CO_2} torr	pHa	Blood Press	100 torr	%	75 torr	%	50 torr	%	35 torr	%
15	39.5	34.3-39.8 (33.6)	7.262-7.309 (7.321)	165-175 / 115-130	160		290	81	500	212	875	447
	19.0	35.1-38.2	7.290-7.330	170-185	240	50	340	112	500	212	800	475
	28.0	34.6-38.6	7.274-7.308	165-180 / 105-120	310	94	440	175	700	340	1180	640
16	45.0	28.2-32.4 (23.2)	7.421-7.504	175-190 / 125-135	25		120	380	340	1260	560	2140
	17.0	28.8-33.0	7.464-7.483	188-185 / 105-120	135	440	130	420	425	1600	720	2780
17	47.0	31.4-34.9	7.341-7.390	170-185	60		175	194	500	73	840	1300
	22.0	31.9-36.9	7.273-7.360 (7.243)	125-160 / 90-130	150	150	270	350	565	842		

Table 5A

Statistical Analysis of Carotid Hypoxic Response Curves

Model Cat	Hct	$Y = A/X$ A ± S. D.	$Y = A/X$ Mean Sq. Error	$Y = A/X^2$ A ± S. D.	$Y = A/X^2$ Mean Sq. Error	n	P
11	33	$1.10 \times 10^5 \pm 2.29 \times 10^4$	3.42×10^5	$8.42 \times 10^6 \pm 7.00 \times 10^5$	6.16×10^4	5	
	18	$1.12 \times 10^5 \pm 1.66 \times 10^4$	2.70×10^5	$7.93 \times 10^6 \pm 3.32 \times 10^5$	2.34×10^4	6	> .200
	25	$1.09 \times 10^5 \pm 1.58 \times 10^4$	2.47×10^5	$7.30 \times 10^6 \pm 2.80 \times 10^5$	1.83×10^4	6	> .050
12	43	$1.23 \times 10^4 \pm 2.91 \times 10^3$	1.53×10^4	$6.41 \times 10^5 \pm 7.27 \times 10^4$	4.23×10^3	6	> .050
	16	$2.05 \times 10^4 \pm 2.77 \times 10^3$	2.23×10^4	$7.70 \times 10^5 \pm 3.11 \times 10^4$	2.18×10^3	6	> .200
	24	$2.39 \times 10^4 \pm 1.78 \times 10^3$	1.06×10^4	$8.21 \times 10^5 \pm 5.84 \times 10^4$	9.78×10^3	7	
13	44	$2.11 \times 10^4 \pm 2.39 \times 10^3$	8.87×10^3	$1.22 \times 10^6 \pm 6.07 \times 10^4$	1.80×10^3	5	< .025
	17	$2.10 \times 10^4 \pm 1.79 \times 10^3$	5.5×10^3	$9.20 \times 10^5 \pm 1.26 \times 10^5$	1.35×10^4	6	
14	42	$2.00 \times 10^4 \pm 2.23 \times 10^3$	9.67×10^3	$1.03 \times 10^6 \pm 5.19 \times 10^4$	2.08×10^3	7	< .0250
	17	$2.11 \times 10^4 \pm 7.79 \times 10^2$	1.44×10^3	$7.50 \times 10^5 \pm 1.17 \times 10^5$	2.33×10^4	6	
	29	$1.92 \times 10^4 \pm 5.29 \times 10^2$	9.73 ± 10^2	$5.39 \times 10^5 \pm 9.23 \times 10^4$	2.83×10^4	6	< .200
15	40	$2.70 \times 10^4 \pm 2.69 \times 10^3$	1.96×10^4	$9.40 \times 10^5 \pm 5.09 \times 10^4$	6.04×10^3	7	
	19	$3.17 \times 10^4 \pm 3.25 \times 10^3$	1.90×10^4	$1.33 \times 10^6 \pm 2.20 \times 10^5$	4.54×10^4	7	> .500
	28	$3.60 \times 10^4 \pm 1.65 \times 10^3$	7.16×10^5	$1.55 \times 10^6 \pm 1.01 \times 10^5$	1.42×10^4	8	> .200
16	45	$1.59 \times 10^4 \pm 2.14 \times 10^3$	1.17×10^4	$6.50 \times 10^5 \pm 3.80 \times 10^4$	2.39×10^3	7	
	17	$3.09 \times 10^4 \pm 6.47 \times 10^3$	9.79×10^4	$1.28 \times 10^6 \pm 8.5 \times 10^4$	1.18×10^4	6	< .001
17	47	$2.04 \times 10^4 \pm 2.54 \times 10^3$	1.52×10^4	$1.21 \times 10^6 \pm 8.22 \times 10^4$	2.64×10^3	6	
	22	$2.66 \times 10^4 \pm 2.83 \times 10^3$	1.22×10^4	$1.17 \times 10^6 \pm 8.72 \times 10^4$	6.12×10^3	5	> 0.50

Figure 8

Failure of Dextran-for-blood exchange transfusion and re-infusion of red cells to affect the hypoxic response curve of carotid chemoreceptors, Cat No. 11.

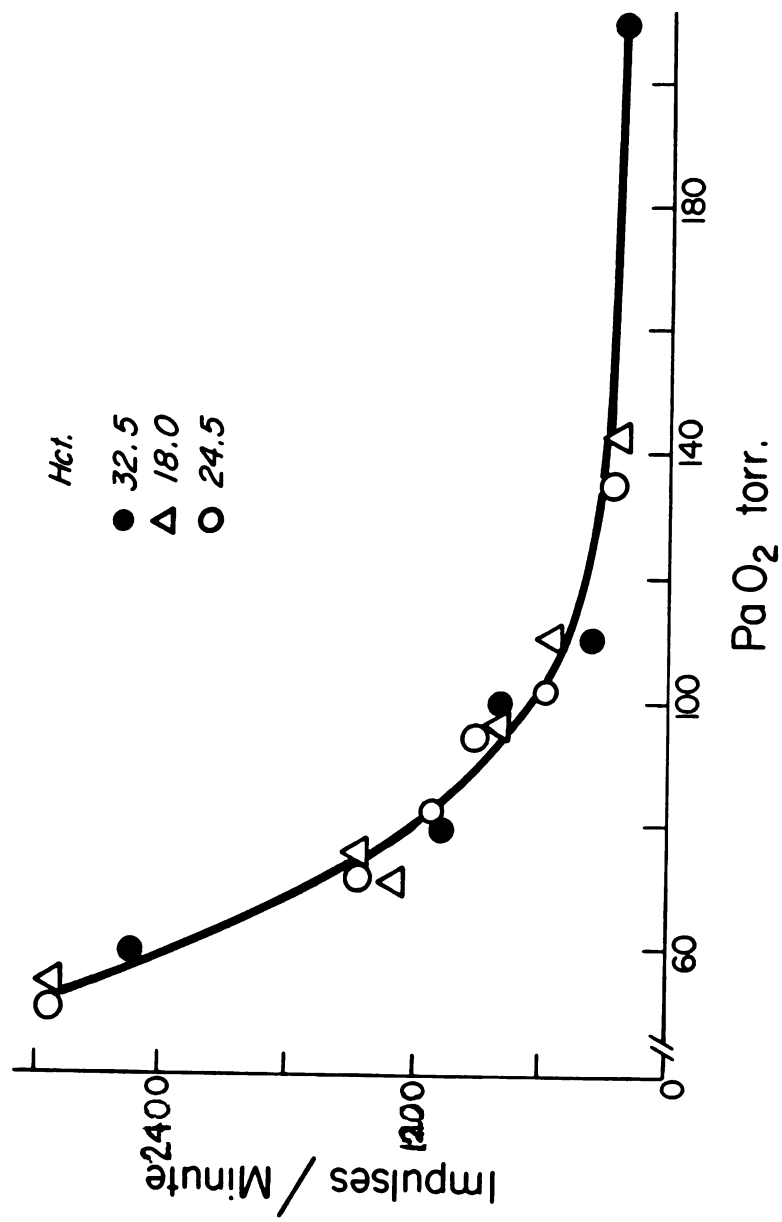

Hct.
32.5
18.0
24.5
Impulses / Minute
2400
1200
0
60
100
140
180
Pa O2 torr.

Figure 8

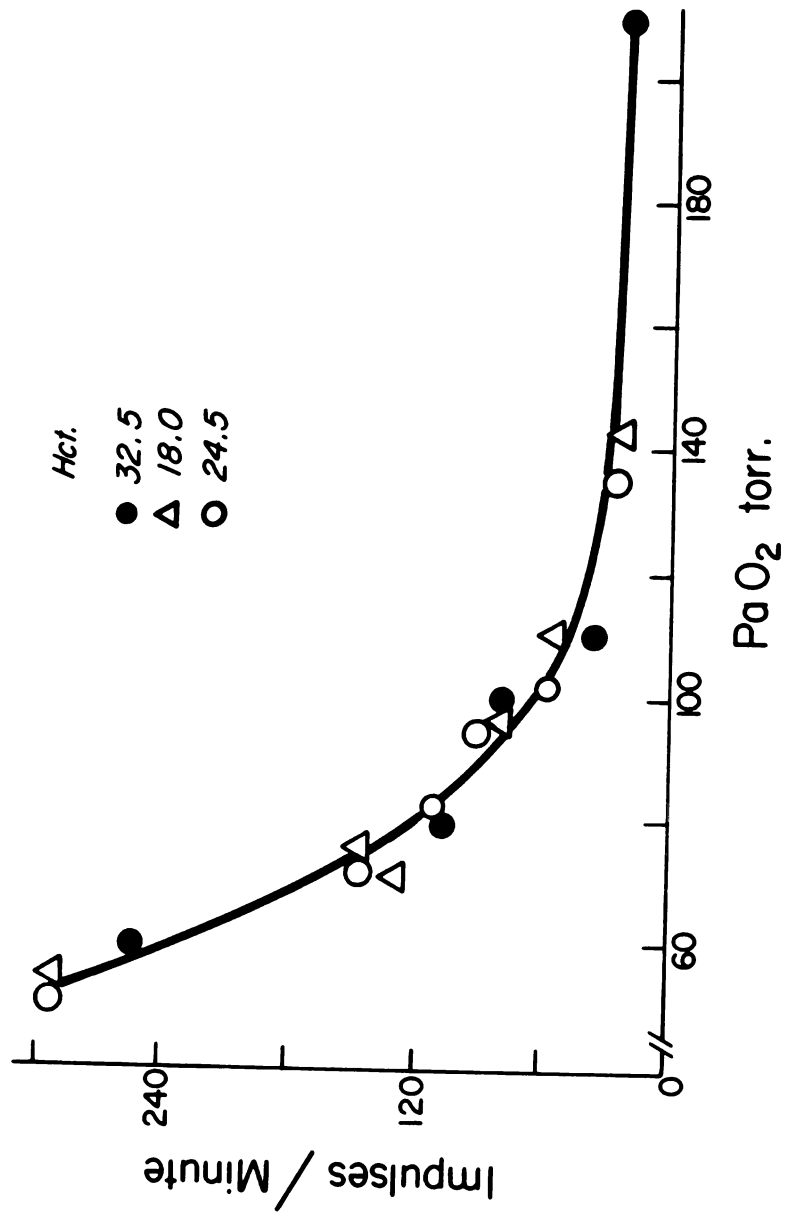

Hct.
32.5
18.0
24.5
Impulses / Minute
240
120
0
60
100
140
180
Pa O2 torr.

Figure 8

Figure 9

Effect of Dextran-for-blood exchange transfusion and red cell re-infusion on hypoxic response curve of carotid chemoreceptors, Cat. No. 14.

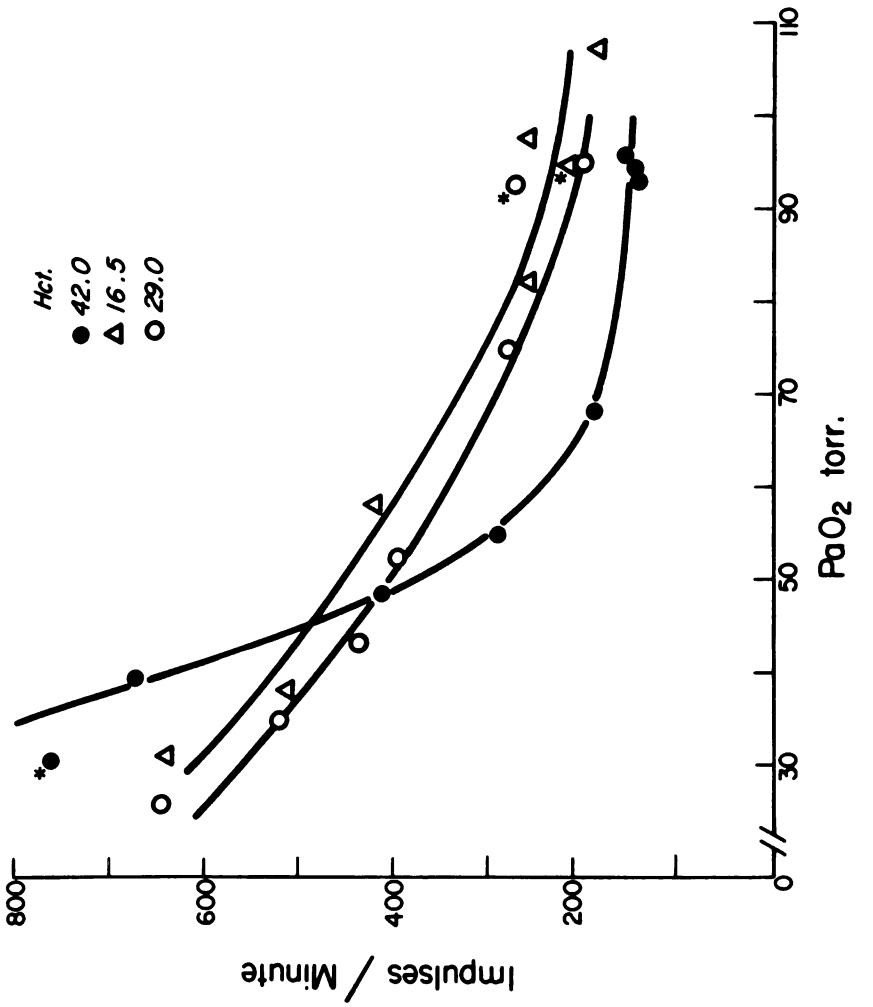

Hct.
● 42.0
△ 16.5
○ 29.0
800
600
400
200
0
30
50
70
90
110
Impulses / Minute
PaO2 torr.

Figure 9

Single-Point Analysis of Effect of Partial Correction of Normovolemic Anemia on Discharge Frequency of Chemoreceptor Fibers

Aortic Chemoreceptors

In six cats in which satisfactory data was obtained, partial correction of the Dextran-for-blood exchange with packed cells resulted in a prompt reduction in impulse activity in aortic chemoreceptor fibers. In cat No. 5 the Pa_{O_2} increased from 72 (anemic) to 80 torr (post-infusion), which could have accounted for a portion of the impulse decrease, however, oxygen response curves on this cat demonstrated the curves were not significantly shifted by hematocrit correction in this oxygen tension range. These data are summarized in Table 6 and illustrated in Figure 104, cat No. 2.

Carotid Chemoreceptors

In the four cats in which satisfactory data was obtained, partial correction of the Dextran-for-blood exchange with packed cells resulted in a variable effect upon discharge rates in carotid chemoreceptor fibers. In cat No. 18 a small decrease in impulse activity was noted (Pa_{O_2} increased 10 torr). In cats No. 12 and 15 the post-infusion discharge rates rose, and in cat No. 14 (Figure 10B) no significant change was noted. These various responses could not generally be related to changes in blood gas tensions or arterial blood pressure. These data are summarized in Table 7.

Table 6

Aortic Chemoreceptor Fibres
Single Point Re-infusion Data

Cat #	Min	Imp/min		Pa_{O_2} torr		Pa_{CO_2} torr		pHa unit		Blood Pressure mm Hg		Hematocrit % rbc	
1	Con	109		77.2		40.6		7.264		185/105		13	
	10	60	-45										
	15	58	-47	73.0	-4.2	45.1	+4.5	7.277	+.013	215/140	+30/+35	25	+12
2	Con	1087		72.3		34.6		7.327		190/75		14.0	
	10	308	-72										
	30	331	-69	75.7	+3.4	34.4	-0.2	7.323	-.004	210/110	+20/+35	28.0	+14.0
3	Con	665		89.1		31.3		7.313		150/75		9.5	
	10	431	-35										
	20	380	-43	88.2	-0.9	32.0	-0.7	7.310	-.003	175/105	+25/+30	14.0	+4.5
4	Con	67		67.0		45.8		7.253		170/80		18.0	
	10	37	-45	71.1	+4.1	46.1	+0.3	7.231	-.022	185/80	+15/0	30.0	+12
5	Con	118		72.0		51.5		7.148		135/80		18.0	
	10	60	-49										
	30	54	-54	80.0	+8.0	46.0	-5.5	7.212	+.064	160/100	+25/+2	28.5	+10.5
6	Con	606		57.2		47.8		7.181		165/100		15.5	
	10	289	-52										
	30	261	-57	59.0	+1.8	47.4	-0.4	7.210	+.029	185/130	+25/+30	22.5	+7.0

Figure 10

A.
Effect of re-infusion of red cells on aortic chemoreceptors, cat previously made anemic by Dextran-for-blood exchange transfusion, Cat No. 2. Open circle is control count. Constant Pa_{O_2}.

B.
Failure of re-infusion of red cells in cat previously made anemic to affect carotid chemoreceptor activity, Cat No. 14, Open circle is control count. Constant Pa_{O_2}.

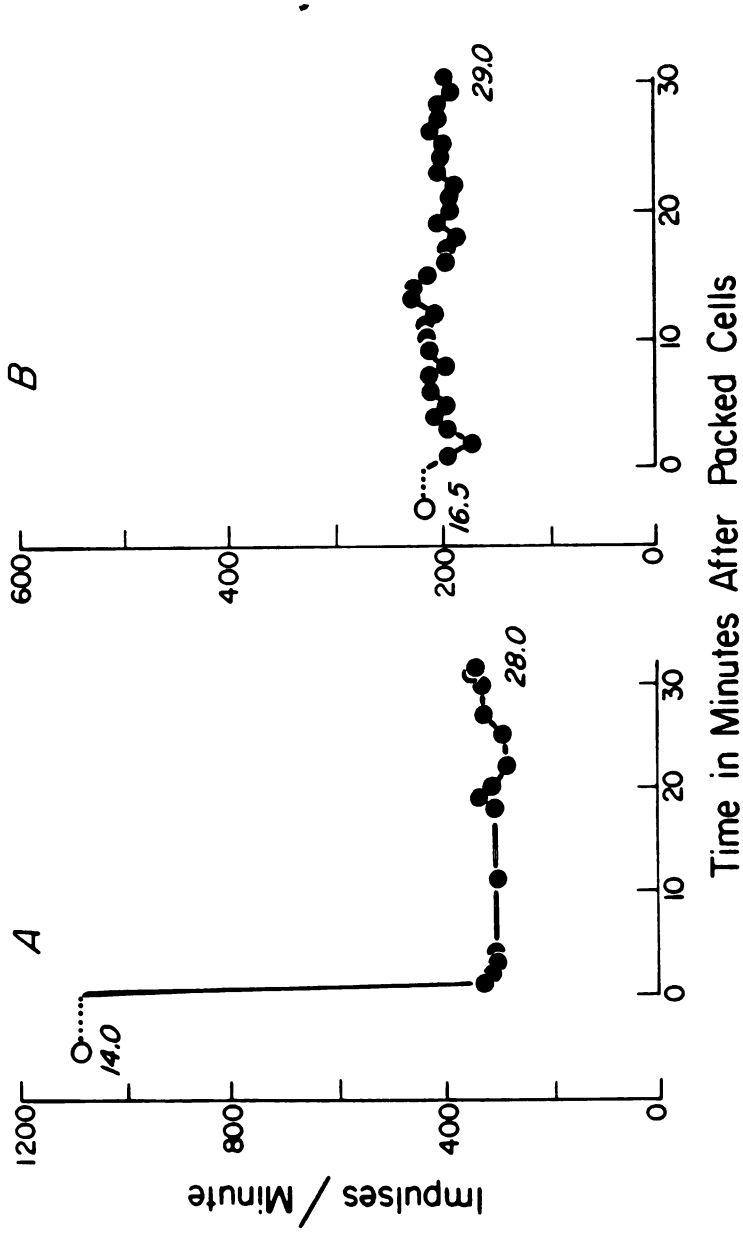

A
B
14.0
16.5
28.0
29.0
1200
800
400
0
600
400
200
0
0
10
20
30
Impulses / Minute
Time in Minutes After Packed Cells

Figure 10

Multipoint Analysis of Effect of Partial Correction of Normovolemic Anemia on Discharge Frequency of Chemoreceptor Fibers

Aortic Chemoreceptors

In the three cats in which satisfactory data was obtained, (No. 1,2 and 5), the oxygen response curves were shifted to a position between that of the control curve and the anemia curve. These data are included in Tables 4, 4A, and 4B and illustrated in Figure 7, cat No. 2.

Carotid Chemoreceptors

In the four cats (No. 11, 12, 14, and 15) in which satisfactory data was obtained, the oxygen response curves were not shifted, or were not shifted in any consistent fashion. These data are summarized in Tables 5 and 5A and illustrated in Figure 8, cat No. 11. In this cat there was no shift of the re-infusion curve, as indeed, there had been no shift of the anemia curve. In cats No. 12 and 14 the post-infusion curve was shifted upwards and to the right. In cat No. 14 the post-infusion curve was intermediate between that of the control curve and the anemia curve above a Pa_{O_2} of 50 torr, then crossed below the control curve. These variations could not be related to changes in arterial blood gas tensions or to changes in arterial blood pressure.

Table 7

Carotid Chemoreceptor Fibres
Single Point Re-Infusion Data

Cat #	Min	Imp/Min	%	Pa_{O_2} torr		Pa_{CO_2} torr		pHa Unit		Blood Pressure mm Hg		Hematocrit % rbc	
18	Con	980		79.5		38.4		7.292		195/110		17.0	
	10	895	-8.6										
	30	779	-20.5	90.0	+10.5	38.4	0	7.308	+.016	180/110	+15/0	23.5	+6.5
12	Con	72		80.9		40.2		7.410		205/130		17.0	
	10	119	+65										
	30	177	+146	82.2	+1.3	41.9		7.394		210/135	+5/+5	23.5	+6.5
14	Con	198		94.7		37.7		7.313		135/90		16.5	
	10	219	+11										
	30	199	0	95.1	+0.4	34.2	-3.5	7.347	+.034	150/100	+15/+10	29.0	+12.5
15	Con	299		90.1		38.2		7.292		185/120		20.0	
	10	412	+38										
	30	372	+24	86.6	-3.5	38.6	+0.4	7.274	-.018	180/115	-5/-5	28.0	+8.0

DISCUSSION

Most investigators consider the high arterial blood flow of carotid and (presumably) aortic chemoreceptors sufficient to meet metabolic requirements with utilization of only dissolved oxygen. Chemoreceptor activity increases have generally been demonstrated in conjunction with a significant decrease in perfusing arterial oxygen tension. It is nontheless widely acknowledged that increases in chemoreceptor activity may result from carotid occlusion (Schmidt, 1932; Euler, et.al., 1939; and Euler and Liljestrand, 1943), hypotension (Landgren and Neil, 1951; Coleridge, et.al., 1949) chemoreceptor warming (Bernthal and Weeks, 1939), increased sympathetic activity (Biscoe and Purvis, 1967), and reduction of arterial oxygen content without reduction of Pa_{O_2} resulting from carboxyhemoglobin (Paintal, 1967; Mills and Edwards, 1968; Edwards and Mills, 1969). Conversely, reductions in chemoreceptor activity can occur not only with increases in Pa_{O_2} above a threshold level (under otherwise normal conditions), but also with hypertension from epinephrine (Wright, 1930; Euler and Liljstrand, 1969; and Gernandt, et.al., 1945), and chemoreceptor cooling (Bernthal and weeks, 1939).

Anemia, with reduced arterial oxygen content but not partial pressure, is not usually characterized by hyperpnea (Comroe, 1965). Duke et.al. (1952) could not demonstrate increased activity in mixed chemoreceptor-baroreceptor multifiber preparations of carotid sinus nerve despite carbon monoxide inhalation, and Chiodi et.al. (1941) found no increase in ventilation in unanes-

thetized man with acute carbon monoxide poisoning. However, Hatcher et al., (1963) have reported increased ventilation in anesthetized dogs made acutely anemic with Dextran-for-blood exchange transfusions.

These observations suggest chemoreceptors are sensitive to their arterial oxygen flux, i.e., oxygen availability per unit time, and that chemoreceptor metabolism to some degree determines the relationship of oxygen flux and chemoreceptor activity. Normal chemoreceptor blood flow is adequate to sustain normal chemoreceptor metabolism with insufficient reduction in sensitive-element oxygen partial pressure to produce other than occassional, random neural activity. This relationship is sufficiently tenuous that changes in blood flow and/or oxygen availability or consumption, of a magnitude usually associated with changes in organ function, also result in neural activity responses from the chemoreceptors. This characteristic is compatible with the oxygen consumption determinations (1.5 ml oxygen per minute per 100 gm tissue) of Fay (1970) and the levels of oxidative metabolism enzymes in chemoreceptor tissue as reported by Lee and Mattenheimer (1964). Type I cells may be perfused by more ordinary flows than is usually accepted.

Very high normal blood flow and very high oxygen consumption would, of course, also satisfy the theoretical hypothesis.

The failure of response of chemoreceptor fibers to reduced oxygen content secondary to carboxyhemoglobin as reported by Duke et al., (1952) has been criticized on the basis of their use of mixed, multi-fiber preparations of sinus nerve and the low signal-to-noise ratio in their recordings. Both these characteristics make their data po-

tentially less reliable than data from more recent few-fiber preparations, which have shown increases in neural activity in both carotid and aortic chemoreceptors with inhalation of carbon monoxide.

To avoid the effects on the shape of the oxyhemoglobin dissociation curve produced by carboxyhemoglobin, we reduced arterial oxygen content by reduction of the quantity of oxyhemoglobin, produced by Dextran-for-blood exchange transfusion. Our study indicates that these acute reductions, as measured by hematocrit, enhance carotid and aortic chemoreceptor activity as determined by increased frequency of impulses in chemoreceptor fibers. One method of assessing the stimulation produced by anemia was the determination, from a control oxygen response curve, the level of Pa_{O_2} which produced an impulse frequency corresponding to a Pa_{O_2} of 100 torr in the post-exchange response (multi-point) curve. A reduction in hematocrit from 39.3 ± 6.0 to 15.2 ± 3.5 percent increases aortic chemoreceptor activity as much as lowering Pa_{O_2} from 100 torr to 62.3 torr at normal hematocrit. Likewise, stimulation of the carotid body by reduction of hematocrit from 41.8 ± 4.7 to 18.3 ± 2.2 percent was equivalent to reducing Pa_{O_2} from 100 torr to 78.4 ± 11.8 torr. Using this method of evaluation, the response of aortic body chemoreceptors exceeded that of carotid chemoreceptor ($p < 0.05$) for similar degrees of anemia. These changes occurred in the absence of the leftward shift or change in shape of the oxyhemoglobin dissociation curve produced by carbon monoxide.

All aortic hypoxic response curves were fitted with the model $Y = A/X^2$. 'A' values were in all cases increased by the dextran exchange transfusion. These increases were significant at the 0.001 level

in 4 of 6 experiments, at less than the 0.025 level in one experiment, and at less than 0.010 level in one experiment. A second statistical test of significance found the slope of the line relating 'A' value to hematocrit to be significantly different from zero at $P < 0.050$ level in 2 of 3 experiments with re-infusion data.

Most carotid hypoxic response curves fit the model $Y = A/X^2$ better than the $Y = A/X$ model, as comparison of mean square errors in Table 5A shows. Factors causing these various responses are not obvious. Anemia did not cause any consistent changes and statistical analysis of 'A' values resulted in a significant increase in 'A' with anemia in one experiment (P less than 0.001), a significant decrease in one experiment (P less than 0.025), and no significant change in 2 experiments, and no significant change in 4 experiments. The slope of the line relating 'A' value to hematocrit was not significantly different from zero in any carotid experiment with re-infusion data.

The shift in the aortic chemoreceptor oxygen response curve upward and to the right, following exchange transfusion in every animal studied indicates a marked increase in the response of the aortic chemoreceptor mechanism. This increased aortic chemoreceptor activity correlates with the degree of anemia; partial correction of the anemia promptly reduces impulse frequency and shifts the oxygen response curve leftward to an intermediate position between the control and post-exchange curves.

While exchange transfusion also increases impulse frequency in carotid chemoreceptor fibers, the curves are not consistently shifted over the range of Pa_{O_2} we studied. The failure of partial correction of the anemia to consistently reduce impulse activity and shift the

oxygen response curve suggests the increased sensitivity of the carotid chemoreceptors was not quantitatively related to the degree of anemia. Perhaps some other variable was operative. Stimulating entities evolving in the stored red cells are a possible explanation, but this seems unlikely inasmuch as such effects were not observed in the aortic chemoreceptors.

Thus, acute exchange anemia induces an increase in aortic chemoreceptor activity which is significantly greater and more consistent than the increases noted for the carotid chemoreceptors. We suggest aortic chemoreceptor afferents primarily determine the increase in cardiac sympathetic activity responsible for the cardiac output increase immediately following exchange transfusion. The carotid chemoreceptors, which are stimulated less, could be responsible for other reports of relatively small changes in respiration in anemia. Our results do not address the role of the peripheral chemoreceptor activity in chronic anemia, where a humoral mechanism may be largely responsible for the cardiac output elevation.

Our study does not allow differentiation of an alternate explanation of the observed results. Stimulation of carotid and aortic sympathetic nerves increases chemoreceptor activity, as previously discussed. Increased systemic sympathetic tone, resulting from anemic stimulation at a site other than chemoreceptors, could conceivably account for the increased activity we noted in anemia, through its effect on chemoreceptor blood flow. On the other hand, if our hypothesis is correct, the sympathetic effect on chemoreceptor blood flow could function as a positive feedback mechanism, augmenting the chemoreceptor response to the reduced oxygen content of its arterial blood supply by

reduction in chemoreceptor blood flow.

Preliminary studies on the effect of aortic chemoreceptor denervation on the cardiovascular responses to anemia in the first 1-2 hours after exchange transfusion is discussed in the following section. Aortic chemoreceptors manifest significant responses to reductions in oxygen flux (availability), such as occur in acute anemia induced by Dextran-for-blood exchange transfusion.

In passing, it is noted that the mathematical model, $Y = A/X^2$, can be interpreted to suggest the chemoreceptor nerve response is a function of two receptors whose activity decreases as Pa_{O_2} increases.

PART III

INTRODUCTION

In the previous section we have reviewed evidence that under certain circumstances, aortic chemoreceptors may be stimulated by reduced oxygen content of their arterial blood supply. The reduction of arterial oxygen content by inhalation of carbon monoxide increases aortic chemoreceptor discharge rate (Paintal, 1967) and has similar effects on carotid chemoreceptor activity (Mills and Edwards, 1968, Edwards and Mills, 1969). Our work, in which arterial oxygen content was reduced by anemia, demonstrated significant aortic chemoreceptor stimulation and evidence of carotid chemoreceptor stimulation to a much lessor degree.

While carboxyhemoglobin is probably rare in natural environments, anemia occurs naturally and is associated with certain cardiovascular compensations. The aortic chemoreceptor stimulation observed in anemia might participate in normal physiologic responses to acute anemia. Before considering this further, we will review cardiovascular reflexes resulting from chemoreceptor stimulation by hypoxia.

CHEMORECEPTOR REFLEXES IN HYPOXIA

Early investigation established that chemoreceptors exert significant effects on cardiovascular function (de Castro, 1926; de Castro, 1928; Heymans and Heymans, 1927; Heymans, Bouckaert, and Dautrebande, 1930). Comroe (1939) noted hypoxia and certain chemical stimulants acted on carotid chemoreceptors in cats and dogs to produce primarily hyperpnea. These stimulants acted on the aortic body to produce hypertension primarily, but considerable overlap of function existed

(Table 8). Comroe noted bradycardia was the primary effect on heart rate of carotid chemoreceptor stimulation. Doi (1921) had reported hypoxia caused tachycardia in the cat, and Bernthal, Green, and Revzin (1951) reported hypoxia caused tachycardia in the dog. These findings led many authors to conclude carotid and aortic chemoreceptors were not responsible for heart rate changes in hypoxia.

Daly and Scott (1958) demonstrated that the primary bradycardia of carotid body stimulation was converted to tachycardia by a secondary reflex antagonizing effect which arose from increased pulmonary stretch reflex activity accompanying hyperventilation. Thus the carotid body, by its reflex increase in ventilation, indirectly produced tachycardia in response to hypoxia. This tachycardia was obliterated by controlled, constant ventilation, by atropine or by lung denervation (Daly and Scott, 1958; Daly and Scott, 1959; Daley and Hazzledine, 1963; Kontos, et.al., 1965).

The primary bradycardia of carotid chemoreceptor stimulation results from an increase in cardiac vagal activity and a simultaneous withdrawal of cardiac sympathetic activity (Downing, Remensynder and Mitchell, 1962; Krasney, 1967).

Doi (1921) noted no increase in cardiac output in hypoxic cats, but the effects of hypoxia on canine cardiac output were less clear. Downing, Remensynder, and Mitchell (1962) demonstrated a decrease in cardiac output in the dog after vascularly-isolated carotid chemoreceptors were stimulated by hypoxemia, but whole-body hypoxia resulted in an increase in cardiac output. Daly and Scott (1963) showed the cardiac output increase in the spontaneously breathing

Table 8

Blood Pressure and Ventilatory Responses to Chemoreceptor Hypoxia in Dogs and Cats

49 Dogs:

24%	carotid hyperpnea > aortic hyperpnea
37%	carotid hyperpnea >> aortic hyperpnea
28%	carotid hyperpnea = aortic hyperpnea
11%	carotid hyperpnea << aortic hyperpnea

36 Dogs:

53%	carotid hypertension < 6 mm Hg
36%	carotid hypertension = 5-30 mm Hg
11%	carotid hypertension > 30 mm Hg
100%	aortic hypertension = 18-126 mm Hg

26 Cats:

27%	carotid hyperpnea > aortic hyperpnea
19%	carotid hyperpnea >> aortic hyperpnea
23%	carotid hyperpnea < aortic hyperpnea
12%	carotid hyperpnea << aortic hyperpnea

25 Cats:

36%	aortic hypertension > carotid hypertension
8%	aortic hypertension >> carotid hypertension
28%	aortic hypertension < carotid hypertension
12%	aortic hypertension << carotid hypertension

Data from Comroe, J.H., Jr., Am. J. of Physiol. 127:176 1939.

hypoxic dog was related to heart rate changes, whereas with constant ventilation there was a reduction of cardiac output in the hypoxic dog. Eucapnia and lung deneration obliterated the differences in cardiac output response to spontaneous and controlled hypoxic ventilation.

Penna, Soma, and Aviado (1962) concluded that the cardiac output increase in hypoxia, when mediated by the sympathetic nervous system, arises entirely from aortic and carotid chemoreceptors. Richardson et.al. (1965) demonstrated increases in cardiac output in humans in response to hypoxia.

The important differences in carotid and aortic chemoreceptors on cardiac function are emphasized by their divergent effects on contractility.. Hypoxic stimulation of carotid chemoreceptors results in lowered venticular contractility which is blocked by vagotomy (de Geest, Levy and Zieske, 1965). Stern and Rapaport (1967) have recently demonstrated increased myocardial contractility, heart rate and peripheral vascular resistance in response to hypoxic stimulation of aortic chemoreceptors in the dog. Increases in vascular resistance and myocardial contractility were blocked by alpha and beta blocking drugs, respectively. Tachycardia and increased cardiac sympathetic activity as reported by others, may result from aortic body stimulation by hypoxia (Krasney, 1967; Downing and Siegel, 1963; Downing, Remensynder and Mitchell, 1962).

After Comroe (1939) established hypertension as a primary aortic body reflex effect in hypoxia other authors reported increased peripheral vascular resistance (PVR) from carotid chemoreceptors hypoxia

(Downing, Remensynder, and Mitchell, 1962; Daly and Scott, 1963; Stern and Rapaport, 1967; Daly and Unger, 1966; James and Daley, 1969; Pelletier, 1972; Browse and Shepherd, 1966; Pelletier and Shepherd, 1972). Many studies have confirmed Comroe's findings in aortic chemoreceptor hypoxia (Daly, Hazzledine and Howe, 1963; Daley Hazzledine and Howe, 1965; Daley and Unger, 1966). Stern and Rapaport (1967) and Calvelo et.al. (1970) have stimulated aortic bodies with nicotine, resulting in increases in peripheral vascular resistance. Calvelo et.al. (1970) also found similar responses with nicotine stimulation of carotid chemoreceptors, but Stern and Rapaport (1967) found little increase in vascular resistance after nicotine stimulation in the carotid body. Browse and Shepherd (1966) noted consistent arterial hypertension with cyanide stimulation of aortic bodies, but variable blood pressure effects from similar stimulation of carotid bodies.

Secondary vascular reflexes resulting from hyperventilation antagonize the increased PVR resulting from hypoxic stimulation of chemoreceptors. Daly, Hazzledine, and Howe (1965) concluded hypocapnia reduced the degree of increase in PVR resulting from aortic body stimulation. Daly and Unger (1966) demonstrated that hypoxic stimulation of aortic bodies resulted in greater increase in PVR that did carotid body stimulation in spontaneously breath dogs, but in dogs with controlled ventilation aortic and carotid body hypoxic stimulation resulted in similar increases in PVR. The lung inflation reflex antagonized the carotid hypertension reflex but not the aortic hypertension reflex; hypocapnia antagonized both the aortic and carotid

hypertension reflexs. These findings are supported by findings of Kontos (1965), Asmussen and Chiodi (1942) and Pelletier (1972).

Studying man and trained dogs, Kontos, et.al. (1967) found similar responses to hypoxia, i.e., both have tachycardia and increased cardiac output. Trained dogs, in contrast to man, manifested hypertension in response to reduction of Pa_{O_2} to 35-40 torr.

Significant changes in venous tone result from chemoreceptor stimulation. Decreases in central blood volume in dogs occurs with hypoxia (Kahler, Goldblatt, and Brannwald, 1962), even in splenectomized animals. This increase in venous tone is partially prevented by prior adrenalectomy and blocked by gangiolic blocking drugs or vagosympathetic trunk interruption. Peripheral venoconstriction (Browse and Shepherd, 1966) and splenic contraction (Pelletier and Shepherd, 1972) can result from carotid chemoreceptor hypoxia.

Generally, direct reflex responses to carotid body hypoxia are:

1. Stimulation of the respiratory center with a resultant increase in respiratory minute volume, and
2. stimulation of the vasomotor center with
    a. withdrawal of sympathetic tone to the heart
    b. an increase in parasympathetic tone to the heart which acts with a) above to slow the heart rate,
    c. an increase in peripheral vascular sympathetic tone resulting in an increase in peripheral vascular resistance,
    d. an increase in venous tone.
3. Stimulation of adrenal medulla by sympathetic fibers with

release of circulating catecholamines,

4. an increase in parasympathetic tone and withdrawal of sympathetic tone of coronary arteries resulting in vasodilatation.

Tachypnea results in two indirect reflex effects:

1. increased stimulation of the pulmonary stretch receptors resulting in vasomotor center increase in sympathetic tone to the heart, increasing heart rate, and
2. hypocapnia resulting in a decrease in sympathetic tone of the peripheral vasculature and a decrease in peripheral vascular resistance and venous tone.

In the spontaneously breathing animal the indirect effects usually predominate. In the animal with controlled, constant ventilation, the direct effects are manifested.

Aortic body hypoxia reflexes include:

1. an increase in sympathetic activity to the heart with a resulting increase in contractility of the myocardium an increase in heart rate,
2. an increase in peripheral vascular resistance secondary to an increase in sympathetic tone.

The aortic body has little influence on the ventilation and its direct reflexes are not significantly affected by the pattern of breathing of the animal; although some authors find hypocapnic alkalosis reduced aortic body reflex activity.

CARDIOVASCULAR ADJUSTMENTS TO ANEMIA

Effects of anemia on cardiac output have been reviewed in Part II, to which the reader is referred. PVR usually decreases in anemia (Brannon et.al., 1945; Case, Berglund and Sarnoff, 1955; Lovegrove, Gowdey and Stevenson, 1957; Parker, 1957; Garvock, 1958; Jennings, 1960; Jennings and Hatcher, 1964; Glick, Planth and Braunwald, 1964; and Chamorro et.al., 1973). Despite the good agreement on decreasing PVR, reports on arterial blood pressure in anemia are conflicting (Brannon et.al., 1945; Lovegrove, Gowdey and Stevenson, 1957; Hatcher et.al., 1954; Neil, Oxendine and Moore, 1969).

Blood flow to selected organs in the anemic state have been studied. Scheinberg (1949) and Scheinberg (1951) reported cerebral blood flow in patients with hemoglobin below 7 gm/100 ml (with pernicious anemia) to be 96 ml/min/100g compared to 65 ml/min/100g in controls. Heyman, Patterson, and Duke (1952) reported similar increases in patients with severe sickle cell anemia.

Dilatation of coronary arteries is an important adaptive mechanism in anemia. Case, Berglund and Sarnoff (1955) have reported marked increases in coronary flow following Dextran-for-blood exchange transfusion. In control dogs 2.4-4.3% of cardiac output perfused the coronary arteries. Reduction of hematocrit to 19% resulted in 11.0-12.4% of cardiac output perfusing coronary arteries. Bing (1951) and Kobelt et.al. (1957) noted increased coronary flow in humans in anemia.

Hepatic blood flow increases in anemia (Myers, 1951; Chamorro et.al., 1973). Renal blood flow is reported to decrease in anemia

(Bradley and Bradley, 1947; Paterson, 1951; Whitaker, 1956; Ingram et.al., 1956; Davis, Goodkind and Ball, 1957).

Muscle blood flow increases (Abramson, Fierst and Flashs, 1943; Hatcher, Sunhara,and Edholm, 1954; Sharpey-Schafer, 1961). Skin blood flow also increases (Stewart, 1912; Abramson, Fierst,and Flashs, 1943; Hatcher, Sunhara and Edholm, 1954). We are not aware of any reports in the literature which have demonstrated a participation of arterial chemoreceptors in the reflex cardiovascular adjustments to anemia.

CARDIOVASCULAR MEASUREMENTS WITH RADIOACTIVE MICROSPHERES

To determine the influence, if any, of arterial chemoreceptors in anemia and, for comparison, hypoxia, we examined the validity of the radionuclide-labeled ceramic microsphere method for determination of organ blood flows and cardiac output in the cat.

Rudolph and Heyman (1967) described a method for accurate and repeated measurements of cardiac output and organ blood flow using insoluble microspheres. These microspheres are available in a variety of diameters (e.g., 15 ± 5μ, 25 ± 5μ) with one of several radionuclides embedded in them. A suitable dose of these microspheres can be injected into the left atrium, where it mixes with arterial blood and distributes to tissues in proportion to organ blood flow. If, during the microsphere injection of activity, I_i, one withdraws an arterial reference sample at a known, constant flow, $\dot{Q}_{ra}$, this reference sample will contain a radioactivity I_{ra}. The cardiac output, C.O., at the time of injection can then be estimated by the relationship C.O. =

$I_i(\dot{Q}_{ra}/I_{ra})$. If, in every case, the microspheres presented to an organ are trapped during their first passage (no re-circulation) these trapped beads will result in an organ radioactivity of I_o. Again, using the activity and flow of the arterial reference sample, the organ blood flow, $\dot{Q}_o$, may be estimated by the relationship $\dot{Q}_o = \dot{Q}r_a(I_o/I_{ra})$. A complete discussion and mathematical derivation of the microsphere methodology are available from several sources (Rudolph and Heymann, 1967; Archie et.al., 1973; Wagner et.al., 1969; and Buckberg et.al., 1971). Multiple measurements of organ blood flow and cardiac output can be obtained by injecting microspheres with different nuclide labels which are suitable for separation by gamma ray spectrometry. Repeated injections of microspheres do not significantly affect organ blood flow (Neutze, Wyler and Rudolph, 1968).

Archie et.al. (1973) have identified three critical requirements for assuring accuracy with this method:

1) Adequate mixing of injected microspheres prior to the first branching of the arterial tree,
2) the number of microspheres injected must be sufficient to give statistical reliability to the measured flows (this has been established to be 400 microspheres for 10% error limits with 95th percentile confidence intervals for an organ),
3) there must be minimal systemic and pulmonary shunting of microspheres (i.e., insignificant circulation).

We have adapted to the cat methods used previously in other

animals. Using the technique of selective aortic chemoreceptor denervation we have conducted preliminary studies to examine the applicability of the microsphere method to cardiovascular adjustments in acute anemia and have tested the physiologic significance of anemic stimulation of aortic chemoreceptors reported in Part II.

METHODS

We studied 10 cats, weighing 3.12 to 6.75 kg, anesthetized with intraperitoneal injections of chloralose (40 mg/kg) and urethane (200 mg/kg). We gave supplemental intravenous doses of these two agents as required. All cats were paralyzed with 20 mg of gallamine intravenously and received 10-20 mg per hour for maintenance of muscle relaxation.

SURGICAL PREPARATION

We cannulated both femoral arteries and one femoral vein and recorded arterial blood pressure continuously with a Statham transducer and Grass Model 5 polygraph. We opened the chest at the left forth intercostal space and placed a 0.69 mm ID catheter in the right atrium with a purse-string suture in cat M001. In all other cats a similar catheter was placed in the left atrium. We closed the chest and evacuated intrapleural air. Throughout the entire study we ventilated cats with a Harvard small-animal respirator with a minute ventilation adjusted to maintain a Pa_{CO_2} of approximately 40 torr. We recorded the tracheal P_{CO_2} with a Beckman Medical Gas Analyzer and a Grass Model 5 polygraph.

In cats M002-M005 we placed an additional catheter in the left brachial artery. In cats M007-M011 we exposed the junction of the superior laryngeal nerve and the vagus nerve through a ventral midline neck incision. We identified the aortic depressor nerve at its point of entrance into the superior laryngeal nerve and, with careful technique to minimize trauma to the nerve, placed a suture loosely

about the aortic depressor nerve bilaterally and closed the neck wound. In the course of the experiment we denervated the aortic chemoreceptors by briefly opening the neck incision and transecting the aortic depressor nerve as it passed through the loose retracting suture.

MICROSPHERE METHODOLOGY

Microspheres, 15 ± 5μ or 25 ± 5μ in diameter, were injected into the left atrium of the cat where they mixed with arterial blood and were distributed with the blood flow. An arterial reference sample was withdrawn from the femoral artery at a constant rate by a withdrawal pump during the microsphere injection.

Pertinent microsphere data are tabulated below:

Isotope	Bead Size Microns	$T_{1/2}$ Days	Energy KEV
Id-125	25	60.2	35.2
Sr-85	25	64.0	513.3
Nb-95	25	35.0	768
Ce-141	15	27.8	144
Cr-51	15	32.5	324

Initial bead activity ranged from 50-100 counts per minute per bead and decreased in accordance with the half-life of the isotope. We injected sufficient isotope activity to assure $1\text{-}2 \times 10^5$ beads per injection. We prepared microspheres by suspending an approximately correct volume in Tween-80 solution and counted the isotope activity in the injector vial using a well scintillation counter and a multiple channel pulse height analyzer. The volume was then altered to more closely approximate the desired bead count, utilizing the calculated

counts per minute per bead. We injected the beads via the atrial catheter over 20-30 seconds, followed with a 5 ml flush of 0.9% saline solution. In cats M001-M005 simultaneous injections of 15 micron and 25 micron spheres were also performed. In these animals a reference arterial sample was withdrawn with a constant-rate withdrawal pump from both a femoral artery and a brachial artery site. In cats M007-M011 we injected either 15 micron or 25 micron spheres and withdrew a single reference sample from a femoral artery. We began withdrawal of the reference arterial sample prior to isotope injection and continued withdrawal for one minute, at least 20 seconds of withdrawal time elapsing after completion of the intra-atrial injection of microspheres. We determined withdrawal time with a stopwatch. The arterial sample was transferred to a weighed vial, its volume determined by weighing, and the withdrawal flow rate calculated. The injected dose was determined by total body counting. Immediately prior to injection of a microsphere batch we withdrew a specimen of arterial blood anaerobically for determination of Pa_{O_2}, Pa_{CO_2}, and pHa. Table 9 summarizes the experimental protocal, which is described below.

Table 9

Microsphere Protocol

Cat	Study
M001	Validation study-right atrial injections 1. normoxic, normal hematocrit 2. hypoxic, normal hematocrit
M002	Validation study-femoral and brachial artery reference sampling, left atrial injections 1. simultaneous 15 and 25μ spheres, normoxic, normal hematocrit 2. simultaneous 15 and 25μ spheres, hypoxic, anemic
M003-005	Validation study-femoral and brachial artery reference sampling, left atrial injections 1. normoxic, normal hematocrit 2. simultaneous 15 and 25 micron spheres, hypoxic 3. normoxic, anemic
M007-008	Aortic denervation and hypoxia, femoral artery reference sample, left atrial injections 1. normoxic, normal hematocrit control 2. hypoxic,normal hematocrit 3. normoxic, normal hematocrit, aortic denervated 4. hypoxic, normal hematocrit, aortic denervated 5. normoxic, normal hematocrit, aortic denervated
M009-011	Aortic denervation and anemia, femoral artery reference sample, left atrial injections 1. normoxic, normal hematocrit control 2. normoxic, anemic 3. normoxic, anemic, aortic denervated 4. normoxic, anemic, aortic denervated

M001: In one cat we placed a right atrial catheter and injected 15 micron and 25 micron spheres simultaneously, ventilating the animal with room air. We then reduced the $F_{I_{O_2}}$ to reduce Pa_{O_2} to 46 torr and injected again 15 and 25 micron beads simultaneously

M002: In a second cat we injected into the left atrium and sampled simultaneously from both femoral and brachial arteries. We reduced the $F_{I_{O_2}}$ to make the cat hypoxic and produced anemia with a Dextran-for-blood exchange transfusion as previously described. A simultaneous injection of 15 micron and 25 micron beads was then administered.

M003-M005: In three cats with left atrial injection sites we obtained simultaneous reference arterial samples from femoral and brachial artery catheters. An initial simultaneous injection of labeled 15 micron and 25 micron beads was made with the animal ventilated with a room air-oxygen gas mixture. We reduced the inspired oxygen to produce hypoxia and again injected 15 micron and 25 micron beads simultaneously. We then returned the inspired oxygen to normal and conducted a Dextran-for-blood exchange transfusion. A third simultaneous injection of labeled 15 micron and 25 micron beads was made.

Additional studies were designed to obtain preliminary data on the effects of denervation of the aortic bodies on the cardiovascular responses to hypoxic and anemia. Fifteen micron and 25 micron beads were used interchangeably in these studies:

M007-M008: In two cats with left atrial injection sites reference arterial samples were withdrawn from a femoral artery catheter. We made an initial control injection of microspheres, ventilating the cat with room air. We then reduced the inspired oxygen to produce hypoxia,

and after a stabilization period, we injected a second microsphere batch. The animal was returned to a normoxic gas mixture and, after a stabilization period, the aortic nerves were sectioned bilaterally and a third microsphere injection performed. The inspired oxygen was then again reduced to make the animal hypoxic and a fourth microsphere injection performed. Finally, we ventilated the animal with a normoxic gas mixture and made a terminal control injection of a fifth population of microspheres.

M009-M011: In three cats with left atrial injection sites a reference arterial sample was obtained from a femoral artery. Ventilating the animal with a room air gas mixture, we made an initial control injection. A Dextran-for-blood exchange transfusion rendered the animal anemic. After a stabilization period we injected a second population of labeled microspheres. We then sectioned the aortic nerves and injected a third population of labeled microspheres. Finally, after the passing of at least an hour's time beyond the exchange transfusion, a fourth injection of labeled microspheres was made.

The reference arterial samples were counted, using standardized techniques, in a Nuclear Chicago well scintillation counter with a multi-channel pulse-height analyzer. Reference sample activity was converted to number of microspheres utilizing the counts per minute per bead data of each isotope as supplied by the manufacturer, with correction for decay. This bead number was related to the flow rate of the arterial withdrawal sample for future use in calculating cardiac output.

At the conclusion of bead injection the animals were sacrificed with intravenous injections of KCl. The abdomen was opened and the following organs or organ groups removed and weighed:

1. left and right kidneys
2. stomach and esophagus
3. small bowel
4. large bowel
5. omentum
6. liver
7. spleen
8. aliquot of abdominal wall muscle

The chest was opened and the following organs removed and weighed:

1. heart
2. right lung
3. left lung

We skinned the animal, including all limbs and the head. The skin, including subcutaneous fat and hair, was weighed. The cranial vault was opened, the brain removed (to the foramen magnum) and weighed. An aliquot of leg muscle and a portion of either femur was obtained and weighed. The carcass was arbitrarily divided into two or three portions to facilitate further handling.

After weighing, all organs were fixed in formalin and carbonized at 200°C for 2-3 days. We manually pulverized organs and pulverized carcass parts with a food blender, placing the resulting powder in counting vials. For large carcass parts, weighed aliquots of the well-homogenized, pulverized carcass were placed in counting vials. All vials were counted simultaneously with reference arterial sample vials, using the multi-channel pulse-height analyzer. Five areas of interest in the energy spectrum were counted and count data was outputed onto IBM computer punch cards. Header cards were added which specified

organ weight, reference flows, isotope identification, bead activity, counting times, and isotope energy window matrix equations. A PL/I program entitled COUNTS was utilized for data analysis running on an IBM 37145 computer. Output of this program was as follows:

1. Counts per minute per organ for each isotope
2. beads per organ for each isotope
3. cardiac output, as ml per minute for each injection, calculated for each reference site and each isotope
4. organ flows, as ml per minute, for all organs and for the following groups of organs, calculated for each isotope injection and each reference withdrawal site:
   a) Portal flow, i.e., arterial flow to all viscera except liver
   b) left and right lungs
   c) left and right kidneys
   d) sum of carcass parts
5. Flows as ml per 100 grams of tissue for all organs and the above-listed organ groups, for each isotope injection and each reference withdrawal site.
6. Flows, as percent of cardiac output, for each isotope injection and each reference withdrawal site.
7. Weight of each organ as percent of body weight.

Other data were calculated by hand. These included:

1. Flows expressed as above for:
   a) Total liver (arterial plus portal)
   b) total body muscle.

2. Percent change of subsequent injections compared to the initial control 25 micron bead data.
3. Cardiac index as ml/min/kg body weight.

The following points deserve emphasis:

Lung flow, as measured, has two components,

1) bronchial arterial flow, and
2) venous systemic flow containing microspheres shunted by systemic organs, i.e., re-circulating microspheres.

It is not possible in this study to separate these two components.

Total liver flow was determined by adding to the measured liver arterial flow the sum of arterial flows to the stomach, esophagus, small and large bowel, spleen, and omentum.

Total body muscle flows were calculated using the average muscle flow per 100 grams of tissue determined on the various muscle aliquots measured. Total body muscle weight was estimated by the following regression equations, derived by Latimer (1944) from studies on 102 cats: For males, total muscle weight, in grams, equals (0.529 $\times$ body weight in grams) - 86.04. For females, total muscle weight, in grams, equals (0.522 $\times$ body weight in grams) - 32.29.

RESULTS

Validation of Labeled Microsphere Method for Determination of Regional Flows in the Cat

General hemodynamic and blood gas data are presented in Table 10. Organ blood flows for cats M002-M005 are presented for femoral and brachial artery reference sites in Tables A-1 through A-12 of the Appendix. Organ blood flows for Cats M007 through M011, with a femoral reference site, are presented in Tables A-13 through A-22 of the Appendix. Cat M009 has not been included in denervation calculations. Hemorrhage during the latter portion of this study resulted in a fall in cardiac output and corresponding reductions in organ blood flows. M009 data are included in the Appendix because of their demonstration of distribution of cardiac output as the blood volume progressively decreased.

Microsphere Mixing

Relationship of Flow Estimate to Arterial Reference Site:

The simultaneous injection of a 15 micron and a 25 micron batch of microspheres and the simultaneous sampling from brachial and femoral artery reference sites tested adequacy of mixing as a function of bead size and sampling site. The agreement between the femoral and brachial artery reference calculations was best for 15 micron beads in the control (normoxic, normal hematocrit) state. In this state flow estimates calculated from the brachial artery reference sample exceeded flows calculated from the femoral artery reference sample by 0.2 ± 2.6% for 15 micron beads, while flows determined

Table 10

Hemodynamic and Blood Gas Data

Cat MO-	Intact Control			Intact Hypoxia			Intact Anemia			Dernervated Control			Denervated Hypoxia			Denervated Anemia			Final Anemia			Final Control		
	BP	HR	HCT	BP	HR	HCT	BP	HR	HCT	BP	HR	HCT	BP	HR	HCT	BP	HR	HCT	BP	HR	HCT	BP	HR	HCT
02	180/160	222	46	200/150	210	22																		
03	140/95	240	38	170/115	264	38	200/120	264	17															
04	180/120	264	43	180/115	252	43	185/100		15															
05	180/145	180	45	190/155	180	45	135/80	240	17															
07	185/110	216	38	170/105	210	38				200/130	240	28	180/100	240	28							175/105	246	28
08	165/130	204	42	180/140	216	38				195/150	192	42	165/120	264	42							120/65	288	42
09	180/145	204	54				205/150	204	22							150/80	228	19	75/50	228				
10	190/120	288	43				210/125	240	20							225/140	228	20	225/140	216	23			
11	155/100	228	39				180/110	216	13							185/110	228	13	185/115	228	15			

Cat MO-	O_2 torr	CO_2 torr	pH 7.	O_2 torr	CO_2 torr	pH 7.	O_2 torr	CO_2 torr	pH 7.	O_2 torr	CO_2 torr	pH 7.	O_2 torr	CO_2 torr	pH 7.	O_2 torr	CO_2 torr	pH 7.	O_2 torr	CO_2 torr	pH 7.	O_2 torr	CO_2 torr	pH 7.
02	148	34	35	36	30	30																		
03	110	23	39	36	35	37	101	23	45															
04	84			32			82																	
05	104	34	23	40	33	26	98	29	37															
07	95	33	29	43	28	41				93	30	34	35	32	33							100	28	30
08	79	28	37	34	27	37				77	28	31	11		28							132	32	19
09	63	28	32				90									121	31	35	125	29	33			
10	97	23	39				92	23	40							94	23	43	95	23	42			
11	106	27	44				94	27	46							96	26	47	94					

Notes: M002 - Second state was combined anemia and hypoxia.
M007 - $NaHCO_3$ added IV to correct acidosis.
M011 - Final control BP fell from 185/115 to 90/45 transiently with bead injection.
Heart rate fell transiently from 228 to 90.

simultaneously with 25 micron beads averaged 14.1 ± 4.4% greater. Lumping all flows in the control state resulted in brachial estimates exceeding femoral estimates by 7.2 ± 8.2%. In the anemic condition flows from brachial artery calculations averaged 2.0 ± 6.6% less than corresponding estimates from the femoral artery for 25 micron spheres.

There was little preferential distribution by bead size to the brachial or femoral reference sites. Accordingly, flow estimates derived from both bead sizes and based on the femoral reference averaged 2.9% less than brachial estimates in the control state (range of -13.2 to +6.0%). In hypoxia the difference averaged 5.6% (range of 1.1% to 9.5%). As expected, the combined state of anemia and hypoxia resulted in the greatest discrepancy between flow determinations done simultaneously with brachial and femoral references. Brachial artery estimates exceeded femoral estimates by as much as 21.4%, using either bead size, in this circulatory state.

Thus, there was generally good agreement between flow estimates derived from femoral reference samples and brachial reference samples in anemia, hypoxia, and the control conditions, but agreement deteriorated with combined anemia and hypoxia.

Relationship of Flow Estimate to Microsphere Size:

In the normoxic, normal hematocrit control state, 15 micron spheres over-estimated organ flow relative to 25 micron spheres by an average of 4.3%, 2.4%, and 12.2%, and under-estimated by 4.7% in one cat. In the hypoxic state, 15 micron spheres exceeded the 25

micron flow estimate by an average of 5.1% and 3.2% in two cats and under-estimated the 25 micron sphere flow by 1.1% in one cat.

Pulmonary Shunting

In a single cat with 15 and 25 micron labeled beads injected into the right atrium there was negligible radioactivity (consistent with 'leeched' counts) in peripheral organs. This was so with normal heamtocrit, with normoxia, and with arterial hypoxemia to 46 torr. In experiments M002-M005 the number of 15 micron beads entrapped in the lungs exceeded the number of 25 micron beads entrapped suggesting the lung is an effective filter of both sizes of beads.

Relationship of Magnitude of Flow on Flow Estimate

Inasmuch as the brain is one of the organs of interest whose flow is derived from arteries with early take-off from the aorta, where mixing is likely to be critical, a more detailed analysis of this organ's flow estimates was made. Figure 11A shows the good correspondence between brain flow estimates derived with 15 micron beads from both reference sites, regardless of flow magnitude. Figure 11B shows the same relationship for 25 micron beads in the control state. It is obvious that the correspondence decreases as a function of increased flow. Figure 11C illustrates the deterioration of the correspondence with hypoxia for 15 micron spheres. Correspondence of the two estimates is still good, however. The correspondence in anemia with grouped 15 and 25 micron sphere data is illustrated in Figure 11D. The spread of 25 micron bead data at high flows during hypoxia is illustrated in Figure 11E. Finally,

the relationship, for both bead sizes, of flow estimates derived from the two reference sites shows good correspondence at all flow levels, although the scatter is less at low flows (Figure 11F). Table 10 lists the regression equations for the linear best-fit least squares regressions shown in Figure 11.

Organ blood flows (mean and standard deviation) for control state and for hypoxia and anemia, in cats with intact chemoreceptors, are presented in Table 11. Organ blood flows in two cats undergoing hypoxia and aortic chemoreceptor denervation are presented in Table 12. Table 13 presents corresponding data for cats with acutely induced anemia and aortic chemoreceptor denervation.

Figure 11

Comparison of estimates of brain blood flow determined simultaneously from femoral and brachial artery reference sites in the control and experimental states for 15 micron and 25 micron beads.

Solid line is line of identity, dashed line is best fit least square linear regression line.

Figure 11. Brain Flow

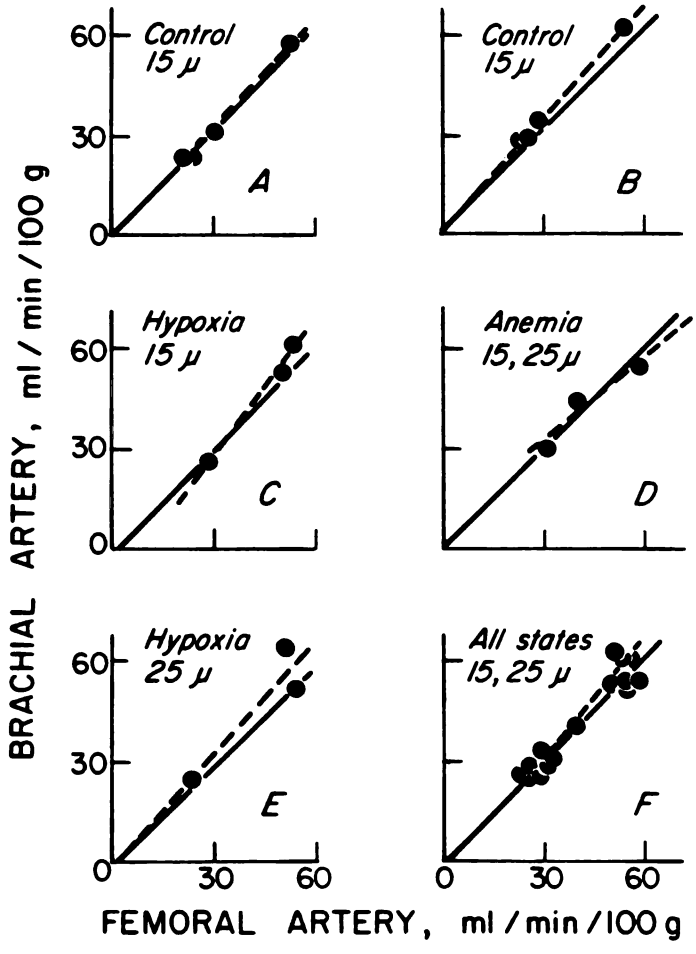


Table 10

Regression Equations and Correlation Coefficients for Brain Blood Flow Estimated From Brachial Artery Reference and Compared to Femoral Artery Reference

Normoxia, 15 micron spheres:
Brachial Art. Flow = (1.05)(Fem. Art. Flow) - 1.55
R = 0.998

Normoxia, 25 micron spheres:
Brachial Art. Flow = (1.26)(Femoral Art. Flow) - 3.43
R = 0.998

Hypoxia, 15 micron spheres:
Brachial Art. Flow = (1.275)(Femoral Art. Flow) - 10.385
R = 0.994

Hypoxia, 25 micron spheres:
Brachial Art. Flow = (1.12)(Femoral Art. Flow) - 1.042
R = 0.809

Anemia, 15 and 25 micron spheres:
Brachial Art. Flow = (0.860)(Femoral Art. Flow) + 5.045
R = 0.950

All states and size spheres:
Brachial Art. Flow = (1.08)(Femoral Art. Flow) - 1.12
R = 0.911

Table 11

Organ Blood Flow in Hypoxia and Anemia

Mean ± Standard Deviation

	Control		Hypoxia		Anemia	
Pa_{O_2} (torr)	98.4 ± 23.8		36.8 ± 4.0		92.8 ± 6.7	
Hct[2] (%)	43.1 ± 5.0		41.3 ± 3.8		17.3 ± 3.3	
n[1]	13(9)		8(5)		6(6)	
Organ	ml/min/ 100 gm	% C.O.	ml/min/ 100 gm	% C.O.	6(6) ml/min/ 100 gm	% C.O.
Brain	31.6 ± 10.1	2.0 ± 1.2	43.7 ± 12.4	1.91 ± 0.4	48.0 ± 9.9	2.1 ± 0.8
Heart	164.2 ± 52.5	4.6 ± 1.4	365.3 ± 151.3	8.8 ± 2.0	300.6 ± 127.7	8.1 ± 1.2
Kidneys	492.8 ± 155.1	22.8 ± 6.8	495.0 ± 208.3	21.7 ± 3.8	518.9 ± 193.4	21.2 ± 2.8
Lungs	86.2 ± 78.5	6.11 ± 4.5	136.2 ± 110.3	9.3 ± 5.5	83.5 ± 111.6	4.42± 5.1
Liver (A+V)[2]	91.1 ± 29.6	23.3 ± 4.2	79.9 ± 31.9	17.3 ± 4.1	119.8 ± 35.4	27.2 ± 4.8
Liver (A)[3]	31.6 ± 9.9	8.0 ± 2.5	31.6 ± 17.5	6.8 ± 2.6	33.5 ± 22.5	7.8 ± 4.7
Spleen	132.3 ± 68.8	3.3 ± 1.6	41.5 ± 16.9	1.0 ± 0.5	143.5 ± 132.0	2.7 ± 1.9
Sto + Eos	15.7 ± 11.6	1.1 ± 0.5	10.6 ± 3.1	0.7 ± 0.2	28.9 ± 16.8	1.6 ± 0.5
Sm Bowel	39.9 ± 31.5	6.8 ± 2.6	33.0 ± 11.8	5.5 ± 1.2	65.5 ± 28.9	9.4 ± 1.6
Lg Bowel	49.8 ± 22.6	2.2 ± 0.7	40.6 ± 7.1	1.9 ± 0.8	89.7 ± 76.6	2.7 ± 1.4
Muscle	4.0 ± 2.7	20.4 ± 8.4	3.3 ± 1.6	17.2 ± 10.2	4.7 ± 2.4	19.3 ± 8.7
Skin	2.6 ± 1.0	4.1 ± 1.0	2.8 ± 1.0	3.8 ± 0.7	4.3 ± 1.2	5.7 ± 1.5
Cardiac Index[4]	104.1 ± 32.3		116.8 ± 30.0		133.6 ± 45.6	

1. Number of measurements; number of cats given in parenthesis.
2. Combined arterial and venous blood flow.
3. Critical flow only.
4. Cardiac index in ml/min/kg body weight.

Table 12

Hypoxia and Aortic Body Denervation

Average Organ Blood Flows, Cats M007 and M008

$P_{A_{O_2}}$ (torr)	87		33		35		33		116	
Organ	Control		Intact Hypoxia		Denevated Control		Denervated Hypoxia		Denervated Final Control	
	ml/min/ 100 gm	% C.O.	ml/min/ 100 gm	% C.O.	ml/min 100 gm	% C.O.	ml/min/ 100 gm	% C.O.	ml/min/ 100 gm	% C.O.
Brain	27.5	2.2	44.2	2.5	48.0	3.4	46.2	5.8	47.3	4.4
Heart	201.3	6.3	425.8	9.2	350.6	9.3	313.1	9.7	345.1	12.5
Kidneys	593.3	24.0	388.6	21.7	312.6	21.4	156.6	15.4	197.1	18.1
Lungs	71.7	8.5	155.2	12.7	102.4	9.7	65.1	7.2	75.9	10.3
Liver (A+V)[1]	94.3	22.8	121.7	20.4	105.7	21.9	76.7	28.3	81.8	23.1
Liver (A)[2]	42.6	10.1	54.7	9.0	46.8	9.5	35.8	13.0	36.4	10.1
Spleen	88.5	2.0	47.5	0.7	67.8	1.3	25.4	0.5	33.6	0.7
Sto & Eso	10.1	1.0	12.0	0.8	11.8	0.9	8.9	1.4	10.1	1.1
Sm Bowel	26.9	6.0	18.1	6.0	32.3	6.0	25.4	8.7	24.7	6.5
Lg Bowel	32.1	2.1	45.8	2.1	41.6	2.4	27.7	3.0	36.6	5.4
Muscle[3]	7.0	30.9	9.4	27.8	6.4	23.2	4.2	23.9	5.3	20.5
Skin	2.3	3.3	3.1	3.1	2.4	2.9	2.3	4.0	1,9	3.1
Cardiac Index[4]	97.0		140.0		116.6		77.8		84.9	

1. Combined arterial and venous blood flow.
2. Arterial flow only.
3. Flow estimated from alignots of total estimated muscle mass.
4. Cardiac indes as ml/min/kg body weight.

Table 13

Anemia and Aortic Body Denervation

Average Organ Blood Flows

Cats M010 and M011

Hematocrit (%)	41.0 Control		16.5 Anemia		16.5 Denervated Anemia		19.0 Final Denervated Anemia	
Organ	ml/min/ 100g	%C.O.	ml/min/ 100g	%C.O.	ml/min/ 100g	%C.O.	ml/min/ 100g	%C.O.
Brain	27.6	1.2	53.7	1.8	49.4	2.4	55.9	2.1
Heart	164.2	4.5	360.0	8.6	247.6	9.5	315.9	8.4
Kidneys	388.0	18.5	267.3	19.3	107.4	11.4	259.2	20.3
Lungs	46.1	4.7	27.2	1.7	16.1	1.8	22.5	1.5
Liver (A&V)[1]	229.5	28.7	240.8	31.8	111.5	37.7	123.2	28.5
Liver (A)[2]	23.9	7.5	20.8	4.5	31.6	11.7	28.9	6.7
Spleen	175.7	3.2	304.6	4.7	180.5	4.3	275.5	4.7
Sto. Eos	31.2	1.5	48.4	2.0	32.8	2.1	38.5	1.7
Sm Bowel	88.9	11.0	149.1	11.2	67.1	11.4	77.0	9.4
Lg Bowel	95.3	2.6	185.2	4.1	126.6	8.4	144.6	3.4
Muscle[3]	5.3	22.5	5.8	22.3	3.9	19.5	4.8	17.7
Skin	2.8	3.3	4.6	4.8	3.9	6.62	3.3	3.86
Cardiac Index[4]	154.3		188.5		121.1		171.9	

1. Combined arterial and venous blood flow.
2. Arterial flow only
3. Flow estimated from alignots of total muscle mass.
4. Cardiac index as ml/min/kg body weight.

DISCUSSION

Prior to the introduction of the microsphere method for determination of regional blood flow in multiple organs simultaneously, such measurements had only been accomplished in rats, using Rb^{86} as a tag, as reported by Sapirstein (1960). Since the introduction by Rudolph and Heymann (1967) of the microsphere method, regional blood flows have been reported in the dog (Kaihara, et.al., 1968), sheep (Makowski, et.al., 1968), rabbit (Neutze, et.al., 1968), and monkey (Forsyth, et.al., 1968; Hoffbrand and Forsyth, 1969; Amory, et.al., 1971; Hoffbrand and Forsyth, 1971; Miller, et.al., 1973 and Hill, et.al., 1973). Comparisons of these data with microspheres and data from the rat with Rb^{86} are presented in Table 14. These flows, as per cent of cardiac output, are shown in Table 15.

It has since been demonstrated that labeled microsphere measurement of cardiac output is the preferred method, resulting in a repeatability and accuracy not found with dye-dilution and other techniques (Archie, et.al., 1973).

As there have been no reports in the literature of labeled microsphere measurements of regional flow in the cat, we undertook to validate the method in this animal. This entailed demonstration of adequate mixing of the microspheres before branching of the arterial tree. By calculating organ flows estimated from reference arterial samples withdrawn from a brachial artery and a femoral artery in a series of cats we were able to demonstrate close correlation between the two estimates, confirming satisfactory mixing. This was done not only in the most physiologic state encountered, i.e., anesthetized but with normal

Table 14

Reported Organ Flow Estimates
with Labeled Microspheres

Reference	1	2	3	4	5
Species	Rabbit	Rhesus	Rhesus	Rhesus	Rhesus
Organ			ml/min/100g Tissue		
Brain	102.8	74	70 ± 25	80 ± 28	75 ± 19
Heart	212.5	328	236 ± 82	324 ± 199	366 ± 142
Lungs	28.6	18	18 ± 12	18 ± 16	34 ± 25
Kidneys	349.1	795	466 ± 171	543 ± 183	1121 ± 544
Liver (Art)	23.1	51	90 ± 41	34 ± 28	41 ± 69
Gut					
Spleen	540.1	224	138 ± 83	267 ± 124	
Splanchnic	68.8	67	43 ± 15	90 ± 34	78 ± 30
Skin	6.39	23		16 ± 8	25 ± 5
Carcass	8.89			23 ± 6	
Stomach	66.1			64 ± 32	
Sm Int.	77.4			93 ± 38	
Lg Int.	64.5			91 ± 47	
Portal				108 ± 30	
Liver (total)	158.4			148 ± 23	
Muscle	7.24	14			29 ± 10

1. Neutze, Wyler and Rudolph, 1968.
2. Miller, Forsyth, Hoffbrand, and Melmon, 1973.
3. Hill, Lees, Malinow, Ochsner and Thomas, 1973.
4. Forsyth, Nies, Wyler, Neutze and Melmon, 1968.
5. Amory, Steffenson and Forsyth, 1971.

Table 15

Reported Organ Flow Estimates
with Labeled Microspheres or Rb^{86}

Reference	1	2	3	4	5	6	6
Species	Rat	Rabbit	Rhesus	Rhesus	Rhesus	Dog	Dog
Organ	Per Cent Cardiac Output						
Brain	1.2 ± 0.31	1.2	6.0 ± 2.2	5.4	6.5 ± 2.0	3.7 ± 2.9	5.9 ± 2.7
Heart	2.9 ± 0.43	3.1	6.2 ± 2.2	4.9	5.2 ± 1.4	3.8 ± 1.2	
Lungs	3.0 ± 0.37	2.6	0.9 ± 0.5	0.9	0.5 ± 0.6	2.6 ± 1.7	17.4 ± 5.6
Kidneys	17.8 ± 2.2	16.2	13.5 ± 4.3	15.7	12.3 ± 3.1	13.3 ± 4.4	13.9 ± 3.6
Liver (Art)	6.7 ± 1.5	3.4	13.8 ± 3.7	4.8	4.6 ± 3.8	8.5 4.9	11.4 ± 7.4
Gut	18.5 ± 2.7						
Spleen	1.1 ± 0.42	1.8	0.8 ± 0.7	2.5	1.9 ± 1.2	2.1 ± 0.8	7.4 ± 2.p
Splanchnic	26.3	24.2	7.0 ± 2.3		11.0 ± 4.1		
Skin	8.3 ± 2.0	7.4		7.8	4.8 ± 2.4		
Carcass	44.3 ± 5.1	39.6			50.8 ± 8.7		
Stomach		3.4		1.1	1.2 ± 0.5		
Sm Int.		11.5		3.1	4.0 ± 1.4		
Lg Int.		3.6		3.4	4.4 ± 2.0		
Portal		26.0			14.9 ± 4.8		
Liver, total		29.4			19.4 ± 5.4		
Muscle		17.2		25.4			

1. Sapirstein, 1960.
2. Neutze, Wyler, and Rudolph, 1968.
3. Hill, Lees, Malinow, Oshsner and Thomas, 1973.
4. Hoffbrand and Forsyth, 1969.
5. Forsyth, Nies, Wyles, Neutze and Melan, 1968.
6. Kaihara, VanHeerden, Migita and Wagner, 1968.

hematocrit and normoxia, but also in the anemic and hypoxic states. Fifteen micron beads showed uniform mixing in controls, 25 micron beads showing somewhat less uniform mixing. Overall difference between the two reference site estimates was 7.2 ± 8.2%. In the anemic state the mixing was improved for 25 micron beads. While hypoxia did not adversely effect the 15 micron bead mixing, it did significantly impair the mixing of the 25 micron beads. As expected, the single worse-case study of combined severe hypoxia and severe anemia resulted in a 21.4% difference in the respective flow estimates. Of course this condition was not one of the experimental states studied.

Because of financial constraints it was necessary to utilize both 15 micron and 25 micron beads in this study. Using simultaneous injections of these two sizes allowed determination of the effect of bead size on the flow estimate. These effects probably represent a worse case than the injection of a normal dose of a single size bead, as the hemodynamic effects of the injection of a double dose are more severe than those of a single dose injection. Nonetheless, in the normal control state, percent differences in flow estimates ranged from 5.97% to -13.2%, averaging -2.9%, brachial to femoral comparison. In hypoxia this range narrowed to 1.1% to 9.46%, averaging 5.56%. Thus, utilization of two bead size populations contributed little to decreased accuracy.

The influence of the magnitude of flow on the accuracy of the flow estimate was studied by detailed analysis of the flow to the brain, chosen because of its small total magnitude of flow, its early take-off from the aorta, and its general importance in regional flow

studies. These analyses showed the method to be satisfactory over a broad range of flows, generally most accurate with 15 micron beads and least accurate with 25 micron beads and hypoxia. Marcus et.al. (1976) have recently studied cerebral blood flow in the dog using microspheres ranging from 7-50 microns in diameter. They concluded that 15 micron spheres were most accurate, with less than 2% shunting Their study also revealed a 25% reduction in cerebral blood flow, attributed to anesthesia with chloralose-urethane. This anesthetic technique did not appear to reduce cerebral vascular reactivity, however.

Generally, the labeled microsphere method in the cat appears to be valid in a broad range of flows, in hypoxia, and in anemia, when the kinetics of the circulation can be expected to be significantly altered from the control state.

The microsphere method of determining regional flows has been criticized because of its failure to measure shunted organ flows. This criticism does not seem valid if one uses a bead size of about 15 microns as the shunted beads will have not passed through capillaries and thus represent blood flow to an organ that was not available for metabolic use by the organ. It can thus be argued that, by using the appropriate sized bead, the measured flow represents 'metabolic' flow, unbiased by the usually-measured shunt flow which comlicates interpretation of most organ flow measurements.

The determinations of control flows, with normal hematocrit and normal arterial oxygen tension, in our study compare well with previously reported values in the literature for other species. This can be seen by comparing mean values from cited studies with values

from this study, as compiled in Table 16. Mean value for brain flow for the rat and rabbit were used, the cerebral flows in monkeys not being comparable as a percent of cardiac output.

Influence of Acute Anemia, Hypoxia, and Aortic Body Denervation on Regional Blood Flow and Cardiac Output in the Cat, Preliminary Studies

Hypoxia resulted in increased flow in the brain and the heart but essentially no change in renal blood flow. While the percent of cardiac output to the brain and kidneys showed little change the percent of cardiac output to coronary arteries increased significantly. The cardiac index showed little change with hypoxia, increasing slightly. While liver arterial flow was unchanged by hypoxia, the flow to all other visceral organs, muscle, and skin were decreased with induction of hypoxia. The percent of cardiac output distributed to these organs was also decreased. These findings, although based on a small number of experiments, are in keeping with the findings of others.

Aortic denervation and hypoxia resulted in a decrease in the cardiac index, a decrease in brain flow towards control, variable changes in coronary flow (closely related to the changes in cardiac index in each animal), and reductions in flow to all other organs except the liver, whose arterial flow remained little changed. Accompanying these changes were an increase in the percent cardiac output to the brain and liver, a decrease to the kidneys and spleen, and little change in distributional percentage of cardiac output to other organs. Of interest is the relationship between coronary flow and cardiac index in hypoxia as compared to the control relationship, as

Table 16

Comparison of Organ Blood Flows

	Literature[1]		This Study	
Organ	Mean C.O.	ml/min/ 100g	Mean C.O.	ml/min/ 100g
Brain	1.2	--	2.0 ± 1.2	31.6 ± 10.1
Heart	4.2	293	4.6 ± 1.4	164.2 ± 52.5
Kidneys	14.2	686	22.8 ± 5.8	492.8 ± 155.1
Liver (A)	7.6	44.5	8.0 ± 2.5	31.6 ± 9.9
Spleen	2.51	310	3.3 ± 1.6	132.3 ± 68.8
Stomach	1.9	65	1.1 ± 0.5[2]	15.7 ± 11.6[2]
Sm Int.	11.5	85	6.8 ± 2.6	39.9 ± 31.5
Lg Int.	3.6	77	2.2 ± 0.7	49.8 ± 22.6
Liver (A&V)	29.4	155	23.3 ± 4.2	91.1 ± 29.6
Muscle	21.3	19	20.4 ± 8.4	4.0 ± 2.7

1. Data averaged from references listed in Tables
2. Data combined stomach and esophagus.

shown in Figure 12. The effects of aortic denervation on two of these cats is also shown. It appears that one animal was able to compensate for the severe hypoxia with an increase in cardiac output: The other decompensated with hypoxia, but recovered normally with increase in the Pa_{O_2}.

Induction of acute anemia resulted in increased flows to the brain, heart, kidneys, liver, and all other visceral organs except the spleen. Muscle flow was essentially unchanged and skin flows were increased. There were increased percentages of cardiac output to the brain, heart, abdominal organs except the spleen, and to the skin. There was a decreased fraction of cardiac output to the spleen, and little change to muscle.

Of note is the large increase in lung trapping of beads with hypoxia and the very small lung trapping in anemia, suggesting large increases in systemic shunts with hypoxia and reduction of these shunt flows with anemia, when compared to the control state. This increase in shunt flows with hypoxia could serve to raise the mixed venous P_{O_2}, thus indirectly raising Pa_{O_2}, to the benefit of critical organs. In anemia, the decrease in shunts may reflect the channeling of most systemic flow through metabolic capillaries. Shunting in anemia, with normal Pa_{O_2}, would serve no purpose.

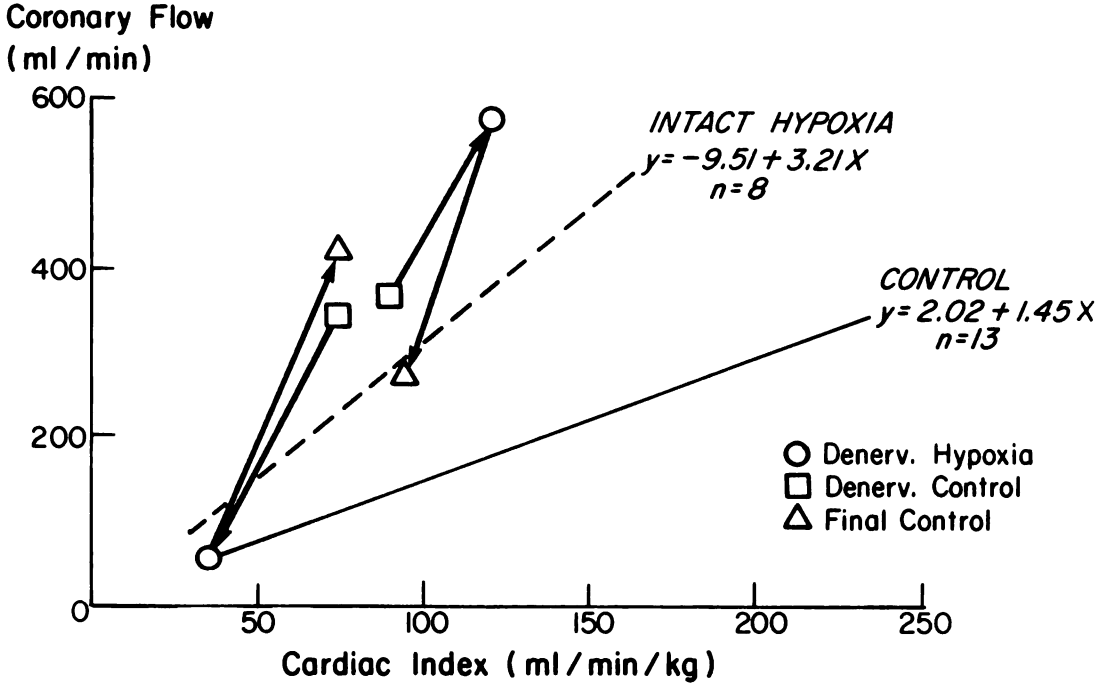
Aortic denervation in the anemic cat markedly reduced the elevation in cardiac output caused by anemia. There were resultant decreases in brain, heart, renal, visceral, and skin flows compared to controls. Muscle flow was little changed. Following aortic denervation the percent of cardiac output to the kidneys, liver, stomach

Figure 12

Least squares linear regression equations for the lines relating the change in coronary flow per 100 g of heart tissue to the cardiac index is shown for control measurements and during hypoxia in cats with intact chemoreceptors. Individual data are presented for two cats which underwent chemoreceptor denervation. Arrows depict temporal relationship of experimental states.

Figure 12

RELATIONSHIP OF CORONARY FLOW TO CARDIAC INDEX IN HYPOXIA



and esophagus, and skin were decreased. Only coronary flow received a greater percentage while percentage to brain, small and large bowel, spleen, and muscle remained essentially unchanged.

One to two hours after aortic denervation in anemic animals cardiac index returned towards normal and flows to brain, heart, liver, stomach, bowel, spleen and muscle increased over control flows. Skin flows continued decreased. Coincident with increased flows was a decreased fraction of cardiac output to the brain, heart, liver, muscle, and skin. Only the spleen received an increased percentage of the cardiac output.

The relationship of cardiac index to coronary flow in the anemic cat compared to the intact cat is demonstrated in Figure 13. The effect of aortic denervation of two animals is also shown. The lesser slope of this relationship in acute anemia compared to hypoxia is probably the result of a lower peripheral vascular resistance in anemia and lower viscosicity of the blood, both of which would tend to decrease the coronary flow required to generate unit cardiac index when compared to the hypoxic state, with its increase in peripheral vascular resistance and normal blood viscosity. This corresponds with the clinically apparent ability of patients to tolerate severe anemia better than severe hypoxia.

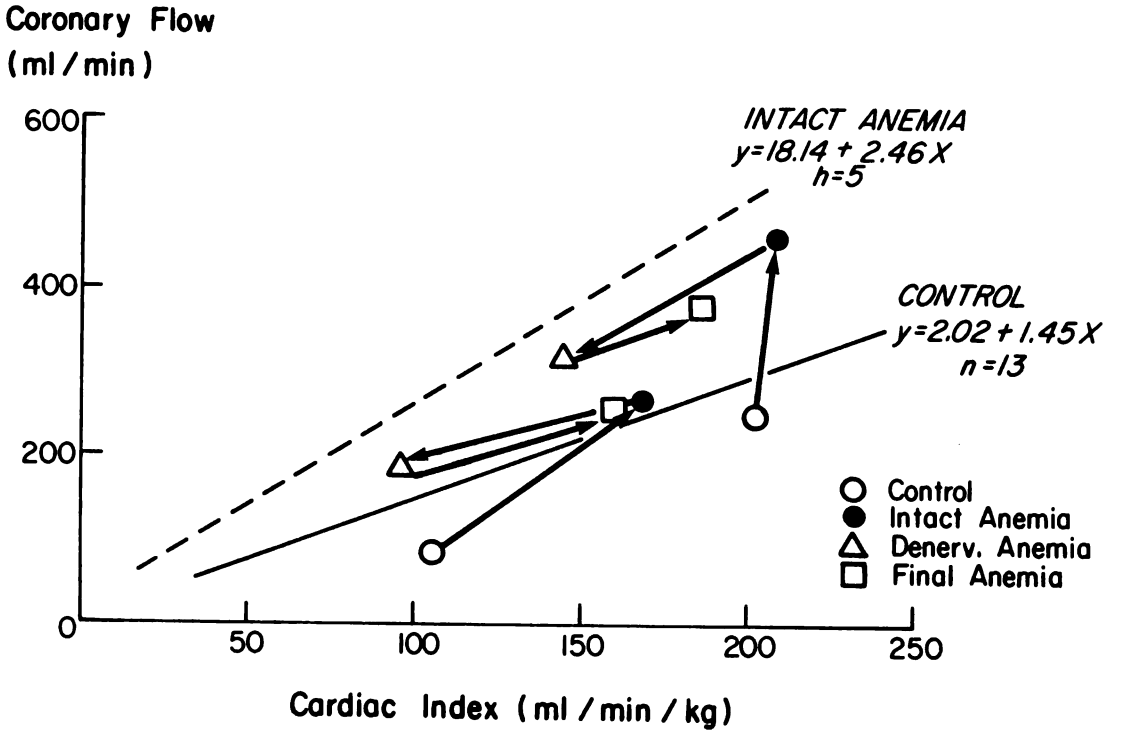
The reduction in cardiac output following aortic chemoreceptor denervation is in keeping with other evidence that the sympathetic nervous system is important in the early increase in the cardiac output after acute anemia is induced. The later increase in the cardiac output in the denervated animal is also consistent with the observa-

Figure 13

Least squares linear regression equations for the lines relating the change in coronary flow per 100 g of heart tissue to the cardiac index is shown for control measurements and after production of acute anemia in cats with intact chemoreceptors. Individual data are presented for two cats which underwent chemoreceptor denervation. Arrows depict temporal relationship of experimental states.

Figure 13

RELATIONSHIP OF CORONARY FLOW TO CARDIAC INDEX IN ANEMIA



tions by Hatcher et.al. (1963); Hatcher and Jennings (1966); Justus, Cornett and Hatcher (1957); and Chiong and Hatcher (1965), that a humoral substance plays an important role in maintenance of the cardiac output in anemia 1-3 hours after its production by Dextran-for-blood exchange transfusion.

Although the number of animals is small and the usual large variations in organ blood flows were seen, these preliminary data suggest the aortic bodies may play a significant role in the cardiovascular adjustments of the cat, not only to hypoxia, but to acutely induced anemia as well.

Our data is consistent with the hypothesis that carotid and aortic chemoreceptors detect acute anemia by the effect of reduced arterial oxygen content on chemoreceptor neural activity. This effect is pronounced in the aortic bodies and significantly less in the carotid bodies. In preliminary studies aortic denervation is followed by marked reductions in coronary flows and cardiac index which recover in several hours. This suggests that aortic chemoreceptors, acting through the sympathetic nervous system, are important in early cardiovascular adjustments to anemia. The role of the aortic bodies in hypoxia is not clear from these studies and larger numbers of animals are necessary for interpretation of the data.

Although we have shown aortic and, at times, carotid chemoreceptors 'sense' anemia we could find no evidence that chemoreceptors play any role in control or synthesis of erythropoietin. They may, however, exert significant indirect effects through their influence on Pa_{O_2} and renal blood flow in hypoxia and in anemia.

APPENDIX

Table A-1

Validation Study
M002
Organ Blood Flows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia, Anemia 15μ	Hypoxia, Anemia 25μ
L Kidney	292.26	304.40	358.52	187.09
R Kidney	430.09	419.86	326.29	254.88
L&R Kidneys	366.58	366.66	341.14	223.64
Brain	52.25	53.76	106.35	80.84
Heart	48.85	60.82	590.68	361.58
L Lung	135.13	150.78	13.35	4.18
R Lung	113.68	125.42	9.00	3.33
L&R Lungs	121.44	134.59	10.57	3.64
Liver	29.51	33.95	47.26	46.24
Liver A&V	56.50	61.52	63.16	69.09
Spleen	63.69	69.29	32.53	15.40
Sto/Eos	9.68	9.77	7.29	7.76
Sm Int	20.08	20.90	38.69	39.21
Lg Int	47.06	50.02	32.05	21.60
Omentum	2.32	2.47	1.54	1.62
Non-Liv Gut	8.94	9.38	10.64	10.20
Skin	1.46	1.45	0.90	1.01
Femur	3.18	3.52	1.15	0.60
Abd Musc	2.02	2.08	1.41	1.73
Hip Musc	2.62	2.76	0.47	0.53
Total Carc	3.61	3.75	2.38	2.03

Table A-2

Validation Study
M002
Organ Blood Flows, ml/min/100g Tissue
Brachial Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia, Anemia 15μ	Hypoxia, Anemia 25μ
L Kidney	347.73	312.70	434.92	227.15
R Kidney	511.72	431.30	395.82	309.47
L&R Kidneys	436.16	376.65	413.83	271.54
Brain	62.16	55.23	129.01	998.16
Heart	58.12	62.48	716.55	439.02
L Lung	160.78	154.89	16.19	5.07
R Lung	135.26	128.84	10.92	4.04
L&R Lungs	144.49	138.26	12.83	4.41
Liver	35.11	34.88	57.33	56.14
Liver A&V	10.64	9.64	12.91	12.39
Spleen	75.73	71.18	39.46	18.70
Sto/Eos	11.52	10.04	8.85	9.42
Sm Int	23.89	21.47	46.93	47.60
Lg Int	55.99	51.38	38.87	26.23
Omentum	2.76	2.54	1.87	1.97
Non-Liv Gut	10.64	9.64	12.91	12.39
Skin	1.74	1.49	1.09	1.22
Femur	3.78	3.61	1.39	0.73
Abd Musc	2.40	2.14	1.71	2.11
Hip Musc	3.12	2.83	0.58	0.64
Total Carc	4.30	3.85	2.89	2.46

Table A-3

Validation Study
M002
Organ Blood Flows, % Cardiac Output
Femoral Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia, Anemia 15μ	Hypoxia, Anemia 25μ
L Kidney	10.32	10.29	7.96	11.67
R Kidney	17.78	16.61	12.69	12.43
L&R Kidneys	28.10	26.90	20.65	24.10
Brain	4.30	4.23	8.02	8.07
Heart	2.08	2.48	18.58	23.22
L Lung	4.02	4.29	0.15	0.37
R Lung	5.96	6.30	0.21	0.44
L&R Lungs	9.98	10.59	0.36	0.80
Liver	6.13	6.75	11.57	9.04
Liver A&V	15.87	17.59	24.86	17.38
Spleen	1.37	1.42	0.40	0.64
Sto/Eos	0.99	0.96	0.96	0.69
Sm Int	3.80	3.79	8.94	6.75
Lg Int	2.98	3.03	1.65	1.87
Omentum	1.61	1.64	1.35	0.99
Non-Liv Gut	9.38	9.42	12.90	10.29
Skin	3.50	3.33	2.91	1.99
Total Muscle	17.41	17.48	7.25	4.76
Total Carc	33.88	33.65	22.93	20.56

Table A-4

Validation Study
M003
Organ Blood Flows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Control 25μ	Control 25μ	Hypoxia, 25μ	Hypoxia, 15μ	Anemia 25μ
L Kidney	256.50	218.21	277.98	281.72	186.99
R Kidney	269.85	236.76	278.18	285.04	178.47
L&R Kidnesy	263.12	227.35	278.08	283.35	182.80
Brain	23.98	23.80	50.29	54.71	40.49
Heart	162.47	169.58	543.99	573.83	258.43
L Lung	14.95	19.38	95.50	112.12	29.22
R Lung	10.33	9.20	74.27	81.87	29.04
L&R Lungs	11.81	12.46	81.06	91.62	29.10
Liver	26.40	24.26	34.04	35.74	28.41
Liver A&V	74.81	69.00	79.90	83.15	84.51
Spleen	141.86	137.25	53.43	60.76	78.11
Sto/Eos	11.61	10.69	13.66	14.07	16.45
Sm Int	39.61	34.97	48.05	48.15	57.93
Lg Int	41.17	36.54	43.43	44.74	58.92
Omentum	9.51	9.69	10.87	11.65	12.25
Non-Liv Gut	32.63	30.16	30.41	31.41	37.94
Skin	3.93	3.56	4.16	4.16	5.63
Femur	0.31	0.34	0.72	0.64	0.31
Abd Musc	3.33	3.44	4.12	4.27	4.83
Hip Musc	11.25	1.26	1.25	1.41	3.19
Total Carc	5.69	5.79	6.77	7.05	5.08

Table A-5

Validation Study
M003
Organ Blood Flows, ml/min/100g Tissue
Brachial Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia, 25μ	Hypoxia, 15μ	Anemia 25μ
L Kidney	288.99	223.09	354.77	312.51	197.33
R Kidney	303.92	242.06	355.03	316.20	188.35
L&R Kidneys	296.34	232.42	354.90	314.33	192.91
Brain	27.01	24.33	64.19	60.69	42.73
Heart	182.98	173.37	694.26	636.56	272.72
L Lung	16.84	19.82	121.88	124.61	30.83
R Lung	11.64	9.41	94.79	90.82	30.65
L&R Lungs	13.30	12.74	103.45	101.63	30.71
Liver	29.74	24.81	43.44	39.65	29.98
Liver A&V	84.25	70.54	101.97	92.24	89.18
Spleen	159.77	140.33	68.19	67.40	82.43
Sto/Eos	13.07	10.93	17.43	15.61	17.36
Sm Int	44.61	35.76	61.33	53.42	61.14
Lg Int	46.37	37.36	55.30	49.63	62.19
Omentum	10.71	9.90	13.87	12.92	12.93
Non-Liv Gut	36.75	30.84	38.81	34.84	40.03
Skin	4.43	3.64	5.31	4.61	5.94
Femur	0.34	0.35	0.92	0.71	0.33
Abd Musc	3.75	3.52	5.26	4.74	5.10
Hip Musc	1.41	1.28	1.60	1.56	3.36
Total Carc	6.41	5.92	8.64	7.82	5.36

Table A-6

Validation Study
M003
Organ Blood Flows, % Cardiac Output
Femoral Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia 25μ	Hypoxia 15μ	Anemia 25μ
L Kidney	15.16	13.66	12.65	12.29	11.13
R Kidney	15.49	14.40	12.29	12.08	10.32
L&R Kidneys	30.65	28.06	24.94	24.37	21.45
Brain	1.05	1.11	1.70	1.77	1.79
Heart	4.46	4.93	11.50	11.63	7.15
L Lung	0.58	0.80	2.87	3.24	1.15
R Lung	0.86	0.81	4.74	5.02	2.43
L&R Lungs	1.44	1.61	7.61	8.25	3.58
Liver	8.93	8.70	8.87	8.93	9.68
Liver A&V	23.45	22.91	19.28	19.24	26.68
Spleen	4.41	4.52	1.28	1.39	2.45
Sto/Eos	0.79	0.77	0.72	0.71	1.13
Sm Int	6.27	5.86	5.85	5.63	9.23
Lg Int	1.64	1.55	1.33	1.32	2.37
Omentum	1.40	1.51	1.23	1.27	1.82
Non-Liv Gut	14.52	14.22	10.42	10.32	17.00
Skin	5.25	5.04	4.28	4.09	7.56
Total Musc	8.58	9.29	7.43	7.67	12.00
Total Carc	31.61	34.10	28.96	28.90	28.44

Table A-7
Validation Study
M004
Organ Blood Flows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia 25μ	Hypoxia 15μ	Anemia 15μ
L Kidney	155.47	139.01	141.68	113.85	202.07
R Kidney	153.07	142.78	166.37	161.77	183.84
L&R Kidneys	154.26	140.92	144.26	138.07	192.86
Brain	29.02	25.76	53.58	50.35	58.04
Heart	184.45	198.52	273.60	297.68	449.52
L Lung	3.41	5.50	14.28	18.24	3.70
R Lung	2.40	4.53	10.55	13.24	2.95
L&R Lungs	2.83	4.94	12.12	15.34	3.26
Liver	21.53	20.90	10.19	11.48	16.88
Liver A&V	85.37	99.67	39.47	36.46	99.67
Spleen	103.17	191.64	17.99	20.48	130.14
Sto/Eos	18.66	18.73	9.40	7.68	29.12
Sm Int	40.97	39.11	23.01	18.23	61.18
Lg Int	44.53	43.83	32.94	30.83	47.99
Omentum	6.80	6.62	4.88	4.23	15.49
Non-Liv Gut	28.38	27.26	13.02	11.11	36.81
Skin	2.95	2.98	2.50	2.37	4.34
Femur	2.54	2.51	1.92	1.81	1.23
Abd Musc	4.65	5.34	4.61	5.24	5.91
Hig Musc	5.71	6.07	4.15	4.53	7.60
Total Carc	8.79	9.09	8.49	9.64	7.75

Table A-8
Validation Study
M004
Organ Blood Fows, ml/min/100g Tissue
Brachial Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia 25μ	Hypoxia 15μ	Anemia 15μ
L Kidney	180.48	136.90	115.09	118.14	188.17
R Kidney	177.69	140.61	157.35	167.72	171.18
L&R Kidneys	179.07	138.77	136.45	143.15	179.59
Brain	33.68	25.37	50.68	52.20	54.15
Heart	214.12	193.53	238.78	308.63	418.59
L Lung	3.96	5.42	13.50	18.91	3.44
R Lung	2.79	4.46	9.98	13.73	2.75
L&R Lungs	3.28	4.86	11.46	15.91	3.04
Liver	25.00	20.58	9.64	11.90	15.72
Liver A&V	99.10	80.96	37.80	80.96	92.81
Spleen	235.85	188.72	17.01	21.23	121.18
Sto/Eos	21.66	18.44	8.89	7.96	27.18
Sm Int	47.56	38.51	21.76	18.90	56.87
Lg Int	51.69	43.17	31.16	31.96	44.68
Omentum	7.90	6.52	4.62	4.39	14.42
Non-Liv Gut	32.95	26.85	12.31	11.52	34.28
Skin	3.43	2.93	2.36	2.46	4.05
Femur	2.95	2.47	1.82	1.88	1.14
Abd Musc	5.40	5.26	4.36	5.44	5.50
Hip Musc	6.63	5.98	3.93	4.69	7.08
Total Carc	10.21	8.96	8.03	10.00	7.21

Table A-9
Validation Study
M004
Organ Blood Flows, % Cardiac Output
Femoral Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia 25μ	Hypoxia 15μ	Anemia 15μ
L Kidney	8.21	7.34	7.33	6.46	9.45
R Kidney	8.26	7.70	10.24	9.37	8.79
L&R Kidneys	16.48	15.04	17.58	15.83	18.24
Brain	0.9[illegible]	0.83	1.98	1.75	1.67
Heart	4.40	4.68	7.44	7.62	9.49
L Lung	0.16	0.26	0.77	0.92	0.15
R Lung	0.16	0.29	0.78	0.92	0.17
L&R Lungs	0.32	0.55	1.55	1.85	0.32
Liver	5.74	5.57	3.10	3.23	3.99
Liver A&V	22.77	21.92	12.01	10.44	23.54
Spleen	4.94	4.65	0.50	0.53	2.80
Sto/Eos	1.25	1.25	0.72	0.55	1.73
Sm Int	6.92	6.60	4.43	3.31	9.13
Lg Int	1.90	1.87	1.61	1.41	1.82
Omentum	2.02	1.97	1.65	1.35	4.07
Non-Liv Gut	17.03	16.35	8.91	7.16	19.55
Skin	5.18	5.14	4.43	4.96	6.69
Total Musc	25.45	27.71	23.48	24.45	29.57
Total Carc	56.52	48.10	51.26	54.78	36.29

Table A-10

Validation Study
M005
Organ Blood Fows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia 25μ	Hypoxia 15μ	Anemia 15μ
L Kidney	185.78	191.96	166.41	187.86	181.76
R Kidney	187.25	186.59	176.40	188.82	235.38
L&R Kidneys	186.50	189.33	171.30	188.33	208.00
Brain	25.73	31.02	24.49	37.87	32.66
Heart	156.36	192.56	168.03	213.26	243.22
L Lung	134.86	177.09	225.53	291.13	157.92
R Lung	226.94	243.48	283.14	341.15	395.66
L&R Lungs	187.83	215.28	258.67	319.91	294.69
Liver	35.58	39.87	24.45	27.56	38.73
Liver A&V	109.64	122.50	75.03	81.63	97.39
Spleen	127.29	148.60	49.83	34.32	34.91
Sto/Eos	14.75	7.17	10.90	5.09	13.72
Sm Int	28.67	32.26	22.71	27.21	30.61
Lg Int	47.20	58.07	35.91	45.45	38.72
Omentum	6.04	7.19	5.07	5.45	5.24
Non-Liv Gut	26.42	29.48	18.04	19.29	20.92
Skin	1.98	2.04	1.69	1.68	3.64
Femur	3.94	5.30	2.67	3.65	0.29
Abd Musc	2.42	2.98	1.56	2.07	2.43
Him Musc	1.54	2.10	1.12	1.22	0.64
Total Carc	4.72	5.88	3.75	4.42	3.14

Table A-11

Validation Study
M005
Organ Blood Flows, ml/min/100g Tissue
Brachial Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia 25μ	Hypoxia 15μ	Anemia 15μ
L Kidney	202.16	187.04	171.61	171.22	173.21
R Kidney	203.76	181.80	181.91	172.09	224.33
L&R Kidneys	202.94	184.48	176.65	171.64	198.24
Brain	28.00	30.22	25.26	25.40	31.13
Heart	170.14	187.62	173.28	194.36	231.80
L Lung	146.74	172.53	232.58	265.33	150.50
R Lung	246.92	237.24	291.99	310.92	377.08
L&R Lungs	204.39	209.76	266.76	291.56	280.85
Liver	38.71	38.84	25.21	25.11	36.92
Liver A&V	119.31	119.36	77.37	74.39	92.89
Spleen	138.51	144.79	51.39	31.28	33.27
Sto/Eos	16.06	6.98	11.24	4.63	13.08
Sm Int	31.19	31.43	23.42	24.80	29.17
Lg Int	51.37	56.58	37.03	41.42	36.90
Omentum	6.57	7.01	5.23	4.97	5.00
Non-Liv Gut	28.75	28.72	18.61	17.58	19.94
Skin	2.15	1.99	1.74	1.53	3.47
Femur	4.29	5.16	2.76	3.33	0.27
Abd Musc	2.63	2.90	1.61	1.88	2.32
Hip Musc	1.67	2.05	1.15	1.11	0.61
Total Carc	5.14	5.73	3.86	4.03	2.99

Table A-12

Validation Study
M005
Organ Blood Fows, % Cardiac Output
Femoral Artery Reference Site

Organ(s)	Control 25μ	Control 15μ	Hypoxia 25μ	Hypoxia 15μ	Anemia 25μ
L Kidney	11.69	10.56	11.99	11.78	11.28
R Kidney	11.29	9.84	12.18	12.35	14.00
L&R Kidneys	22.98	20.39	24.17	23.12	25.29
Brain	1.44	1.51	1.57	1.55	1.80
Heart	5.39	5.80	6.63	7.32	8.27
L Lung	2.78	3.19	5.32	5.98	3.21
R Lung	6.34	5.94	9.06	9.49	10.90
L&R Lungs	9.12	9.14	14.38	15.47	14.11
Liver	7.87	7.71	6.20	6.08	8.46
Liver A&V	24.08	23.51	18.86	17.86	21.10
Spleen	4.23	4.32	1.90	1.14	1.14
Sto/Eos	1.15	0.49	0.98	0.40	1.06
Sm Int	6.70	6.59	6.08	6.34	7.06
Lg Int	3.03	3.26	2.64	2.91	2.45
Omentum	1.27	1.33	1.23	1.15	1.09
Non-Liv Gut	16.40	15.99	12.82	11.93	12.81
Skin	3.59	3.23	3.51	3.04	6.52
Total Musc	12.15	13.84	9.16	9.89	9.68
Total Carc	30.87	33.62	38.04	28.82	20.24

Table A-13

Hypoxia and Aortic Body Denervation
M007
Organ Blood Fows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Intact Control 25μ	Intact Hypoxia 15μ	Dener. Control 25μ	Dener. Hypoxia 25μ	Final Control 15μ
L Kidney	307.92	372.82	350.60	287.08	221.50
R Kidney	275.42	358.07	309.13	203.72	330.97
L&R Kidneys	292.46	365.80	330.87	247.43	221.25
Brain	24.71	35.76	30.82	53.64	32.83
Heart	168.70	390.03	361.01	572.37	270.65
L Lung	100.55	273.48	229.53	173.09	157.85
R Lung	79.88	135.66	136.78	110.32	93.89
L&R Lungs	85.43	172.62	161.65	127.16	111.04
Liver	50.80	62.36	51.71	46.04	35.85
Liver A&V	92.48	117.10	108.83	95.24	72.13
Spleen	22.81	35.49	44.43	41.11	33.58
Sto/Eos	9.91	12.41	14.60	10.45	9.81
Sm Int	27.91	36.29	38.44	32.81	22.46
Lg Int	31.40	39.57	37.82	33.30	30.25
Omentum	2.80	3.99	3.69	3.61	2.28
Non-Liv Gut	12.41	16.30	17.01	14.66	10.80
Skin	2.37	2.80	3.00	3.70	2.33
Femur	4.59	5.94	4.71	2.70	2.37
Abd Mus	2.93	5.11	4.30	4.24	3.23
Hip Mus	3.83	5.29	3.58	1.67	1.66
Total Carc	5.39	7.57	7.34	4.92	4.40

Table A-14

Hypoxia and Aortic Body Denervation
M007
Organ Blood Flows, % Cardiac Output
Femoral Artery Reference Site

Organ(s)	Intact Control 25μ	Intact Hypoxia 15μ	Dener. Control 25μ	Dener. Hypoxia 25μ	Final Control 15μ
L Kidney	12.57	10.56	10.61	10.16	10.04
R Kidney	10.20	9.20	8.48	6.54	9.09
L&R Kidneys	22.78	19.77	19.09	16.70	19.13
Brain	2.20	2.21	2.04	4.15	3.35
Heart	5.04	8.08	7.99	14.82	8.98
L Lung	3.33	6.29	5.63	4.97	5.81
R Lung	7.22	8.51	9.16	8.64	9.42
L&R Lungs	10.55	14.79	14.79	13.61	15.23
Liver	11.27	9.60	8.50	8.85	8.83
Liver A&V	20.51	18.02	17.89	17.77	18.02
Spleen	0.32	0.34	0.45	0.49	0.52
Sto/Eos	0.83	0.72	0.91	0.76	0.91
Sm Int	5.49	4.95	5.60	5.59	4.91
Lg Int	1.48	1.30	1.32	1.36	1.58
Omentum	1.13	1.12	1.10	1.26	1.02
Non-Liv Gut	9.25	8.43	9.36	9.47	8.94
Skin	3.49	2.86	3.28	4.73	3.81
Total Musc	34.48	35.14	27.26	20.38	22.80
Tot 1 Carc	31.82	30.99	32.11	25.18	18.84

Table A-15

Hypoxia and Aortic Body Denervation
M008
Organ Blood Fows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Intact Control 25μ	Intact Hypoxia 15μ	Dener. Control 25μ	Dener. Hypoxiz 25μ	Final Control 15μ
L Kidney	322.73	416.53	296.34	67.98	161.61
R Kidney	280.47	406.41	292.43	63.60	184.36
L&R Kidneys	301.71	411.49	294.39	65.80	172.92
Brain	30.21	52.70	65.08	38.83	61.72
Heart	233.83	461.60	340.12	53.90	419.56
L Lung	70.60	178.57	53.84	3.66	51.32
R Lung	50.07	112.73	36.59	2.63	34.37
L&R Lungs	57.89	137.82	43.17	3.02	40.83
Liver	34.42	46.96	41.85	25.52	36.88
Liver A&V	96.21	126.35	102.58	58.21	91.39
Spleen	154.20	59.44	91.15	9.72	33.67
Sto/Eos	10.31	11.53	8.95	7.31	10.32
Sm Int	25.84	40.51	26.24	18.02	26.98
Lg Int	32.80	52.02	45.43	22.09	43.02
Omentum	5.17	8.55	5.75	2.71	4.72
Non-Liv Gut	18.32	23.54	18.01	9.69	16.16
Skin	2.13	3.34	1.77	0.88	1.45
Femur	0.04	0.00	0.03	0.00	0.01
Abd Musc	19.46	26.44	16.85	10.97	16.08
Hip Musc	1.79	0.94	0.75	0.06	0.05
Total Carc	3.88	4.87	3.62	1.42	2.68

Table A-16

Hypoxia and Aortic Body Denervation
M008
Organ Blood Flows, % Cardiac Output
Femoral Artery Reference Site

Organ(s)	Intact Control 25μ	Intact Hypoxia 15μ	Dener. Control 25μ	Dener. Hypoxia 25μ	Final Control 15μ
L Kidney	13.51	12.06	12.04	7.31	8.00
R Kidney	11.62	11.64	11.76	6.77	9.03
L&R Kidneys	25.13	23.70	23.79	14.08	17.02
Brain	2.26	2.72	4.72	7.46	5.46
Heart	7.58	10.35	10.70	4.49	16.08
L Lung	2.99	5.23	2.21	0.40	2.57
R Lung	3.44	5.36	2.44	0.46	2.79
L&R Lungs	6.43	10.58	4.65	0.86	5.36
Liver	8.95	8.44	10.56	17.05	11.33
Liver A&V	25.03	22.71	25.88	38.87	28.09
Spleen	3.61	0.96	2.07	0.58	0.93
Sto/Eos	1.13	0.87	0.95	2.05	1.33
Sm Int	6.58	7.13	6.48	11.79	8.12
Lg Int	2.63	2.89	3.54	4.55	4.09
Omentum	2.12	2.42	2.28	2.85	2.28
Non-Liv Gut	16.07	14.27	15.32	21.83	16.75
Skin	3.08	3.34	2.48	3.25	2.48
Total Musc	27.33	20.50	19.08	27.48	18.26
Total Carc	27.84	24.16	25.23	26.21	22.76

Table A-17

Anemia and Aortic Body Denervation
M009
Organ Blood Flows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Intact Control 25μ	Intact Anemia 25μ	Dener. Anemia 25μ	Final Anemia 15μ
L Kidney	330.56	440.92	198.18	52.65
R Kidney	243.62	435.16	166.03	60.11
L&R Kidneys	336.79	438.17	182.84	56.21
Brain	35.92	49.47	41.07	32.28
Heart	87.13	132.58	205.94	138.81
L Lung	170.39	116.52	67.03	4.19
R Lung	212.17	121.66	79.00	8.81
L&R Lungs	194.45	119.48	73.92	6.85
Liver	45.82	76.25	47.23	22.73
Liver A&V	104.72	136.70	83.20	34.17
Spleen	103.38	8.57	13.31	5.44
Sto/Eos	19.92	17.25	9.52	2.03
Sm Int	30.64	45.46	24.76	9.11
Lg Int	24.20	22.07	18.65	3.13
Omentum	12.97	9.73	6.31	1.75
Non-Liv Gut	25.56	26.24	15.61	4.97
Skin	3.51	2.90	1.26	0.14
Femur	1.39	1.22	0.18	0.14
Abd Musc	1.36	3.41	1.72	1.13
Hip Musc	2.65	2.43	0.46	0.50
Total Carc	2.99	4.16	1.73	1.07

Table A-18

Anemia and Aortic Body Denervation
M009
Organ Blood Flows, % Cardiac Output
Femoral Artery Reference Site

Organ(s)	Intact Control 25μ	Intact Anemia 25μ	Dener. Anemia 25μ	Final Anemia 15μ
L Kidney	11.62	12.38	10.17	5.62
R Kidney	11.03	11.16	7.78	5.86
L&R Kidneys	22.65	23.54	17.95	11.47
Brain	3.40	3.75	5.68	9.29
Heart	3.64	6.10	12.56	17.61
L Lung	3.79	2.07	2.18	0.28
R Lung	6.40	2.93	3.48	0.81
L&R Lungs	10.19	5.00	5.66	1.09
Lieer	11.99	15.94	18.04	18.05
Liver A&V	27.39	28.47	31.78	27.14
Spleen	2.31	0.15	0.41	0.35
Sto/Eos	1.90	1.32	1.33	0.59
Sm Int	7.28	8.63	8.59	6.57
Lg Int	1.51	1.10	1.69	0.59
Omentum	2.41	1.45	1.72	0.99
Non-Liv Gut	15.41	12.64	13.74	9.09
Sking	6.04	3.98	3.17	0.72
Total Musc	26.04	30.19	20.25	41.57
Total Carc	23.41	26.00	19.72	25.46

Table A-19

Anemia and Aortic Body Denervation
M010
Organ Blood Flows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Intact Control 25μ	Intact Anemia 25μ	Dener. Anemia 25μ	Final Anemia 15μ
L Kidney	207.58	301.62	184.30	276.38
R.Kidney	205.71	293.69	158.02	264.07
L&R Kidneys	206.68	297.79	171.61	270.44
Brain	30.85	57.48	42.93	58.39
Heart	247.02	457.50	313.72	376.91
L Lung	9.02	12.25	7.84	9.99
R Lung	5.31	7.59	3.88	7.81
L&R Lungs	6.56	9.16	5.22	8.55
Liver	17.77	17.20	20.53	33.44
Liver A&V	164.32	181.73	124.89	138.67
Spleen	281.43	323.98	211.36	242.03
Sto/Eos	51.74	56.51	43.29	44.70
Sm Int	142.40	209.67	91.30	82.30
Lg Int	107.70	209.68	105.29	147.00
Omentum	71.86	61.78	49.75	43.51
Non-Liv Gut	113.63	110.69	80.92	81.59
Skin	4.15	5.63	4.92	3.91
Femur	5.26	4.45	2.03	2.81
Abd Musc	8.50	9.62	5.62	6.59
Hip Musc	7.79	4.83	4.14	3.38
Total Carc	13.92	1.41	7.41	10.83

Table A-20

Anemia and Aortic Body Denervation
M010
Organ Blood Flows, % Cardiac Output
Femoral Artery Reference

Organ(s)	Intact Control 25μ	Intact Anemia 25μ	Dener. Anemia 25μ	Final Anemia 15μ
L Kidney	7.28	10.27	9.01	10.51
R Kidney	6.73	9.33	7.21	9.37
L&R Kidneys	14.01	19.60	16.22	19.87
Brain	1.06	1.92	2.06	2.18
Heart	4.68	8.42	8.29	7.74
L Lung	0.15	0.20	0.18	0.18
R Lung	0.17	0.24	0.18	0.28
L&R Lungs	0.32	0.44	0.36	0.46
Liver	3.54	3.32	5.70	7.22
Liver A&V	32.72	35.14	34.68	29.94
Spleen	4.72	5.27	4.94	4.40
Sto/Eos	2.31	2.45	2.69	2.16
Sm Int	14.69	10.98	13.12	9.17
Lg Int	2.52	4.77	3.44	3.73
Omentum	4.96	4.14	4.79	3.27
Non-Liv Gut	29.18	27.60	28.98	22.72
Skin	3.06	4.03	5.06	3.13
Total Musc	29.48	28.73	23.59	19.19
Total Carc	39.96	31.81	29.68	33.73

Table 4-21

Anemia and Aortic Body Denervation
Organ Blood Flows, ml/min/100g Tissue
Femoral Artery Reference Site

Organ(s)	Intact Control 25μ	Intact Anemic 25μ	Dener. Anemic 25μ	Final Anemic 15μ
L Kidney	180.66	236.72	55.35	241.54
R Kidney	182.12	236.81	39.52	254.17
L&R Kidneys	181.41	236.77	47.25	248.00
Brain	24.25	49.99	45.80	53.38
Heart	81.37	262.54	181.47	254.84
L Lung	107.58	61.02	34.64	47.81
R Lung	77.53	39.31	24.27	32.18
L&R Lungs	85.59	45.14	27.05	36.37
Liver	30.06	23.47	42.57	24.33
Liver A&V	65.16	118.50	98.11	107.66
Spleen	69.90	285.12	149.62	308.90
Sto/Eos	10.75	40.38	22.25	32.33
Sm Int	35.41	88.49	42.80	71.69
Lg Int	82.95	160.72	147.92	142.14
Omentum	12.11	42.84	30.66	37.77
Non-Liv Gut	32.76	88.88	51.85	77.93
Skin	1.40	3.47	2.95	2.74
Femur	7.65	3.75	1.67	5.70
Abd Musc	3.17	5.82	3.27	4.68
Hip Musc	1.77	3.12	2.42	4.66
Total Carc	6.18	9.77	4.54	9.52

Table A-22

Anemia and Aortic Body Denervation
M011

Organ Blood Flows, % Cardiac Output
Femoral Artery Refernce

Organ(s)	Intact Control 25μ	Intact Anemia 25μ	Dener. Anemia 25μ	Final Anemia 15μ
L Kidney	11.21	9.27	3.76	9.89
R Kidney	11.85	9.72	2.81	10.91
L&R Kidneys	23.06	18.98	6.58	20.80
Brain	1.35	1.76	2.79	1.96
Heart	4.41	8.79	10.77	9.11
L Lung	3.09	1.11	1.09	0.91
R Lung	6.08	1.94	2.08	1.66
L&R Lungs	9.17	3.05	3.17	2.57
Livrr	11.47	5.64	17.77	6.12
Liver A&V	24.77	28.38	40.81	26.99
Spleen	1.60	4.11	3.75	4.66
Sto/Eos	0.67	1.59	1.52	1.33
Sm Int	7.26	11.44	9.61	9.70
Lg Int	2.77	3.38	5.41	3.13
Omentum	0.99	2.22	2.75	2.04
Non-Liv Gut	13.30	22.74	23.04	20.87
Skin	3.55	5.54	8.18	4.56
Total Musc	15.54	15.87	15.44	16.17
Total Carc	30.52	30.44	24.52	31.01

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