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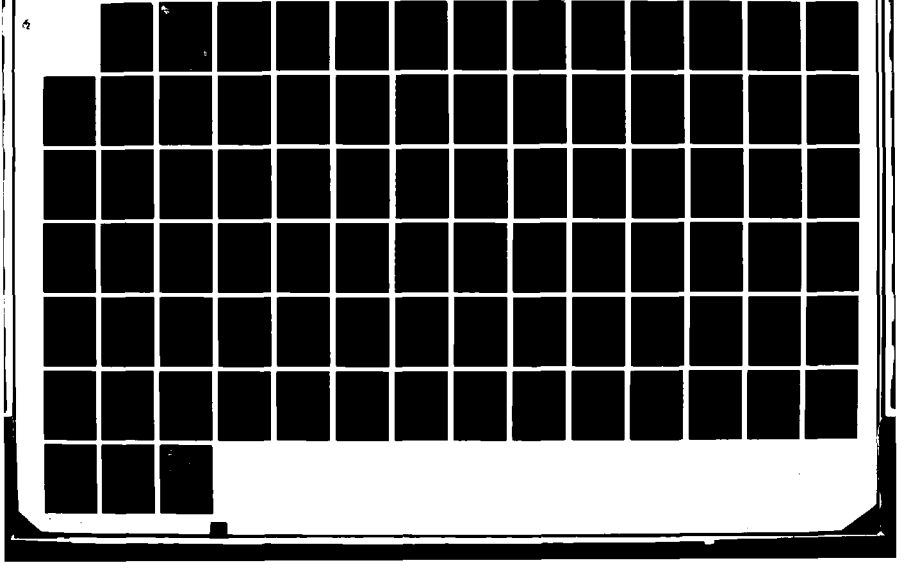

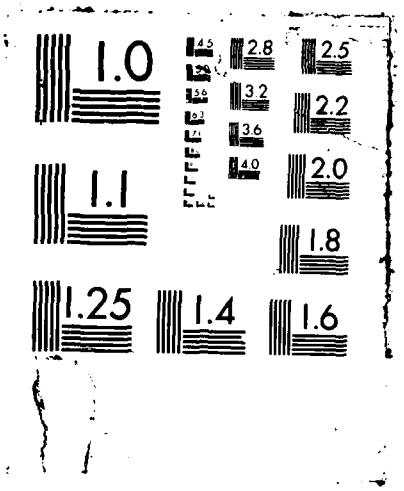
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Institute Report No. 242

The Blood Volume of the Guinea Pig: Effects of Epinephrine and Isoproterenol Upon the Red Cell and Plasma Volumes, Heart Rate, and Mean Arterial Pressure

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DIVISION OF MILITARY TRAUMA RESEARCH

SEPTEMBER 1987

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

The Blood Volume of the Guinea Pig: Effects of Epinephrine and Isoproterenol upon the Red Cell and Plasma Volumes, Heart Rate, and Mean Arterial Pressure--Millnamow

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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Edwin S Beatrice 30 Sept 87

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SECURITY CLASSIFICATION OF THIS PAGE

AD-A189403

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704 0188
Exp Date Jun 30, 1986

1a REPORT SECURITY CLASSIFICATION: Unclassified	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT: This document has been approved for public release and sale. Unlimited.
2b DECLASSIFICATION/DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S): Institute Report No. 242	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION: Letterman Army Institute of Research; 6b OFFICE SYMBOL (If applicable)	7a NAME OF MONITORING ORGANIZATION: US Army Medical Research and Development Command
6c ADDRESS (City, State, and ZIP Code): Division of Military Trauma Research, LAIR Presidio of San Francisco, CA 94129-6800	7b ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701
8a NAME OF FUNDING/SPONSORING ORGANIZATION; 8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c ADDRESS (City, State, and ZIP Code)	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO 61102A; PROJECT NO 3M1611-02BS10; TASK NO BA; WORK UNIT ACCESSION NO 256

11 TITLE (Include Security Classification)
Institute Report No. 242, The Blood Volume of the Guinea Pig: Effects of Epinephrine and Isoproterenol Upon the Red Cell and Plasma Volumes, Heart Rate, and Mean Arterial Pressure.

12 PERSONAL AUTHOR(S)
Gregory A. Millnamow, MA

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Institute Report	FROM N/A TO N/A	1987 September	74

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB GROUP	guinea pigs, splenectomy, epinephrine, isoproterenol, hematocrit, red cell volume, blood volume, heart rate, mean arterial pressure

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Following anesthetization with ketamine hydrochloride and xylazine hydrochloride, mature guinea pigs, both intact and splenectomized, were fitted with acute, indwelling carotid artery catheters. Autologous red blood cells were labeled with $Na_2{}^{51}CrO_4$ and reintroduced. Whole blood samples and heart rate (HR) and mean arterial pressure (MAP) measurements were taken at 5-minute intervals to determine basal circulating values. An intra-arterial injection of either epinephrine (0.15 mg/kg) or isoproterenol (0.15 mg/kg) was administered and measurements repeated at 2.5, 5, 10, 20, 40, and 80 minutes post injection. The gamma emissions of the labeled red cells were counted and the red cell volume (RCV) was deteremined. Plasma volume (PV) and whole blood volume (WBV) were calculated using the hematocrit. Basal averages of the intact group were: Hct = 38.6%; RCV = 17.5 ml/kg; PV = 29.2 ml/kg; WBV = 46.6 ml/kg; HR = 184 beats/min; MAP = 49.1 mm Hg. Basal averages of the splenectomized group were: Hct = 41.2%; RCV = 19.2 ml/kg; PV = 29.0 ml/kg; WBV = 48.2 ml/kg; HR = 155 beats/min; MAP = 47.5 mm Hg. Intra-arterial injection of epinephrine

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	
22a NAME OF RESPONSIBLE INDIVIDUAL: Gregory A. Millnamow	22b TELEPHONE (Include Area Code): (415) 561-5817
	22c OFFICE SYMBOL: SGRD UL MT

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

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produced significant increases in heart rate and mean arterial pressure and significant decreases in circulating plasma and whole blood volumes. Although the circulating red cell volume of the intact animals experienced a significant increase following administration of epinephrine, that of the splenectomized subgroup was not significantly altered. Intra-arterial injection of isoproterenol produced a significant increase in heart rate, circulating plasma and whole blood volumes and a significant decrease in mean arterial pressure and hematocrit. The circulating red cell volume of the intact subgroup was not significantly altered while that of the splenectomized subgroup experienced a significant decline. It may be concluded that sequestration, or mobilization, of red blood cells by the guinea pig spleen may significantly alter circulating blood volume and that epinephrine and isoproterenol may alter the circulating blood volume independently of splenic stimulation.

ABSTRACT

Following anesthetization with ketamine hydrochloride and xylazine hydrochloride, mature guinea pigs, both intact and splenectomized, were fitted with acute, indwelling carotid artery catheters. Autologous red blood cells were labeled with $Na_2{}^{51}CrO_4$ and reintroduced. Whole blood samples and heart rate (HR) and mean arterial pressure (MAP) measurements were taken at 5-minute intervals to determine basal circulating values. An intra-arterial injection of either epinephrine (0.15 mg/kg) or isoproterenol (0.15 mg/kg) was administered and measurements repeated at 2.5, 5, 10, 20, 40, and 80 minutes post injection. The gamma emissions of the labeled red cells were counted and red cell volume (RCV) was determined. Plasma volume (PV) and whole blood volume (WBV) were calculated using the hematocrit (Hct). Basal averages of the intact group were: Hct = 38.6%; RCV = 17.5 ml/kg; PV = 29.2 ml/kg; WBV = 46.6 ml/kg; HR = 184 beats/min; MAP = 49.1 mm Hg. Basal averages of the splenectomized group were: Hct = 41.2%; RCV = 19.2 ml/kg; PV = 29.0 ml/kg; WBV = 48.2 ml/kg; HR = 155 beats/min; MAP = 47.5 mm Hg. Intra-arterial injection of epinephrine produced significant increases in heart rate and mean arterial pressure and significant decreases in circulating plasma and whole blood volumes. Although the circulating red cell volume of the intact animals experienced a significant increase following administration of epinephrine, that of the splenectomized subgroup was not significantly altered. Intra-arterial injection of isoproterenol produced a significant increase in heart rate, circulating plasma and whole blood volumes and a significant decrease in mean arterial pressure and hematocrit. The circulating red cell volume of the intact subgroup was not significantly altered while that of the splenectomized subgroup experienced a significant decline. It may be concluded that sequestration, or mobilization, of red blood cells by the guinea pig spleen may significantly alter circulating blood volume and that epinephrine and isoproterenol may alter the circulating blood volume independently of splenic stimulation.

key words: guinea pigs, splenectomy, epinephrine, isoproterenol, hematocrit, red cell volume, blood volume, heart rate, mean arterial pressure

PREFACE

The research reported herein was conducted at Letterman Army Institute of Research, Presidio of San Francisco and was performed in partial fulfillment of the requirements for the degree Master of Arts in Biology: Physiology at San Francisco State University. All funding and resources were supported by the United States Army, Research and Development Command, Letterman Army Institute of Research.

ACKNOWLEDGMENTS

I thank Colonel Edwin S. Beatrice, MD, United States Army, Commander of Letterman Army Institute of Research, for the privilege of conducting my research and writing this report at the Institute; Lieutenant Colonel William G. Rodkey, DVM, United States Army, Chief of the Division of Military Trauma Research, for his technical advice; Virginia L. Gildengorin, Ph.D., for her gracious help with the statistical analysis of the data; Captain Stephen P. Bruttig, Ph.D., United States Army, for reviewing the scientific and technical content of this paper; and Sandra L. Young for her many helpful editorial suggestions.

Special thanks to John Patrick Hannon, Ph.D., for his support and example throughout the duration of this project; his sage humor distinguished his ability to impart the beauty of basic physiological research.

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INTRODUCTION

The common measures of *in vivo* whole blood volume (WBV) employ the dilution principle: the volume of distribution of a known indicator is equal to the injected dose (ID) divided by the concentration of the tracer after complete mixing. For example, erythrocytes labeled with radiochromium are commonly used to estimate circulating red cell volume (RCV) (1), and radioiodine-labeled human serum albumin (HSA) is commonly used to estimate plasma volume (PV) (2). Whole blood volume is usually calculated using one of three methods: measured red cell volume divided by the red cell concentration (hematocrit or Hct), measured plasma volume divided by the plasma concentration (1 - Hct), or the addition of the simultaneous independent measurements of red cell volume and plasma volume (3). Since plasma volume labels exchange with the extravascular space at the capillaries (4), blood volumes calculated from plasma volume measures must correct for label that has left the system between the time of the injected dose and sampling. Alternatively, direct measures of red cell volume avoid this methodological problem since erythrocytes are not lost from the circulation; hence, they have the same dilution space as blood, per se (5).

While the quantity of circulating blood varies primarily as a function of the intravascular plasma volume, mobilization of splenetically sequestered red cells is capable of causing significant changes in the circulating red cell volume in some animals, such as pigs (6) and dogs (7). The volume of red cells released into the circulation upon splenic contraction is a function of the degree of contraction and the capacity of the spleen to sequester red cells.

A search of the hematological literature reveals estimations of guinea pig blood volume calculated primarily from plasma volume measures and Hct. In addition, the capacity of the guinea pig spleen to sequester red cells has not been investigated. The study reported here was undertaken to assess the circulating whole blood volume of the guinea pig by direct measures of the red cell volume and hematocrit. The influence of splenic mobilization of sequestered erythrocytes upon the WBV was investigated following administration of the catecholamine epinephrine. In addition, the effect of cardiovascular stimulation, independent of splenic contraction, upon the circulating WBV was investigated following administration of the

catecholamine isoproterenol.

The spleen of *Cavia porcellus* (the guinea pig) is flat and oblong, convex on its dorsal surface with a hilus on the ventral surface for blood vessel and nerve access (8). The organ is encased in a connective tissue capsule and compartmentalized by thin-walled trabeculae of smooth muscle (9). The splenic microcirculation is anatomically specialized to rapidly filter red blood cells and is therefore considered a modified lymph gland (8). As the cells enter via the splenic artery, they undergo immunological screening for antigens by the lymphatic nodules of the "white pulp" which ensheathe the vessel. After passing through the white pulp, arterioles deposit the blood in the "red pulp," a reticulum of branched connective-tissue corpuscles, which the RBC's traverse as flow progresses towards the venous sinuses. The pulp cords filter the blood, with some of the cells getting shunted into slow circulation annuli where phagocytes remove dysfunctional erythrocytes and foreign matter. Some RBC's appear to be sequestered, at least temporarily, in the annuli, but the magnitude of sequestration relative to the total circulating RCV of the guinea pig is unknown.

Splenic sequestration and contraction are mediated by the autonomic nervous system and blood-borne agents (10). Sympathetic nerve fibers from the *truncus sympathicus* and parasympathetic neurons of the *nervus vagus* (cranial nerve X) innervate the celiac plexus (8, 11). A subdivision of the celiac plexus, the splenic plexus, follows the course of the splenic artery to the spleen where the splenic arterioles and venules are innervated (12). The neurotransmitters in these two systems differ at the postganglionic fiber-effector organ synaptic junctions: the sympathetic system's adrenergic fibers release norepinephrine, and the parasympathetic system's cholinergic fibers release acetylcholine. Norepinephrine is a member of the catecholamine family of compounds.

The blood-borne, hormonal sympathomimetic splenic agonist epinephrine is secreted by the adrenal medulla chromaffin cells in response to splanchnic nerve stimulation and has been shown to stimulate smooth muscle contractions of the splenic capsule (13). Therefore, epinephrine was administered in the present study to validate its potential to induce splenic contraction in guinea pigs. The adrenal medulla, which developmentally arises from sympathetic ganglia cells derived from the neural crest, is the site of endogenous catecholamine synthesis from the amino acid tyrosine. The aromatic portion of the acid is hydroxylated, and the aliphatic

portion is decarboxylated and then hydroxylated during its conversion to norepinephrine. The amine group of norepinephrine undergoes methyl substitution to complete epinephrine synthesis (14). Following synthesis, epinephrine is stored in the medullary chromaffin granules where its release is mediated by the sympathetic nervous system. Epinephrine has a wide variety of clinical uses, especially in respiratory and myocardial dysfunctions and as a topical hemostatic (15, 16).

In addition to norepinephrine and epinephrine, the catecholamine group also includes the synthetic structural analog isoproterenol. Among the catecholamines, isoproterenol is distinguished by its terminal N-isopropyl group, whereas norepinephrine and epinephrine have an N-hydrogen and an N-methyl endgroup, respectively. Isoproterenol was first studied by Konzett (17) and has been the subject of extensive animal and clinical pharmacological research, especially as a bronchodilator for types of respiratory dysfunction and as a cardiac stimulant after myocardial infarction and septicemic shock (15).

Blood-borne catecholamines mimic the actions of the sympathetic nervous system via stimulation of sites called "adrenergic receptors," located at the nerve terminal-effector organ synaptic junction. These adrenoceptors were initially classified as α and β by Ahlquist (18) based on his observations of the effects of the catecholamines epinephrine, norepinephrine, and isoproterenol upon smooth muscle tissue. Receptor subgroups were later categorized as follows: α_1, predominately located at the postsynaptic effector sites of smooth muscle and gland cells, they initiate excitation of the target organ upon stimulation; α_2, proposed to exist on adrenergic nerve terminals, they are believed to mediate feedback inhibition of neural release of norepinephrine (19); β_1, chiefly located in cardiac tissue, they mediate the force (inotropic effect) and frequency (chronotropic effect) of cardiac contraction upon stimulation; β_2, located primarily in smooth muscle and gland cells, upon stimulation they generally produce an inhibitory effect (20). It should be noted that catecholamines, in general, may produce excitation or inhibition depending on the agonist, site, dose, and method of induction chosen (21).

In the present study, the blood volume of the guinea pig was assessed by direct measures of Hct and dilution samples of autologous red blood cells labeled with sodium chromate. In addition, experimental measures were performed following the administration of the

sympathomimetic catecholamine epinephrine to assess the potential influence of splenic mobilization of red cells upon the circulating mass. Doses were selected to elicit maximum contraction of the splenic smooth muscle and discharge of red blood cells. Isoproterenol was administered in identical doses to a separate set of subjects to assess the pharmacological effects of this potent cardiovascular stimulant upon the circulating blood volume. Mean arterial pressure (MAP) and heart rate (HR) were monitored to assess the hemodynamic responses of the system to these agonists. Likewise, measures were performed upon splenectomized individuals for comparison of the systemic effects of the catecholamines.

METHODS

Experimental Animals and Protocol

Twenty Hartley guinea pigs, both boars and sows, weighing 0.83 ± 0.16 kg (mean ± standard deviation), were used in this study. They were obtained from a commercial breeder (Simonsen Labs, Inc., Gilroy, CA) and were maintained in a hygienic habitat where they were fed a commercial ration (Purina Guinea Pig Chow®, Ralston Purina Co., St. Louis, MO) and received water *ad libitum*.

The animals were randomly assigned to one of two groups, each with two treatment subgroups of n = 5. The first group was composed of intact animals with one subgroup administered epinephrine (0.15 mg/kg) and the other isoproterenol (0.15 mg/kg); the second group consisted of splenectomized individuals with the subgroups receiving the same dose of one or the other of these catecholamines.

The experimental protocol called for surgical embedment of an arterial catheter with subsequent control measures of red cell mass, hematocrit, heart rate, and mean arterial pressure to be followed by administration of an intra-arterial dose of the respective catecholamine with postinjection measures taken at 2.5, 5, 10, 20, 40, and 80 minutes and analyzed exactly as the control values were.

Surgical Procedures

After an overnight fast, the animals upon whom splenectomies were performed were transported to the laboratory and anesthetized with an intraperitoneal (IP) bolus injection of 100 mg/kg ketamine hydrochloride (Vetalar®, Parke-Davis, Morris Plains, NJ) and 10 mg/kg xylazine hydrochloride (Rompun®, Miles Laboratories, Inc., Shawnee, KS) (22). All IP injections were performed with 0.75-inch, 23-gauge disposable needles (Becton, Dickinson, and Co., Parsippany, NJ). As a precaution against anesthetic-induced hypothermia, the animal was placed in a supine position upon a circulating water blanket (38° C) and its core body temperature monitored using a telethermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) with a rectal probe.

Following surgical preparation, a parasagittal

incision was made in the left hypochondriac region and a celiectomy performed, with the spleen excised following double ligation of all afferent and efferent vessels. All celiotomies were closed with two layers of absorbable surgical suture (4-0 Dexon®, Davis and Geck, Inc., Manati, Puerto Rico). The animal was allowed to recuperate for at least two weeks prior to study, with resumption of normal eating habits and weight gain used as criteria for judging recovery.

The preparation for experimentation was the same for all the guinea pigs. On the day of study, the animal was brought into a quiet laboratory following an overnight fast. Using the same anesthetics and surgical preparations employed for the splenectomies, a ventral midline neck incision was made and the left common carotid artery exposed. While temporarily constricting the flow, an incision was made in the wall of the vessel and a catheter inserted. Upon release of the constraint, the catheter was routed to the level of the aortic arch. It was secured in place by ligatures around the artery. The catheter consisted of a 5-cm polyethylene tip (1.14 mm inside diameter, 1.57 mm outside diameter) (Intramedic®, Clay Adams, Parsippany, NJ) joined to a 20-cm length of Silastic® tubing (1.02 mm inside diameter, 2.16 mm outside diameter) (Dow Corning Corp., Midland, MI). This arrangement provided the rigidity necessary for relatively easy catheter insertion into the artery with the flexibility needed to loop the system away from the animal with a minimum of length (to minimize any dampening of arterial pressure while isolating the sampling site to avoid handling-induced artifacts (23)). A three-way stopcock (Pharmaseal, Inc., Toa Alta, Puerto Rico) was connected to the distal end of the Silastic® tubing with an 18-gauge Luer-Stub Adapter (Intramedic®). A Gould P23Gb (Gould Inc., Cleveland, OH) pressure transducer was attached to the stopcock by means of a 30-cm pressure-monitoring injection line (Cobe Laboratories, Inc., Lakewood, CO). The transducer height was adjusted to match the heart level of the recumbent animal by suspending it with clamps from a ring stand. The entire system was filled with heparinized saline (10 units/ml) to maintain patency, with care taken to eliminate any trapped air that would distort pressure readings. The transducer output was graphically monitored with a Gould pressure-processor amplifier and 2400S physiological recorder.

The guinea pig was not disturbed for at least 30 minutes before commencement of experimentation.

Red Blood Cell Labeling and Sampling

Red cell volume was determined by the dilution of autologous radiolabeled erythrocytes. The technique of labeling erythrocytes with sodium chromate ($Na_2{}^{51}CrO_4$) was pioneered by Sterling and Gray (24). One milliliter of blood was withdrawn via an indwelling carotid artery catheter and mixed in a 12 x 75 mm disposable test tube (Falcon 2058, Becton, Dickinson, and Co., Parsippany, NJ) with 5 µCi sodium chromate (New England Nuclear, Boston, MA) and 0.2 ml citrate-phosphate-dextrose (CPD) anticoagulant solution (Sorenson Research Co., Salt Lake City, UT). The mixture was allowed to stand for 30 minutes at room temperature with occasional gentle inversion to facilitate red cell tagging. The solution was then centrifuged at 1000 g for 10 minutes and the plasma portion discarded. The red cell pellet was washed twice to remove any unbound radiochromium: the pellet was dispersed in cold physiologic saline (0.9% NaCl), followed by gentle agitation, centrifugation, and then removal of the supernatant. Following this procedure the erythrocytes were resuspended in 1.1 ml isotonic saline; thus, the total volume, including the cells, was ≈1.5 ml.

Using a positive-displacement pipette (Scientific Manufacturing Industries, Emeryville, CA), exactly 1.0 ml of the tagged red cell suspension was injected intra-arterially and flushed in with 1.0 ml of 0.9% saline. Duplicate aliquots of exactly 0.2 ml of the injected dose were transferred using a SMI positive-displacement pipette to 12 x 75 mm test tubes and diluted with 2.0 ml distilled water. Water was used to dilute the ID aliquots as well as the subsequent dilution samples since osmotically hemolyzing the red cells yielded a more homogeneous solution for analysis.

In order to assure acquisition of dilution samples from fresh circulating blood sample, a 1.0-ml syringe (Tuberculin Monoject®, Sherwood Medical Industries, St. Louis, MO) was used to withdraw the 0.25 ml isotonic saline contained in the catheter plus 0.5 ml of "flush blood." A second calibrated syringe was then used to obtain 0.3 ml of blood sample for counting of the radiolabel dilution and measuring Hct. Immediately after removal of the sample, the flush blood was reintroduced and followed by 0.3 ml of fresh heparinized saline injected to replace the sample volume and reirrigate the catheter. Removal of the 0.5 ml of "flush blood" was performed as a precaution against sampling from a pool of cells at the tip of the catheter that might not accurately reflect a whole body dilution; thus, this volume was returned to the animal prior to

reirrigation of the catheter.

From the tip of the blood-sampling syringe, a heparinized microhematocrit capillary tube (Propper Manufacturing Co., Long Island City, NY) was immediately filled and subsequently centrifuged at 12,000 g for 5 minutes before analysis using an International Microcapillary Reader. The remaining whole blood specimen (≈0.225 ml) was then mixed with 0.005 ml heparin (1000 units/ml), whereupon exactly 0.2 ml was transferred into a fresh 12 x 75 mm test tube using a SMI positive-displacement pipette and diluted with 2.0 ml of dH_2O prior to spectrophotometric analysis.

All tagged red cell samples were assayed for their counts per minute in a well-type scintillation counter (Packard Auto Gamma® 500c, Downers Grove, IL). The peak gamma radiation emission for ^{51}Cr occurs in the 325 keV range; thus, the scintillation counter's energy detection window range was set at 240-400 keV to maximize counting efficiency (25). Two test tubes filled with 2.0 ml dH_2O each were included with the experimental samples for determination of and correction for background radiation. Duplicate runs of all injected dose and dilution samples were performed and averaged prior to calculating erythrocyte volume.

Volume Calculations

Red blood cell volume, as determined using ^{51}Cr-labeled cell dilution, was calculated as:

$$\text{RCV (ml/kg)} = \frac{(CPM_{ID})\ (Hct_A)\ (0.97)}{(CPM_B)\ (Wt)}$$

where CPM_{ID} is the counts per minute (CPM) in the injected dose, Hct_A is the arterial hematocrit, 0.97 is a correction factor (26) used to compensate for the plasma trapped in the erythrocyte column of the hematocrit capillary tube, CPM_B is the counts per minute per ml blood in the dilution samples, and Wt is the body weight in kilograms.

Whole blood volume was calculated as:

$$\text{WBV (ml/kg)} = \frac{\text{RCV}}{(Hct_A)\ (0.97)}, \quad \text{given } (Hct_A)\ (0.97) = \frac{\text{RCV}}{\text{WBV}}.$$

Plasma volume was calculated by taking the difference between the RCV and the WBV.

Heart Rate Measurement

The heart rate of the guinea pig was calculated from recordings of the intra-arterial pressure waveforms. Approximately thirty seconds before whole blood sampling, a 15- to 20-second recording of the waveforms was made (chart speed 10 mm/sec) on 1.0-mm ruled recorder paper. The number of waveform peaks over a 6.0-cm interval (equal to six seconds of data) were counted, with the result multiplied by a factor of ten to extrapolate to beats/min (BPM).

Mean Arterial Pressure Measurement

The mean arterial pressure was constantly monitored using a pressure transducer and the data simultaneously registered on a physiological recorder. The values are a calculated mean derived from the formula:

$$MAP = \text{diastolic} + \frac{(\text{systolic} - \text{diastolic})}{3}$$

where systolic and diastolic are blood pressures in mm Hg. The MAP measures for the various sampling times were taken just prior to the blood sampling, thus minimizing any artifacts due to specimen extraction.

Instrument Calibration

Pipette Accuracy

The SMI positive-displacement pipettes used in this study were gravimetrically tested for accuracy with both distilled water and blood. In the latter case, blood density was first obtained by weighing exactly 100 ml of porcine blood in a volumetric flask. The same blood was then used to determine the weights and calculated volumes delivered by the 0.2- and 1.0-ml pipettes.

Spectrophotometer Accuracy

The accuracy of the Packard scintillation counter was tested by constructing standard dilution curves. After tagging a sample of porcine blood with fresh sodium

chromate, various aliquots were counted and the results plotted versus their respective activities in microcuries. The resultant curves were examined for deviations from the expected linear relationship. The spectrophotometer's optimum accuracy range was used as a guide to determine the activity to be used in tagging the autologous red blood cells. The activity of ^{51}Cr per unit volume varies as a function of how many half-lives (^{51}Cr half-life = 27.8 days) have elapsed since the manufacturer's dated assay (25). Therefore, the volume of sodium chromate equal to 5 µCi was calculated on a daily basis.

Pressure Transducer and Recorder Accuracy

The accuracy of the graphic display of the pressure transducer output signal produced by the recorder was confirmed prior to each experiment with a manometer. The speed at which the chart paper passed under the recorder pen was checked by running the recorder at an indicated chart speed of 10.0 mm/sec and comparing the electronically generated timing marks to the ruled chart paper.

Data Analysis

Means and representative standard deviations were calculated for each group and treatment subgroup at each time point for all measured variables. The means reported for the control values are actually the average of the last two of the control samples recorded, the first sample value having been discarded. The control values of the intact and splenectomized groups were compared using a Student's t-test for independent groups. The respective subgroups were evaluated using a variety of analyses of variance (ANOVA): a single-factor ANOVA for repeated measures over time for each subgroup; separate two-factor analyses for time and condition (intact vs. splenectomized guinea pigs) for the respective treatments; separate two-factor ANOVA's for time and treatment (epinephrine or isoproterenol) for the respective splenic conditions; and a three-factor ANOVA for time, treatment, and condition. A Newman-Keuls comparison of the control and experimental sample means was performed for each treatment subgroup. A statistically significant difference was assumed when $P<0.05$ (27).

RESULTS

Instrument Calibration

Positive-Displacement Pipettes

Gravimetric testing of the positive-displacement pipettes demonstrated excellent accuracy and reproducibility with both water and blood (Table 1). For a given pipette size, there were no significant differences observed between volume replications.

Spectrophotometer

The standard dilution curve for ^{51}Cr showed a limited linear relationship between the scintillation counter's CPM and the activity calculated using the manufacturer's dated assay (Figure 1). Deviations from linearity started to occur in the 220,000 to 240,000 CPM range. The activity of the chromate used to label the erythrocytes, 5 µCi, was chosen so that the resultant sample counts would be well below 100,000 CPM (the samples with the highest activity were the ID aliquots, each 0.2 ml of the ≈1.5 ml tagged cell suspension).

Basal Measures

The mean control values and standard deviations of the intact and splenectomized groups are summarized in Table 2. The only significant difference between the groups was in their basal heart rates.

Treatment Measures

In reporting the results for each variable, the three-factor ANOVA will be discussed first, then the two-factor ANOVA's for the respective treatments and splenic states, followed by the one-factor ANOVA by subgroup. Where appropriate, the results of the Newman-Keuls comparison of the sample means will be discussed. The term "condition" will be used in reference to the splenic state (i.e. intact/splenectomized), not to be confused with the degree of splenic sequestration or mobilization.

The heart rate and arterial pressure means reported for the intact/epinephrine subgroup are based upon measures

from only three of the five subjects in this set. The degrees of freedom used for the t-distribution and F-distribution statistical analyses were adjusted accordingly.

Hematocrit Measures

The effects of time, treatment, and splenic condition upon the guinea pigs' hematocrits are summarized in Figures 2 and 3 (by treatment) and Figures 4 and 5 (by condition). The three-factor analysis of variance, ANOVA, shows a significant interaction between the splenic groups, treatments, and time (Table 3). Table 3 also indicates significant interactions for the treatments vs. time and the conditions vs. time.

The subgroups which received epinephrine experienced an immediate postinjection increase in Hct values that peaked after 2.5 minutes (Figure 2). The values then returned to basal levels, although at differing rates, as reflected in the two-factor ANOVA's significant time effects and condition vs. time interaction (Table 4). The Hct of the intact/epinephrine subgroup (n = 5) had a significant elevation from the control value of 39.3 to the 44.0% range for the 2.5- and 5-minute samples (Table 5). Samples taken 10 to 80 minutes post injection were not significantly different from the control values, all means being between 39.6 and 40.0%. The splenectomized/epinephrine subgroup (n = 5) experienced a more prolonged, significant Hct elevation, rising from a basal value of 40.1 ± 3.7 to 46.2 ± 4.1% at 2.5 minutes before beginning a gradual descent in magnitude. The Newman-Keuls analysis indicated that the splenectomized subgroup's Hct values recorded at 2.5, 5, 10, and 20 minutes after injection significantly exceeded the control values (Table 5).

Isoproterenol administration led to an abrupt post-treatment decrease in Hct for the splenectomized animals, while the intact set's measures were relatively more stable (Figure 3). This relative difference was apparently responsible for the significant condition and time interaction in the two-factor ANOVA's significant time effect (Table 6). The intact/isoproterenol animals (n = 5) showed little Hct deviation in the postinjection sampling, with an insignificant decrease from the basal value of 37.8 to 36.0% after 40 minutes, where it held steady. The splenectomized/isoproterenol animals (n = 5) experienced a sharp drop in Hct, with all postinjection measures significantly different than the control (Table 5). This subgroup's Hct decreased from a basal level of 42.2% to the 38.5% range for the 5-, 10-, and 20-minute specimens before

resuming its descent to 36.8% at 80 minutes.

The two-factor analyses of variance for treatment and time with respect to the intact (Table 7) and splenectomized (Table 8) groups show significant time effects and significant interactions between the catecholamines and time. Figures 4 (intact group) and 5 (splenectomized group) clearly illustrate these treatment differences for the splenic conditions over time.

The single-factor analysis of variance for Hct measures over time (Table 9) reflects the significant shifts in the magnitude of the Hct over the course of the experiment for each of the treatment subgroups as was indicated by the three- and two-factor ANOVA's.

Red Cell Volume Measures

Erythrocyte volume data are summarized by treatment in Figures 6 and 7 and by condition in Figures 8 and 9. The three-factor analysis of variance, Table 3, for the condition, treatment, and time effects indicates that there were significant interactions between these factors.

The intact/epinephrine subgroup experienced a small but significant elevation of circulating erythrocyte volume from the 17.4 ± 2.4 ml/kg control value to a plateau in the 17.8 ml/kg range that persisted throughout the sampling period (Figure 6) and accounts for the two-factor ANOVA's significant time effect (Table 4). The RCV of the splenectomized/epinephrine set, with a basal value of 19.4 ± 2.1 ml/kg, showed no significant postinjection changes as a function of time as revealed by the Newman-Keuls test (Table 5). The different RCV responses of the subgroups administered epinephrine are reflected in the significant two-factor ANOVA splenic condition vs. time interaction (Table 4).

The intact/isoproterenol subgroup experienced an insignificant increase in erythrocyte volume from the control level of 17.6 ± 2.6 ml/kg to the 80-minute value of 17.9 ± 2.6 ml/kg (Figure 7). The splenectomized/ isoproterenol animals had an average basal RCV value of 19.0 ± 1.7 ml/kg. A postinjection decrease in RCV to the significantly different measure of 18.4 ± 1.7 ml/kg at 10 minutes was followed by a gradual return to the control value by the 80-minute specimen (Table 5). The apparent decline in the RCV of this subgroup is reflected in the significant time effect and interaction between the condition and time seen in the two-factor ANOVA for the isoproterenol treatment (Table 6).

The RCV's two-factor ANOVA for time and treatment effects with respect to the intact group (Table 7) shows only a significant time effect. Examination of Figure 8 reveals that the intact animal treatment subgroups each experienced a slight elevation in their circulating red cell mass over time. Conversely, the two-factor ANOVA for time and treatment effects with respect to the splenectomized animals (Table 8) reveals no time effect, only a significant time and treatment interaction. Figure 9 illustrates the differing effects the treatments had upon the RCV of the splenectomized treatment subgroups.

The single-factor ANOVA for repeated measures of RCV over time (Table 9) reveals a significant time effect that was condition dependent for each treatment. The animals with spleens that received epinepharine experienced a significant change in RCV, but such was not the case for the splenectomized subgroup. The isoproterenol subgroups experienced the converse: the splenectomized animals had a significant RCV change while the intact subgroup did not.

Plasma Volume Measures

The plasma volumes for each treatment subgroup are summarized in Figures 10 through 13, first by treatment and then by condition. The three-factor analysis of variance, Table 3, shows significant interactions between the splenic condition, treatment, and time. In addition, significant interactions between the condition and time as well as treatment and time are present.

The result of an intra-arterial injection of epinephrine on plasma volume was similar in the intact and splenectomized animals in that there was an initial PV decline followed by a return to the control range, which resulted in a significant time effect (Table 4). The plasma volume of the intact/epinephrine set declined from the control level of 28.1 ± 1.3 ml/kg to significantly lower values in the 24 ml/kg range for the 2.5- and 5-minute samples; by the 10 minute sample PV was back to the control level where the measures plateaued (Figure 10). The circulating plasma volume decline was even greater in the splenectomized epinephrine individuals, an initial decline from 30.5 ± 1.9 ml/kg to 24.1 ± 1.8 ml/kg after 2.5 minutes was followed by a return to the control range by the 20-minute sample (Table 5). The more profound effect seen in the splenectomized animals, in both the magnitude and duration of PV decrease, accounts for the significant splenic condition vs. time interaction seen in the two-factor ANOVA (Table 4).

Isoproterenol administration resulted in an elevation of PV to levels significantly higher than those of the controls in both condition subgroups (Figure 11). This rise was maintained throughout sampling, resulting in a significant PV change over time (Table 6). The intact/isoproterenol group had a control plasma volume of 30.2 ± 2.2 ml/kg that rose to the 33.0 ml/kg range by the 10-minute sample before finally reaching significantly different values for the last two samples (Table 5). Isoproterenol administered to splenectomized animals caused an initial sharp rise in PV followed by a less steep PV-expansion slope: all treatment measures were significantly different than the controls. From the basal value of 27.4 ± 2.0 ml/kg, the PV value rose to 31.4 ± 1.7 ml/kg at 5 minutes before continuing its upward movement to 34.1 ± 2.2 ml/kg at 80 minutes. The two-factor ANOVA for splenic condition vs. time interaction for the animals given isoproterenol indicates a significant interaction between these factors, which appears to be a result of the greater expansion of PV seen in the splenectomized subgroup (Table 6).

The two-factor ANOVA's for the intact (Table 7) and the splenectomized groups (Table 8) reveal significant differences between the treatment subgroups, time effects, and treatment vs. time interactions for each of the splenic conditions. These treatment effects are illustrated in Figures 12 and 13 for the intact and splenectomized animal subgroups, respectively.

The single-factor ANOVA for PV measures over time, Table 9, shows significant time effects in each of the four treatment subgroups, as was indicated by the three- and two-factor ANOVA's.

Whole Blood Volume Measures

Summaries of each treatment subgroup's whole blood volume values by treatment and splenic condition appear in Figures 14 through 17. The three-factor ANOVA indicates a significant interaction between the splenic groups, treatments, and time (Table 3). In addition, Table 3 also indicates that there were significant interactions for the conditions vs. time and treatments vs. time.

The animals receiving epinephrine all experienced a postinjection decrease in WBV followed by a steady return to control levels; the net result was a significant time effect (Table 4). The WBV of the intact/epinephrine set dropped following catecholamine administration from the control 45.5 ± 1.1 ml/kg to 41.3 ± 1.9 ml/kg after 2.5

minutes (Figure 14). This decrease was short-lived though, since by the 10-minute sample the volume was back to the control range, where it stabilized (Table 5). The splenectomized/epinephrine treatment subgroup experienced a sharp drop from its control value, 49.9 ± 1.9 ml/kg, to the 44 ml/kg range for the 2.5- and 5-minute samples before eventually returning to the control level by the 40-minute sample. The differences in magnitude and duration of these subgroups' WBV response curves account for the two-factor ANOVA's significant condition effect and condition vs. time interaction (Table 4).

The two condition subgroups that received isoproterenol each experienced a statistically significant rise in whole blood volume in the postinjection period that remained unabated after 80 minutes of sampling; this elevation is reflected in the significant time effect seen in the two-factor ANOVA for isoproterenol (Table 6). The intact/isoproterenol guinea pigs' blood volume rose from the basal level of 47.8 ± 3.8 ml/kg to 50.6 ± 5.7 ml/kg at 10 minutes and ultimately reached 51.5 ± 5.9 ml/kg after 80 minutes (Figure 15). The splenectomized/isoproterenol group's WBV increase was more profound, with all postinjection sample means significantly greater than the control: a rise from the control value of 46.4 ± 2.4 ml/kg to 50.2 ± 2.3 ml/kg after 5 minutes, ultimately reaching 53.1 ± 2.3 ml/kg at 80 minutes.

The two-factor ANOVA summaries for the respective condition groups' WBV, Tables 7 and 8, indicate significant interactions for time effects and treatment vs. time interactions. These treatment effects are illustrated in Figures 16 and 17 for the intact and splenectomized animal groups, respectively.

The single-factor analysis of variance for repeated WBV measures over time shows significant changes for each of the four treatment subgroups (Table 9).

Heart Rate Measures

Figures 18 and 19 summarize the heart rate means and their standard deviations by treatment, and Figures 20 and 21 by group. The three-factor ANOVA for heart rate indicates a significant treatment vs. time interaction (Table 3).

Both sets of animals that received epinephrine underwent a profound increase in rate, as indicated by the significant time effect seen in the two-factor ANOVA (Table 4). The intact animals had an increase in rate from a

control 201 ± 36.4 to 317 ± 55.1 BPM at 2.5 minutes, and then began a steady decline back to the control level that was completed by the 20-minute sample (Figure 18). The splenectomized animals' HR increased from the control value of 146 ± 16.4 BPM up to 265 ± 24.5 BPM at 2.5 minutes, followed by a return to the control range for the 40-minute sample (Table 5). The two-factor ANOVA for epinephrine indicates a significant time effect, but no condition vs. time interaction.

The guinea pigs that received isoproterenol had nearly identical heart rate responses following stimulation (Figure 19). The intact animals experienced a rise from 173 ± 27.2 to a peak of 312 ± 20.1 BPM at 2.5 minutes, followed by a gradual return to 204 ± 37.0 BPM by the 80-minute recording. The rates of the splenectomized guinea pigs rose from 163 ± 18.9 to a peak of 302 ± 17.0 BPM at 2.5 minutes before dropping back to 190 ± 10.3 BPM for the concluding sample. These profound isoproterenol-induced increases in HR, with a return to the control range only by the 80-minute measure, are reflected in the two-factor ANOVA's significant time effect (Table 6).

The two-factor ANOVA for the intact group shows a significant time effect and treatment vs. time interaction (Table 7). The intact subgroups' HR responses to the two catecholamines are illustrated in Figure 20. The treatments produced significant treatment and time effects in addition to a treatment vs. time interaction in the splenectomized subgroups (Table 8) as illustrated in Figure 21.

The single-factor analysis summary for repeated HR measures over time indicates a significant effect upon heart rate for each of the four subgroups (Table 9).

Mean Arterial Pressure Measures

The mean arterial pressure readings are illustrated in Figures 22 and 23 (by treatment) and Figures 24 and 25 (by group). The three-factor ANOVA, Table 3, shows a significant interaction between the treatment and time effects.

All animals administered an intra-arterial injection of epinephrine experienced a sharp increase in mean arterial pressure that peaked at 2.5 minutes, followed by a gradual return to the control range by the 20-minute sample (Figure 22). The intact animals experienced an escalation of MAP from a basal level of 52.3 ± 8.5 to 107.7 ± 10.7 mm Hg at 2.5 minutes, while the splenectomized subjects

experienced a nearly identical rise in the same [illegible], from a basal 48.9 ± 6.7 to 107.8 ± 11.6 mm Hg at 2.5 minutes. Following the initial excitation, both subgroups experienced a return to control levels by the 10-minute sample (Table 5). The two-factor ANOVA for the epinephrine subgroups reflects the observed changes in MAP with a significant time effect, with no significant condition effect (Table 4).

The effect isoproterenol elicited was very similar in both the intact and splenectomized subgroups (Figure 23). The intact animals experienced an initial drop from the control value of 47.2 [illegible] 5.6 mm Hg at 2.5 minutes, with a return to the control range by the 10-minute sample (Table 5). The splenectomized subgroup's response was very similar: a drop from a basal level of 46.1 ± 3.1 mm Hg to 28.2 ± 1.6 mm Hg at 2.5 minutes and then back to the control range by the 10-minute sample. There was a significant time effect seen in the two-factor ANOVA for the MAP of the animals administered isoproterenol, but there was no significant effect attributable to the splenic condition (Table 6).

The two-factor ANOVA for the intact group indicates significant treatment and time effects in addition to treatment vs. time interactions (Table 7). Likewise, the two-factor ANOVA for the splenectomized animals reveals significant treatment and time effects and treatment vs. time interactions (Table 8). Figures 24 and 25 illustrate the different responses each of these condition subgroups had to the treatments.

The single-factor ANOVA for repeated measures of MAP over time indicates significant changes in the magnitude of the pressures for each of the treatment subgroups (Table 9).

DISCUSSION

The experiments described in this study evince application of surgical procedures and analytical methodologies for measuring a number of hemodynamic variables. Hematocrit, red cell volume, plasma volume, whole blood volume, heart rate, and mean arterial pressure were successfully measured in anesthetized animals, both with their spleens intact and splenectomized, under basal conditions and following stimulation with cardiovascular agonists. There were, however, areas of significant concern, including potential skewing of the hemodynamic measures resulting from side effects of the anesthetics and potential error arising from the extrapolation of measures of red cell volume to plasma and whole blood volume using the large vessel hematocrit. In all, the attractive features of these procedures include ease of sampling (of both blood and intravascular pressure waveforms), improved accuracy of red cell volume measures resulting from use of direct measures of autologous erythrocytes labeled with ^{51}Cr, relatively small sample volumes (0.3 ml blood per dilution sample), and simple calculation formulae for indirect measures.

Basal Measures

Prior to administration of the treatments, control measures were performed upon all animals. These "basal" measures were, in fact, performed upon anesthetized animals, following catheterization. The anesthetics of choice, ketamine and xylazine, have been shown to be cardiovascular agonists (22); their potential hemodynamic side effects will be discussed in the Critique of Methods section. Despite potential anesthetic-induced skewing of the measures, the anesthetized model was deemed preferable to potential uncontrolled error arising from random environmental and handling-induced excitation of a conscious animal (23).

Since the spleen has been shown to influence the circulating blood volume in other animal models, splenectomized subjects were also administered the treatments and the effects were observed. The validity of comparing intact and splenectomized models is predicated on the assumption that the surgical removal of the spleen does not have secondary effects that fundamentally alter the physiological characteristics of the cardiovascular system.

making any comparisons irrelevant. Thus, the animals were monitored for any outward signs of debility during the fortnight recuperative period following splenectomy. Comparison of their basal measures to those of the intact group did not indicate any gross differences in function that appeared to be attributable to splenectomy.

Hematocrit Measures

The basal blood volume measures of the intact and splenectomized sets were quite similar. The slight, nonsignificant tendency for a lower total blood volume in the splenectomized group was attributable to a greater volume of circulating erythrocytes. Since the plasma volumes of the two groups were nearly identical, the average Hct of the splenectomized group was higher than that of the intact group, though not significantly. A similar, but significant, postsplenectomy rise in red cell volume and Hct has been reported by Wachtel and McCahan (6) and Hannon et al. (26) using porcine models. Discharge of splenetically sequestered red cells during surgery, postoperative erythropoietic activity or the loss of the splenic phagocytic function may have contributed to the observed effects.

The relative concentration of red blood cells in the present study, ≈39%, was within the range of published values for guinea pig hematocrits. Edmondson and Wyburn (28) reported 36%, Sewell et al. (29) and Baker and Schaefer (30) 37%, Bosse and Wittmann (31) 42%, Sanderson and Phillips (32) 44%, Kutscher (33) 47% and Constable (34) 48%. Analytic methodology, as related to sampling extraction and centrifugation, could account for much of the variance in this range of values. When measuring Hct via centrifugation, some investigators use a correction factor for plasma trapped in the red cell portion of the capillary tube. Of the studies cited above, the present study and that of Constable (34) corrected the Hct via multiplication by a 0.97 factor, while Baker and Schaefer (30) used a 0.96 factor. The rationale for, and applicability of, a trapped plasma correction factor will be discussed in the Design of Metabolism section.

Blood Volume Measures

Guinea pig blood volumes calculated from direct red cell volume measures using ^{51}Cr and Hct have been reported by other investigators. Edmondson and Wyburn (28) reported RCV = 20 ml/kg, PV = 44 ml/kg, and WBV = 64 ml/kg and Baker and Schaefer (30) found RCV = 22 ml/kg, PV = 38 ml/kg, WBV = 60 ml/kg. The plasma and whole blood volumes reported in

the present study, 29.2 and 46.6 ml/kg, are depressed relative to those cited by the other investigators in part due to their use of a "whole body" Hct correction factor. This factor is used in an attempt to correct for the higher plasma concentration in the microcirculation which is not directly apparent in the large vessels; Edmondson and Wyburn used a 0.82 factor, Baker and Schaefer a 0.91 factor. Developmental theory and applicability of this Hct correction factor, and why it was not employed in the present study, will be discussed in the Critique of Methods section. Nevertheless, for comparative purposes, factors of 0.70, 0.80, and 0.90 were applied to the intact group's basal arterial Hct (Table 2), and the PV and WBV were calculated from the RCV. For the given RCV measure of 17.5 ml/kg, the PV = 49.1, 40.8, and 34.2 ml/kg, and the WBV = 66.6, 58.3, and 51.8 ml/kg for the 0.70, 0.80, and 0.90 factors, respectively.

The guinea pig blood volumes calculated from RCV and Hct measures in this experiment were lower than those reported in the literature based upon PV and Hct measures. Using ^{125}I-HSA as the plasma diluent and Hct, Edmondson and Wyburn (28) reported PV = 38 ml/kg and WBV = 54 ml/kg, and Baker and Schaefer (30) found PV = 54.4 ml/kg and WBV = 87 ml/kg. Using an alternate method, dilution of albumin stained with Evans Blue dye, Kutscher (33) calculated PV = 38 ml/kg and WBV = 57 ml/kg. Constable (34) reported PV = 30 ml/kg and WBV = 59 ml/kg, and Ancill (35) found WBV = 72 ml/kg. Potential errors contributing to overestimation of intravascular PV measured via dilution of labeled plasma moieties will be addressed in the Critique of Methods section.

The discrepancy between the blood volumes calculated in this study and those cited in the literature is not only a function of application of correction factors to Hct values and potential error in defining the intravascular plasma volume via dilution of plasma labels, but also of population variability. Smaller animals, in general, have a higher percentage of lean body mass, hence more blood per unit mass, than larger animals of the same mammalian species (34). Also, the guinea pig, for the most part, is not as homogeneous a population as some laboratory animal populations (22). The studies cited above were inconsistent in their reporting of the sample populations' vital statistics. From the information provided, the subjects appear to have come from a variety of strains, with weights ranging from 0.25 to 1.0 kg. The animals in the present study were all mature Hartley guinea pigs (mean body weight = 0.83 kg).

Heart Rate Measures

The heart rates of the intact animals were significantly higher than those of the splenectomized animals. While the experimental preparation procedure was the same for all animals, between-animal response variations to the potent cardioactive anesthetics could well have contributed to the large variance seen in the present study.

The wide range of basal heart rate means observed in this study is at least partially attributable to the potent negative cardiac chronotropic effects of the anesthetics, whose effects appear to be dose related. Hayes (36) reported a guinea pig mean heart rate of 221 BPM using doses of ketamine and xylazine equal to 40 and 50%, respectively, of those used in the present study. Higher rates, in the 250-260 BPM range, were reported by Hart et al. (37) for guinea pigs anesthetized with ketamine and xylazine at doses equal to 25 and 1.5%, respectively, of those used in this experiment.

Guinea pig pulse rates reported in the literature for unanesthetized subjects are quite a bit higher than those recorded in this study. Conscious guinea pig models using some form of restraint had mean rates of ≈260 BPM in studies by Richtarik et al. (38) and Petelenz (39), while Zeman and Wilber (40) reported a mean value of 335 BPM. Fara and Catlett (41) reported a value of 276 BPM employing an implanted telemetry device that allowed for a relatively free-ranging model. The conscious animal model has a potential for environmental and handling-induced anxiety that may augment the level of, and contribute to variance in, the baseline values recorded; alternatively, the anesthetized model used in this study may have experienced a sedative effect due to undisturbed recumbency upon a heated circulating water blanket prior to sampling.

Maturation-related differences in heart rate have been reported for the guinea pig and may have contributed to the relatively depressed mean pulse rates reported in this study. An inverse relationship between heart rate and body weight has been reported for guinea pigs by Mikiskova and Mikiska (42) and in 31 species of small mammals by Dawe and Morrison (43). A majority of the animals cited above were less mature than the sample population of this study: the anesthetized guinea pigs cited had body weights ranging from 0.25-0.35 kg (36) and 0.50-0.80 kg (37); the conscious animals had body weights ranging from 0.3 to 0.60 kg (39-41) and 0.82 to 1.06 kg (38). Regardless, the apparent heart rate depression seen in this study is probably less a

function of maturation than of the potential cardiac effects of the anesthetics.

Mean Arterial Pressure Measures

The basal mean arterial pressures recorded here were almost identical for intact and splenectomized groups: ≈49 and 48 mm Hg, respectively. These means are similar to the 50-55 mm Hg range reported by Hart et al. (37) for guinea pigs anesthetized with ketamine and xylazine and measured at approximately the same time, 90-105 minutes after administration of anesthetics, as in the present study. Marshall and Hanna (44) reported a MAP value of 57 mm Hg for animals anesthetized with either ether, pentobarbital, or procaine (range of body weights 0.2-1.0 kg). All the foregoing values are depressed relative to the basal MAP (≈67 mm Hg) recorded in conscious, unanesthetized animals by Hart et al. (37).

Treatment Measures

Measures of guinea pig blood volume reported in the literature have not addressed the potential effect of splenic sequestration, or mobilization, of erythrocytes upon the circulating blood volume. In the present study, epinephrine was administered in an effort to induce splenic smooth muscle contraction and observe the subsequent effect upon the circulating WBV. Since this agonist is not spleen-specific, splenectomies were performed upon half of the subjects in an attempt to observe this treatment's effect upon the blood volume in the absence of splenic influence. In an effort to observe the influence of β adrenergic stimulation upon blood volume, in general, and whether there was an anesthetic-induced increase in the tone of the splenic smooth muscle, in particular, like measures were performed upon a separate group following administration of isoproterenol.

Hemodynamics

Intra-arterial injection of sympathomimetic catecholamines produced significant changes in the profile of the circulating blood volume of the guinea pig. Epinephrine administration produced a temporary decrease in the circulating blood volume, whereas isoproterenol produced an unabated expansion in WBV over the time course of experimental measures.

The rapid intra arterial injection of epinephrine, 0.15 mg/kg, in this study produced an increase of the heart

rate and mean arterial pressure that abated over time. These effects are believed to have resulted principally from a coupling of direct stimulation of myocardial β_1 adrenergic receptors with stimulation of both α and β_2 vasculature adrenoceptors. The distribution of these receptors is not uniform; quite often both α and β are found in the same region. The net effect elicited upon simultaneous stimulation is believed, in general, to be dependent upon the relative density of the respective receptors (45).

Epinephrine directly stimulates coronary β_1 receptors of the sino-atrial node and the Purkinje fibers, resulting in positive cardiac inotropic and chronotropic effects. Epinephrine-induced, α adrenoceptor-mediated vasoconstriction of cutaneous precapillary sphincters and subpapillary venules has been shown to reduce blood flow to the extremities (46). In addition, α stimulation has also been shown to result in contraction of splenic capsule smooth muscle, increased tone of the venous vascular smooth muscle, and decreased renal blood flow (47). Concomitant stimulation of β_2 adrenoceptors by epinephrine appears to be responsible for vasodilation of skeletal muscle precapillary sphincters and capillary beds with a resultant increase in blood flow and optimization of flow distribution, thus facilitating exchange between the tissue and blood (48). Hepatic blood flow has also been shown to increase following administration of epinephrine (45).

Epinephrine stimulation of the heart illustrates its behavior as a dual agonist and the cardiovascular system's ability to respond to contradictory stimuli. Following administration of epinephrine one would expect to observe vasoconstriction of coronary blood vessels, where there is a predominance of α adrenoceptors, but such is not the case. It appears that epinephrine's concomitant β_1-mediated increases in chronotropic and inotropic activity result in relative cardiac hypoxia, which in turn initiates local production of extremely potent vasodilatory metabolites, such as adenosine. These local metabolites effect the preservation of the tissue's integrity by overriding the potentially toxic effects of myocardial ischemia (15). The pathological ramifications of a dysfunction in the heart's homeostatic mechanisms are seen in humans with Prinzmetal's variant angina. These patients suffer from a supersensitivity to the α component of sympathetic nerve discharge with consequent arterial vasospasms resulting in angina pectoris and even myocardial infarction at rest (49).

The amalgamation of more frequent and forceful cardiac

contractions, vascular shunting of blood away from the periphery, and an increase in systemic venous tone is believed to have increased venous blood return. Increased venous return augments cardiac output, secondary to the direct cardiac β_1 stimulation, as promulgated by Starling (50) in his "law of the heart": the force of cardiac contraction is a simple function of the length of the myocardial fibers, which, in turn, is directly proportional to the diastolic filling.

The observed increase in arterial hydrostatic pressure is believed to be primarily responsible for the post-treatment decline of the circulating plasma volume. This is believed to have been due to an efflux of vascular plasma from the patent vessels as described by Starling's hypothesis.

Ernest Starling, a British physiologist, was the first to elucidate the relationship between the transmural pressure dynamics and plasma flux at the small vessels (arterioles, capillaries, and venules). Starling (51) described capillary plasma flux in terms of the relative hydrostatic and colloid osmotic (oncotic) pressures of the blood and the tissue. The former pressure is determined by arterial and venous blood pressures. The latter is determined by the semipermeability of the capillary endothelium to plasma components; plasma moieties of larger molecular size (e.g. proteins) are restricted to the vasculature, allowing an oncotic pressure gradient to form across the capillary endothelium. As the blood enters the capillary, its net hydrostatic pressure is greater than its net oncotic pressure, resulting in the movement of fluid (water and solutes, but only a small fraction of plasma protein) from the vasculature to the interstitium (52). The hydrostatic pressure undergoes a steady decline due to friction and the loss of volume to the tissue as the blood proceeds through the capillary. This fluid loss concomitantly leads to an increase in intravascular oncotic pressure relative to the hydrostatic pressure, resulting in the flow of fluid from the tissue back into the circulation at the venous end of the capillary. The small protein portion of the plasma filtrate that enters the interstitium at the arterial end of the capillary is removed from the interstitium by the lymphatic system and returned to the plasma via the lymph ducts, thus perpetuating the oncotic gradient and preventing edema (53, 54). Furthermore, the lymphatic ducts respond actively to elevations in the volume of interstitial fluid by facilitating flow along the lymph channels by muscular contraction and tissue movements (55).

Electron micrographs of capillary beds have been used to demonstrate that capillary permeability to plasma solutes is largely a function of morphology, e.g., liver capillaries allow passage of higher molecular weight solutes into the lymph ducts relative to the solutes found in muscle capillary effluence (56, 57). The efflux of fluid from the system described by Starling is not confined to the systemic capillary beds; Weiner (15) reported that an overdose of epinephrine may produce fatal pulmonary edema resulting from elevated pulmonary capillary filtration pressure.

The loss of plasma from the system following administration of epinephrine was reflected in a significant increase in the hematocrit of all the animals given this treatment. For the intact animals, the extent of this rise due to plasma loss could not be determined with certainty because of the apparent epinephrine-induced splenic contraction and mobilization of sequestered red cells to the circulating blood which resulted in a significant increase in red cell volume. The volume of red cells apparently mobilized was slight, on the order of 0.5 ml. Due to the potential influence of the anesthetic xylazine upon the tone of splenic smooth muscle, it is not possible to determine the splenic sequestration capacity with certainty using this model. While the splenectomized animals also experienced a significant epinephrine-induced rise in Hct, they experienced no significant change in the circulating RCV over the time course of the experiment; therefore, this effect appears to be solely a product of Starling effects.

The response of the animals administered an intra-arterial dose of isoproterenol is believed to have been mediated by the β adrenergic receptors of the heart and vasculature (15). Following injection, a positive chronotropic response was observed, apparently as a result of β_1-mediated stimulation of the sino-atrial node and the Purkinje fibers; this increase in rate was likely coupled to a positive inotropic effect (15). Since isoproterenol almost exclusively stimulates the β_2 adrenoceptors in the vasculature, the generic response is vasodilation (due to a lowering of vascular smooth muscle tone), especially in the skeletal muscle but also in the renal and mesenteric vascular beds (15). Thus, the increased force and frequency of cardiac contraction increased the systolic pressure, but the lower vascular resistance resulted in such a drop in diastolic pressure that the mean arterial pressure was lowered. Nevertheless, cardiac output has been shown to rise as a result of an increase in venous return (15). The loss of tone in the systemic vascular

beds apparently led to the observed increase in the circulating plasma volume and concomitant drop in the relative red cell concentration.

In addition to these cardiac and vascular effects, isoproterenol also has been shown to cause β_2-mediated relaxation of organ smooth muscle (when tone is high) in the bronchi, gastrointestine, and the splenic capsule (21). Since the circulating RCV of the intact group showed almost no deviation following administration of isoproterenol, it is speculated that there was no anesthetic-induced increase in splenic capsule tone. The seemingly paradoxical significant difference in the red cell volume over time of the splenectomized animals receiving isoproterenol (Table 9) is attributable to error associated with a lone sampling time point. Comparison of the splenectomized subgroup's sample means via the Newman-Keuls test revealed that only the 10-minute sampling point was significantly different from the basal mean (Table 5).

An isoproterenol treatment (0.15 mg/kg) model for guinea pigs has been reported by Hayes (36) with doses of ketamine and xylazine equal to 40% and 50%, respectively, of those used in the present study. In addition to reporting a positive inotropic effect, Hayes observed an increase in heart rate of ≈40% (the intact set in the present study had a heart rate increase of ≈80%). Since the peak rates were almost identical (Hayes (36) reported a range of 290-310 BPM, while the intact animals in the present study had peak values of 312 ± 20.1 BPM), the percentage increase was greater in this study. The relatively lower basal rates observed in this study were apparently due to higher doses of the anesthetics.

Critique of Methods

Surgical Preparation

The preparation of choice for introduction of stimuli and sampling, an acute surgically embedded catheter, necessitated anesthetization of the subject. While a chronic catheter preparation that would allow for sampling of conscious animals was feasible, it was deemed inappropriate for the present study. A conscious guinea pig would be likely to respond to environmental stimuli and any resulting excitation would not only affect the measures but also could result in dislodgment of the catheter.

The anesthetics utilized for the surgical preparation and experimentation, ketamine hydrochloride and xylazine

hydrochloride, form a common combination that has been used effectively in a variety of animals ranging from reptiles and birds to mammals (58). Ketamine induces a cataleptoid state, with profound analgesia, but unfortunately may also enhance the muscle tone. Xylazine complements ketamine by acting as a muscle relaxant while synergistically deepening the level of anesthesia (59). D'Alleinne and Mann (22) found that when using this combination of anesthetics, the duration of anesthesia produced is apparently dependent upon the dose of ketamine, with xylazine having a relatively slower onset and shorter half-life. The most profound sedation in this study is believed to have coincided with the catheterization procedure (15 to 45 minutes after the IP injection), after which the animals were not directly manipulated. The duration of the anesthesia at the dose administered is approximately 2.5 to 3.0 hours (22), a time frame that approximates the duration of the present study.

When ketamine and xylazine are used in tandem, they have been shown to produce depression of heart rate and mean arterial pressure (47, 60). Using an isolated, perfused guinea pig heart model, Chamberlain et al. (61) demonstrated myocardial depression of contractile rate and coronary blood flow following administration of ketamine. Xylazine has been shown to decrease the guinea pig's heart rate, apparently via a decrease in sympathetic input to the heart and an increase in vagal activity (37, 62).

An increase in splenic smooth muscle tone following administration of xylazine has been demonstrated by Hubbell and Muir (47) using a canine model. Using adrenoceptor agonist blocking agents, these investigators demonstrated that the spleen is sensitive to xylazine; dose-dependent splenic contraction was observed following xylazine stimulation of α_1 adrenergic receptors. The duration and degree of potential xylazine-induced splenic contraction in the present study is not known; therefore, the epinephrine-induced rise in circulating RCV cannot be interpreted as reflecting the total splenic sequestration capacity. At the same time, it is speculated that there was no significant anesthetic-induced increase in splenic tone, since isoproterenol administration did not demonstrate a β_2-mediated decrease in splenic tone that would have precipitated a decline of circulating RCV of the intact animals.

Hematocrit

The hematocrit, as determined by centrifugation of whole blood, must be corrected for plasma trapped in the

red cell column of the microhematocrit capillary tube. Correction factors have evolved since Chapin and Ross (63) first proposed their usage as the development of capillary tubes and high-speed centrifuges refined the separation of the red cells from the plasma component. The trapped plasma correction factor used in this study, 0.97, was derived by Hannon et al. (26) using a procedure similar to that developed by Baker (64).

The Hct was used to calculate all blood volumes. The direct measure of red cell volume protocol used in this study called for injection of tagged autologous RBC's and, following complete dilution, extraction of an arterial blood dilution sample. The Hct (corrected for trapped plasma) of this sample was used to determine the volume of red cells in the aliquot used for spectrophotometric analysis. The activity per unit volume of this aliquot was divided into the injected dose activity to determine the whole body RCV, from which PV and WBV were calculated.

When calculating plasma and whole blood volume from direct red cell volume measures, some investigators employ a correction factor to large vessel Hct measures to account for the ratio "F_{cells}." F_{cells} is defined as the ratio of the whole body hematocrit (Hct_{WB}) to the large vessel hematocrit (Hct_{Lv}) (65). The whole body hematocrit is commonly calculated following simultaneous, independent, double-isotope measurements of the red cell and plasma volumes by division of the RCV by the sum of the two volumes.

The Hct_{WB} is consistently found to be smaller than Hct_{Lv} due to a higher plasma concentration in the microcirculation relative to the large vessel circulation. Generally, the hematocrit decreases as the vessel diameter becomes smaller, a phenomenon first described by Fahraeus and Lindqvist (66). The decreasing of vessel diameter (capillary diameters are on the order of 5 μm, which is smaller than that of the RBC) contributes to an increase in shear stress and shear rate which results in dispersal of any rouleaux (67) and deformation of the red cells (68). Lowering of the red cell concentration decreases the viscosity of blood, which in turn increases its fluidity. As per Poiseuille's law, flow volume of a fluid in a tube is *(a)* directly proportional to the pressure drop along the length of the tube and to the fourth power of the radius of the tube, and *(b)* is inversely proportional to the length of the tube and the viscosity of the fluid. Thus, while hydrostatic pressure and geometric limitations of the vasculature are the primary determinants of flow, the resultant stresses upon the blood effect transitory,

dynamic viscosity alterations which in turn facilitate flow. Lipowsky et al. (69) found that the ratio of the cat microvessel Hct to Hct_{Lv} reached a nadir of 0.21 in the 10.0-μm diameter postcapillaries. Parenthetically, it should be noted that while the overall microvascular Hct is lower than the Hct_{Lv}, it reflects a heterogeneous distribution of RBC's; a "plasma skimming" mechanism has been proposed whereby Hct's may range from naught to theoretical unity in different parts of the same capillary network at a given instant (70).

Elucidation of a correction factor for the ratio F_{cells} is complicated due to methodological problems related to defining plasma volume. Elution of labeled plasma from the system and possible intravascular binding of the labeled species (especially proteins) appear to contribute to PV estimations that exceed the dilution space of blood, per se, the intravascular space. Swan and Nelson (71) reported an inverse relationship between the size of the diluent and the plasma volume calculated: Evans Blue PV or ^{131}I-HSA PV > ^{131}I-globulin PV > ^{131}I-fibrinogen PV > ^{51}Cr-RBC and Hct_{Lv}-derived PV. Likewise, Baker and Wycoff (72) reported ratios for F_{cells} that increased when larger plasma labels were utilized. Labeled plasma moieties have characteristic diluent concentration regression curves that require retrograde extrapolation to determine a "zero time concentration." This procedure appears to contribute to methodological error due to difficulty in choosing a sample point or points representative of the transitory concentration at which there is complete dilution and negligible effective loss of label from the system.

In the present study it was decided not to employ a correction for Hct_{WB}. Experimental elucidation of the guinea pig ratio F_{cells} via simultaneous, independent, double-isotope measures was beyond the scope of this project. A guinea pig ratio F_{cells} of 0.82 has been reported in the literature by Edmondson and Wyburn (28). It was decided not to utilize the correction factor calculated by Edmondson and Wyburn in the present study since *(a)* their ratio was not calculated from simultaneous measures (separate groups were used to measure the PV and RCV) and *(b)* their report does not address how their venous Hct samples were analyzed and whether they corrected for trapped plasma. Baker and Schaefer (30) measured guinea pig blood volume with simultaneous, independent measures (^{125}I-HSA and ^{51}Cr-RBC's) and calculated the Hct_{WB}, which proved to be greater than the measured Hct_{Lv} (corrected for trapped plasma). Had they used these data to calculate F_{cells}, the resulting ratio would have been greater than 1.0, which indicates that there was potential

methodological or reporting error. It was deemed best not to utilize these results in the present study due to the interpretational difficulties cited.

Ratio F_{cells} values reported in the literature derived from studies on other animal models were deemed inappropriate for use in the present study. There is a wide range of correction factors cited in the literature, apparently a result of interspecies and methodological variation. For human subjects, Mayerson et al. (73) reported a Hct_{WB}: Hct_{LV} ratio of 0.915 and Chaplin et al. (74) 0.91, with both investigations using ^{32}P-labeled RBC's and Evans Blue as the plasma diluent. Canine values ranging from 0.85 to 0.97 were reported by Baker and Wycoff (72), using ^{51}Cr-labeled RBC's and Evans Blue and ^{131}I-fibrinogen as the respective plasma labels. For pigs, Bossone (75) calculated a ratio value of 0.70 and Hannon et al. (26) 0.76, with both investigations using ^{51}Cr-labeled RBC's and ^{125}I-HSA as the plasma diluent.

The foregoing discussion of some of the problems related to the determination of whole body red cell concentration is indicative of potential problems that arise in the literature as a result of variations in analytical methodology. For this reason, it was deemed best for this study to rely upon measures that provided ease of sampling, reproducible results, and minimal sources of error. Use of large vessel Hct in this experiment was based on the belief that Hct_{LV} is reflective of the relative Hct of the whole body, and as such would be useful for comparing the systemic erythrocyte concentrations at rest and following catecholamine stimulation. Any underestimation of absolute PV and WBV arising from omission of a correction factor for Hct_{WB} was acceptable, since, as mentioned above, elucidation of the F_{cells} was beyond the scope of the experimental objectives, and the use of correction factors derived from other animal models was considered inappropriate.

Red Cell Volume

The labeling of erythrocytes with ^{51}Cr was first reported by Gray and Sterling (76), who in turn employed the technique to determine circulating red cell volume (24). The red cell's hemoglobin molecule undergoes dissociation into free alpha and beta globin subunits in a mildly acidic solution (77), and when so disposed, ^{51}Cr preferentially combines with the beta chains (78). Thus, CPD, a mildly acidic anticoagulant solution, was included as part of the radiochromium and erythrocyte mixture during incubation. Using the same labeling procedure as that of

this study, Bossone (75) reported a ^{51}Cr-RBC labeling efficiency of 84%, with less than 1% of the ^{51}Cr used for incubation found to be present in the supernatant of the second wash. For this reason, in this study the cells were washed twice following incubation to remove any unbound tag. The quantity of this label eluted over time course of this experiment is negligible (5).

Following injection of the labeled RBC's into the animal, sampling was conducted at 5-minute intervals to determine the basal circulating values. The first of these samples was discarded due to erratic measures. This potential error is believed to have been a result of recirculation of the tracer in the vasculature close to the site of injection or, in the intact animals, possibly a function of red cell splenic transit time. During the period of indicator dilution in the spleen, the circulating indicator concentration declines. Values calculated from samples taken prior to complete dilution would tend to either under- or overestimate the actual circulating RCV, depending on whether the sample concentration was higher or lower than the "complete" systemic dilution concentration.

The red cell volume was diminished by approximately 0.12 ml each time a 0.3-sample of whole blood was extracted; the cumulative RCV depletion for nine samples was approximately 1.08 ml. The Hct and volume measures, as reported, do not include any correction for this sampling loss. The isotonic saline used to replenish the sample volume is believed to have left the vasculature rather rapidly; therefore, the Hct values are believed to reflect only negligible error due to saline introduction.

TABLE 1.

Measurement accuracy of 0.2 and 1.0 ml positive-displacement transfer pipettes.

Trial*	0.2 ml		1.0 ml	
	dH_2O	Blood	dH_2O	Blood
1	0.1986	0.1995	1.0017	1.0038
2	0.2013	0.2013	1.0046	1.0065
3	0.1987	0.1996	0.9963	0.9969
4	0.2004	0.2004	1.0024	1.0048
5	0.2016	0.2020	0.9972	0.9995
mean ± SD	0.2001 ± 0.0014	0.2006 ± 0.0011	1.0004 ± 0.0035	1.0023 ± 0.0040

* Each trial, in ml, represents the average of three measurements. Replacement pipette capillary tips were used for each trial. Within each trial the same tip was used to measure the dH_2O and, following thorough room-air drying, the porcine blood.

TABLE 2.

Comparison of the control means of the intact and splenectomized animals.

Measurement	Spleen Intact mean ± SD	Splenectomized mean ± SD	t value
Hematocrit*	38.6 ± 3.9	41.2 ± 3.4	1.59
Red Cell Volume*	17.5 ± 2.3	19.2 ± 1.8	1.90
Plasma Volume*	29.2 ± 2.1	29.0 ± 2.4	0.22
Whole Blood Volume*	46.6 ± 2.9	48.2 ± 2.7	1.25
Heart Rate^	184 ± 31.8	155 ± 19.0	2.24#
Mean Art. Pressure^	49.1 ± 6.2	47.5 ± 5.2	0.60

Hematocrits are percentages; volumes are ml/kg; heart rates are beats/min; mean arterial pressures are mm Hg.

* df = 18 (n = 10 for each group).

^ df = 16 (n = 8 intact, n = 10 splenectomized animals).

# Indicates a significant difference ($P<0.05$) when df = 16 $t \geq 2.12$, df = 18 $t \geq 2.10$.

TABLE 3.

Three-factor analysis of variance summary for condition, treatment, and time.

	CON	TRT	T	CON x TRT	CON x T	TRT x T	CON x TRT x T
				F Ratios			
Hct	1.21	4.83*	34.69*	<0.01	5.97*	29.73*	4.93*
RCV	2.50	0.17	2.59*	0.17	1.75	2.08	3.50*
PV	<0.01	11.97*	35.08*	0.53	5.16*	20.43*	2.85*
WBV	1.22	8.28*	33.43*	0.95	4.59*	16.28*	2.24*
HR	5.89#	4.77#	80.79#	1.83	0.51	7.44#	0.87
MAP	2.66	189.8#	20.54#	1.20	0.29	59.65#	0.27

* Indicates a significant effect ($P<0.05$):

CON, $F_{1,16} \geq 4.49$; TRT, $F_{1,16} \geq 4.49$; T, $F_{6,96} \geq 2.20$;

CON x TRT, $F_{1,16} \geq 4.49$; CON x T, $F_{6,96} \geq 2.20$;

TRT x T, $F_{6,96} \geq 2.20$; CON x TRT x T, $F_{6,96} \geq 2.20$.

# Indicates a significant effect ($P<0.05$):

CON, $F_{1,14} \geq 4.60$; TRT, $F_{1,14} \geq 4.60$; T, $F_{6,84} \geq 2.22$;

CON x TRT, $F_{1,14} \geq 4.60$; CON x T, $F_{6,84} \geq 2.22$;

TRT x T, $F_{6,84} \geq 2.22$; CON x TRT x T, $F_{6,84} \geq 2.22$.

TABLE 4.

Two-factor analysis of variance summary for the condition (intact vs. splenectomized) and time responses to epinephrine.

Measurement	Condition *F* Ratio	Time *F* Ratio	CON x T *F* Ratio
Hematocrit	0.50	33.56*	5.28*
Red Cell Volume	2.06	2.31*	2.85*
Plasma Volume	0.39	30.45*	4.82*
Whole Blood Volume	6.47*	27.99*	4.28*
Heart Rate	3.28	15.88#	0.65
Mean Art. Pressure	1.95	31.55#	0.22

* Indicates a significant effect ($P<0.05$):

CON, $F_{1,8} \geq 5.32$; Time, $F_{6,48} \geq 2.30$;

CON x T, $F_{6,48} \geq 2.30$.

# Indicates a significant effect ($P<0.05$):

CON, $F_{1,6} \geq 5.99$; Time, $F_{6,36} \geq 2.37$;

CON x T, $F_{6,36} \geq 2.37$.

TABLE 5.

Newman-Keuls comparison of the basal and treatment means.

Measurement Subgroup	Postinjection Treatment Measures					
	Minutes post treatment					
	2.5	5	10	20	40	80
Hematocrit						
Intact, Epinephrine	*	*	-	-	-	-
Splen., Epinephrine	*	*	*	*	-	-
Intact, Isoproterenol	-	-	-	-	-	-
Splen., Isoproterenol	*	*	*	*	*	*
Red Cell Volume						
Intact, Epinephrine	-	*	-	*	*	*
Splen., Epinephrine	-	-	-	-	-	-
Intact, Isoproterenol	-	-	-	-	-	-
Splen., Isoproterenol	-	-	*	-	-	-
Plasma Volume						
Intact, Epinephrine	*	*	-	-	-	-
Splen., Epinephrine	*	*	*	-	-	-
Intact, Isoproterenol	-	-	-	-	*	*
Splen., Isoproterenol	*	*	*	*	*	*
Whole Blood Volume						
Intact, Epinephrine	*	*	-	-	-	-
Splen., Epinephrine	*	*	*	*	-	-
Intact, Isoproterenol	-	-	*	-	*	*
Splen., Isoproterenol	*	*	*	*	*	*
Heart Rate						
Intact, Epinephrine	*	*	*	-	-	-
Splen., Epinephrine	*	*	*	*	-	*
Intact, Isoproterenol	*	*	*	*		-
Splen., Isoproterenol	*	*	*	*	*	-
Mean Arterial Pressure						
Intact, Epinephrine	*	*	*	-	-	-
Splen., Epinephrine	*	*	*	-	-	-
Intact, Isoproterenol	*	*	-	-	-	-
Splen., Isoproterenol	*	*	-	-	-	-

* Indicates a significant difference from the basal mean.

- Indicates no significant difference from the basal mean.

TABLE 6.

Two-factor analysis of variance summary for the condition (intact vs. splenectomized) and time responses to isoproterenol.

Measurement	Condition *F* Ratio	Time *F* Ratio	CON x T *F* Ratio
Hematocrit	0.73	27.81*	6.00*
Red Cell Volume	0.66	2.36*	2.42*
Plasma Volume	0.22	22.81*	2.51*
Whole Blood Volume	<0.01	19.72*	1.99
Heart Rate	2.12	132.09*	0.35
Mean Art. Pressure	0.30	27.80*	0.18

* Indicates a significant effect ($P<0.05$):

CON, $F_{1,8} \geq 5.32$; Time, $F_{6,48} \geq 2.30$;

CON x T, $F_{6,48} \geq 2.30$.

TABLE 7.

Two-factor analysis of variance summary for the treatment (epinephrine vs. isoproterenol) and time responses in the intact group.

Measurement	Treatment *F* Ratio	Time *F* Ratio	TRT x T *F* Ratio
Hematocrit	2.26	12.96*	8.79*
Red Cell Volume	<0.01	3.58*	1.48
Plasma Volume	6.54*	12.67*	5.32*
Whole Blood Volume	4.73	12.94*	4.41*
Heart Rate	0.17	36.54#	3.96#
Mean Art. Pressure	62.47#	6.82#	22.02#

* Indicates a significant effect ($P<0.05$):

TRT, $F_{1,8} \geq 5.32$; Time, $F_{6,48} \geq 2.30$;

TRT x T, $F_{6,48} \geq 2.30$.

# Indicates a significant effect ($P<0.05$):

TRT, $F_{1,6} \geq 5.99$; Time, $F_{6,36} \geq 2.37$;

TRT x T, $F_{6,36} \geq 2.37$.

TABLE 8.

Two-factor analysis of variance summary for the treatment (epinephrine vs. isoproterenol) and time responses in the splenectomized group.

Measurement	Treatment *F* Ratio	Time *F* Ratio	TRT x T *F* Ratio
Hematocrit	2.61	27.20*	25.29*
Red Cell Volume	0.44	1.12	3.77*
Plasma Volume	5.82*	27.09*	17.43*
Whole Blood Volume	4.20	24.19*	13.39*
Heart Rate	16.44*	46.12*	4.42*
Mean Art. Pressure	159.48*	15.72*	40.79*

* Indicates a significant effect ($P < 0.05$):

TRT, $F_{1,8} \geq 5.32$; Time, $F_{6,48} \geq 2.30$;

TRT x T, $F_{6,48} \geq 2.30$.

TABLE 9.

Single-factor analysis of variance summary for repeated measures over time.

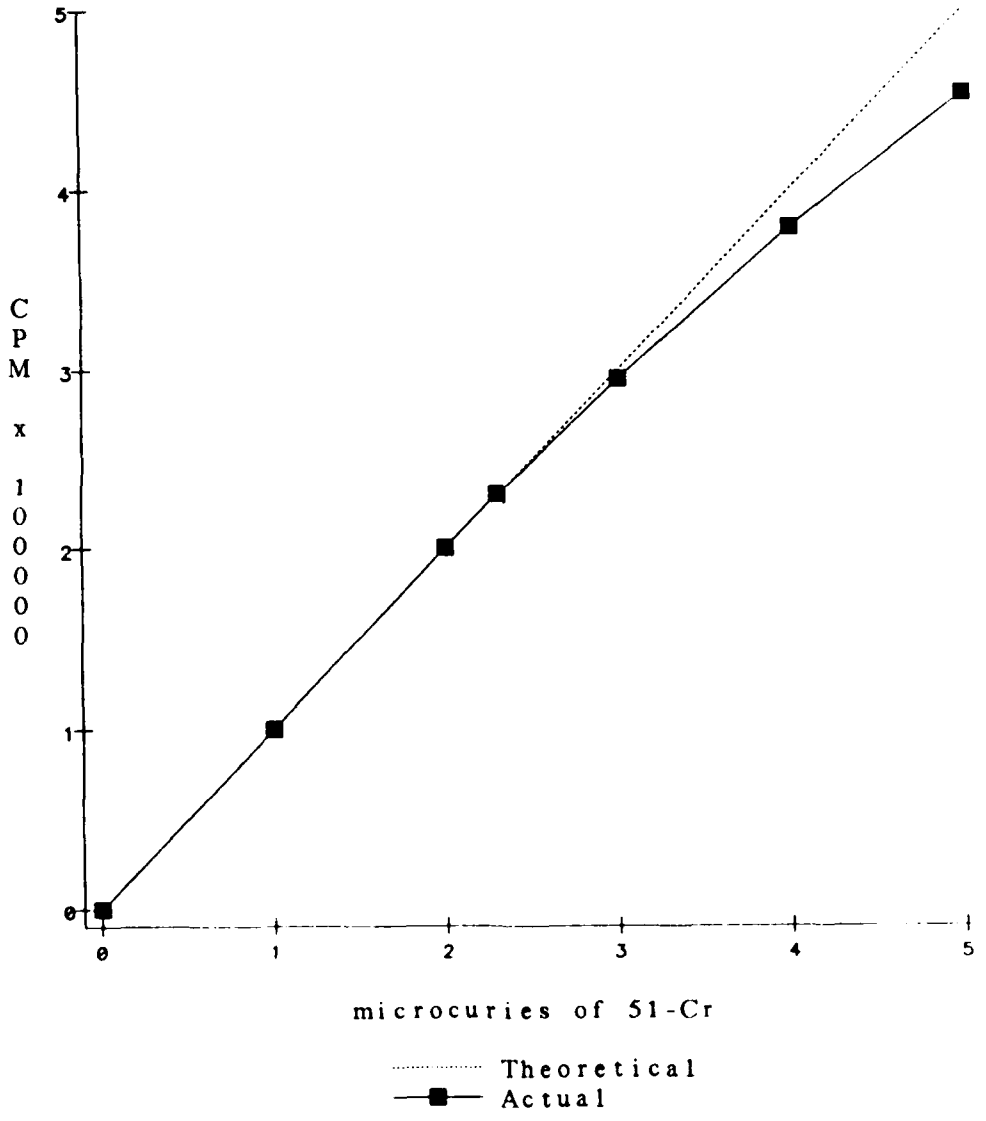
Measurement	Subgroup (Splenic Condition, Treatment)	Time *F* Ratio
Hematocrit	Intact, Epinephrine	13.55*
	Splenectomized, Epinephrine	24.23*
	Intact, Isoproterenol	4.19*
	Splenectomized, Isoproterenol	34.96*
Red Cell Volume	Intact, Epinephrine	3.14*
	Splenectomized, Epinephrine	2.06
	Intact, Isoproterenol	1.80
	Splenectomized, Isoproterenol	2.75*
Plasma Volume	Intact, Epinephrine	11.90*
	Splenectomized, Epinephrine	21.69*
	Intact, Isoproterenol	5.41*
	Splenectomized, Isoproterenol	24.26*
Whole Blood Volume	Intact, Epinephrine	11.49*
	Splenectomized, Epinephrine	19.07*
	Intact, Isoproterenol	5.59*
	Splenectomized, Isoproterenol	18.12*
Heart Rate	Intact, Epinephrine	5.78#
	Splenectomized, Epinephrine	11.77*
	Intact, Isoproterenol	66.16*
	Splenectomized, Isoproterenol	66.28*
Mean Art. Pressure	Intact, Epinephrine	8.53#
	Splenectomized, Epinephrine	28.05*
	Intact, Isoproterenol	12.46*
	Splenectomized, Isoproterenol	31.40*

* Indicates a significant effect ($P<0.05$): $F_{6,24} \geq 2.51$.

# Indicates a significant effect ($P<0.05$): $F_{6,12} \geq 3.00$.

FIGURE 1

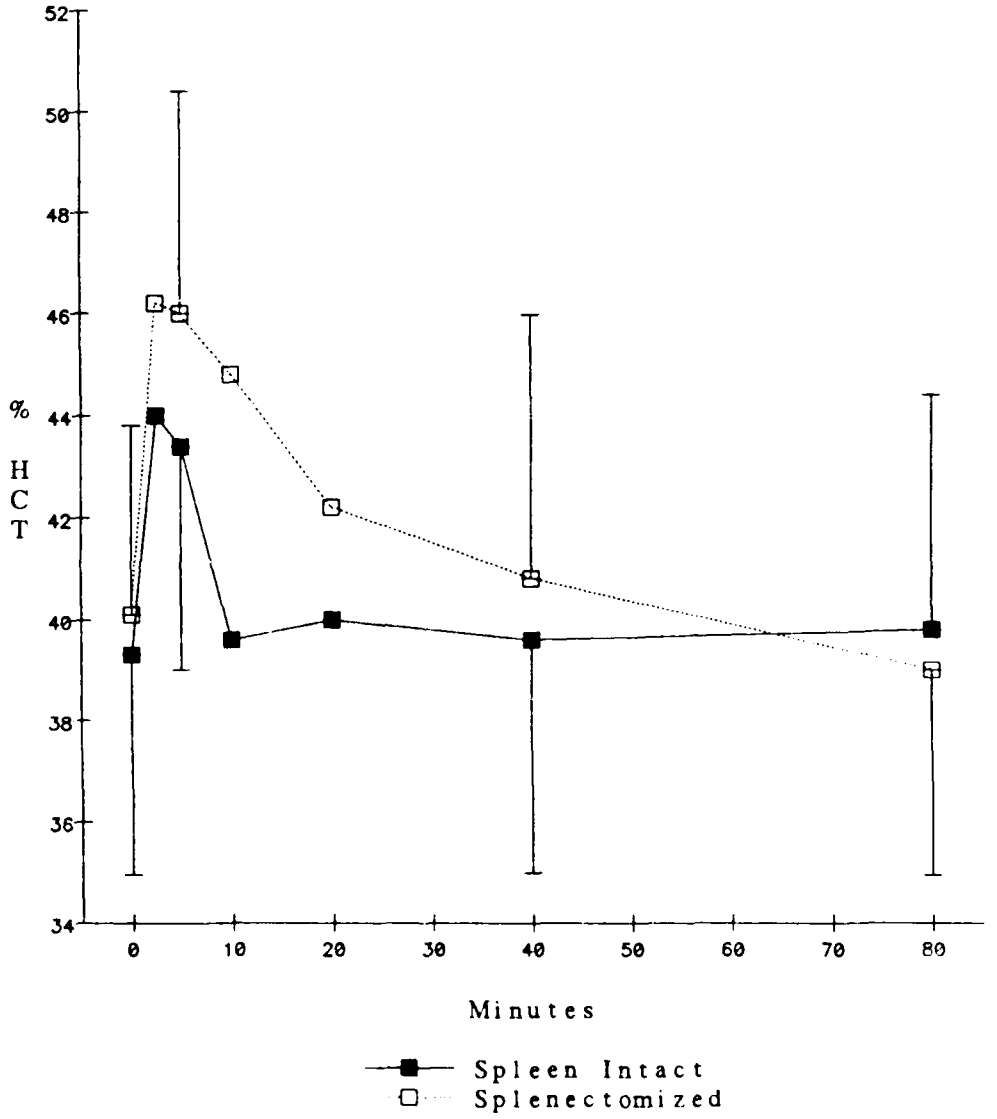
SPECTROPHOTOMETER ACCURACY



Actual and theoretical ^{51}Cr counting accuracy at increasing radioactivity levels of the Packard Auto Gamma® 500c scintillation counter.

FIGURE 2

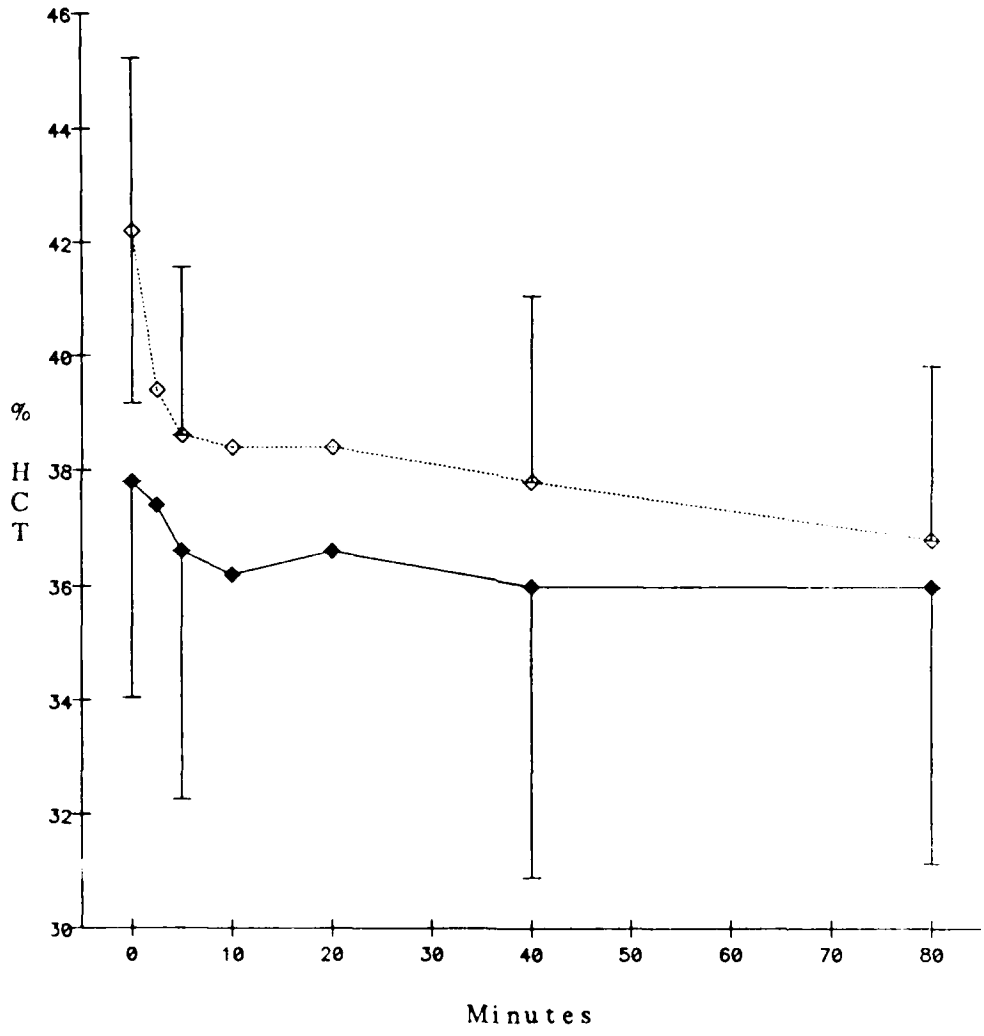
HEMATOCRIT: EPINEPHRINE TREATMENT



Effect of epinephrine upon the hematocrit of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

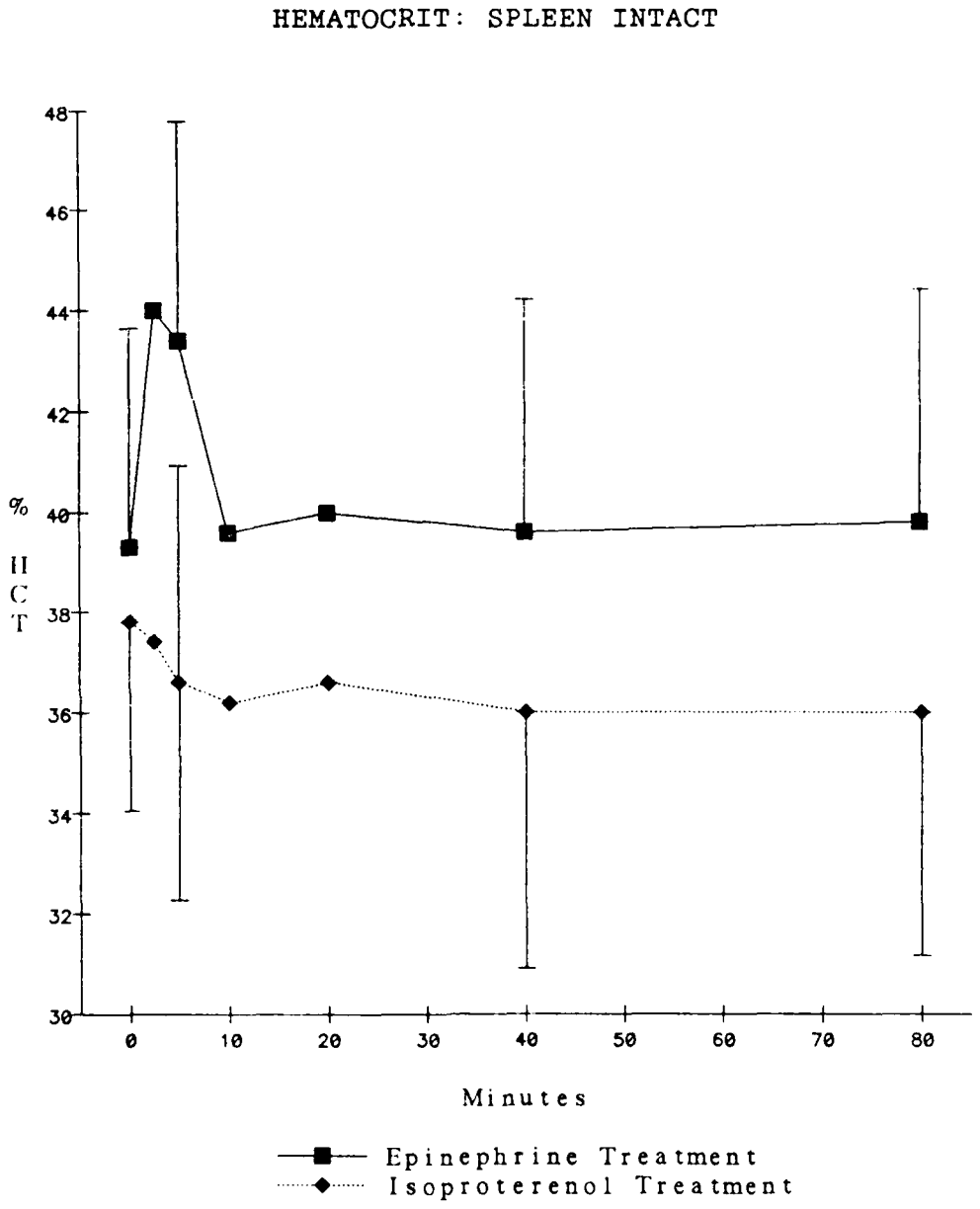
FIGURE 3

HEMATOCRIT: ISOPROTERENOL TREATMENT



Effect of isoproterenol upon the hematocrit of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

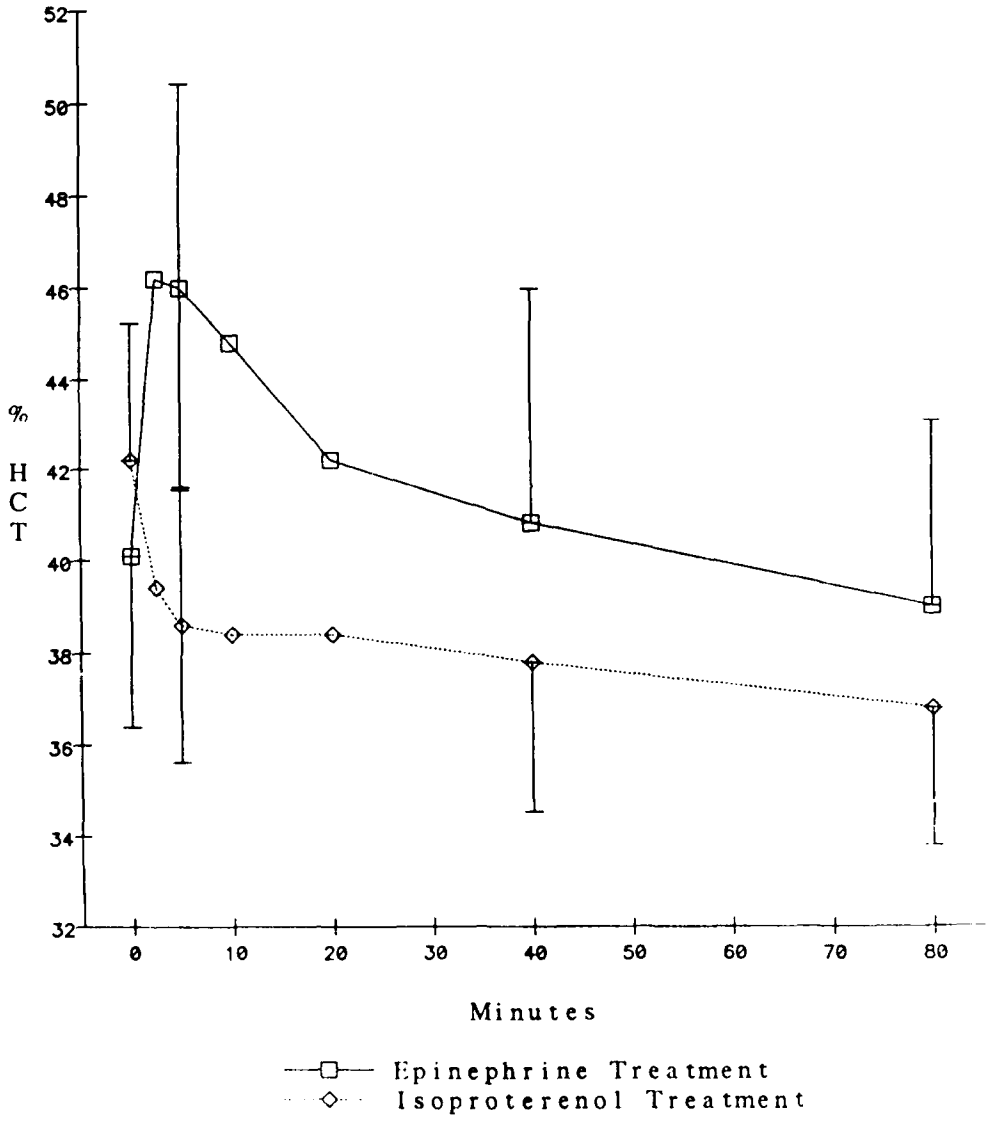
FIGURE 4



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the hematocrit of intact, anesthetized guinea pigs (mean ± standard deviation).

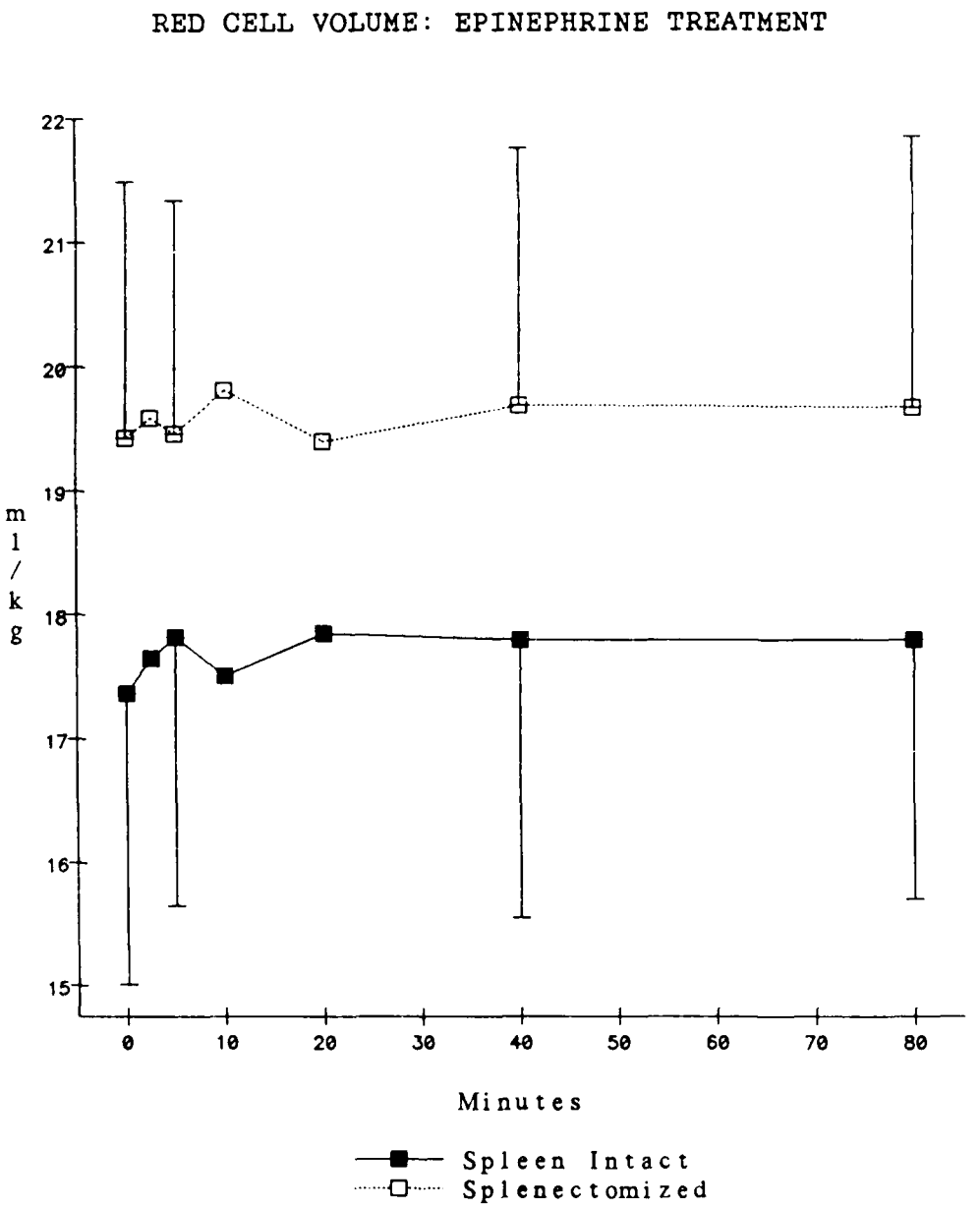
FIGURE 5

HEMATOCRIT: SPLENECTOMIZED ANIMALS



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the hematocrit of splenectomized, anesthetized guinea pigs (mean ± standard deviation).

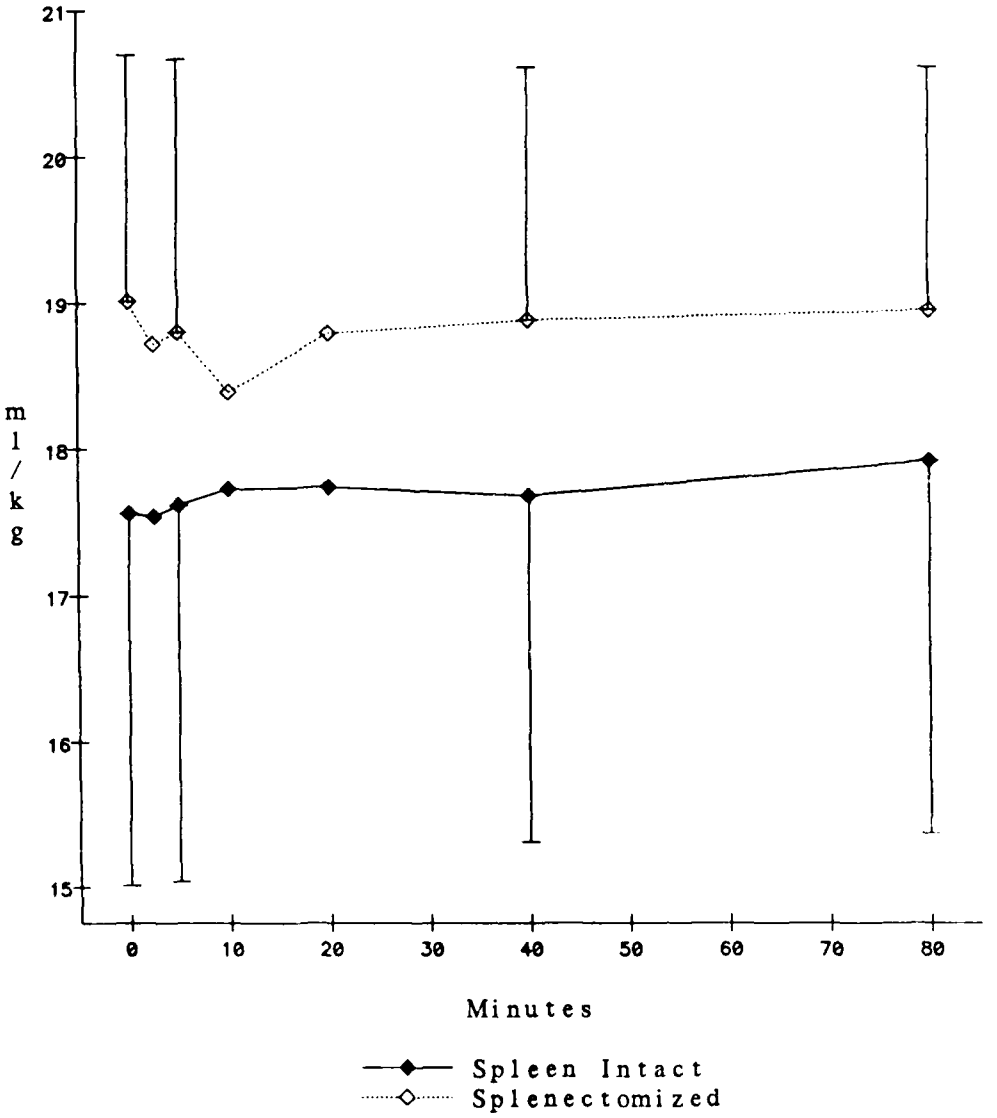
FIGURE 6



Effect of epinephrine upon the red cell volume of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 7

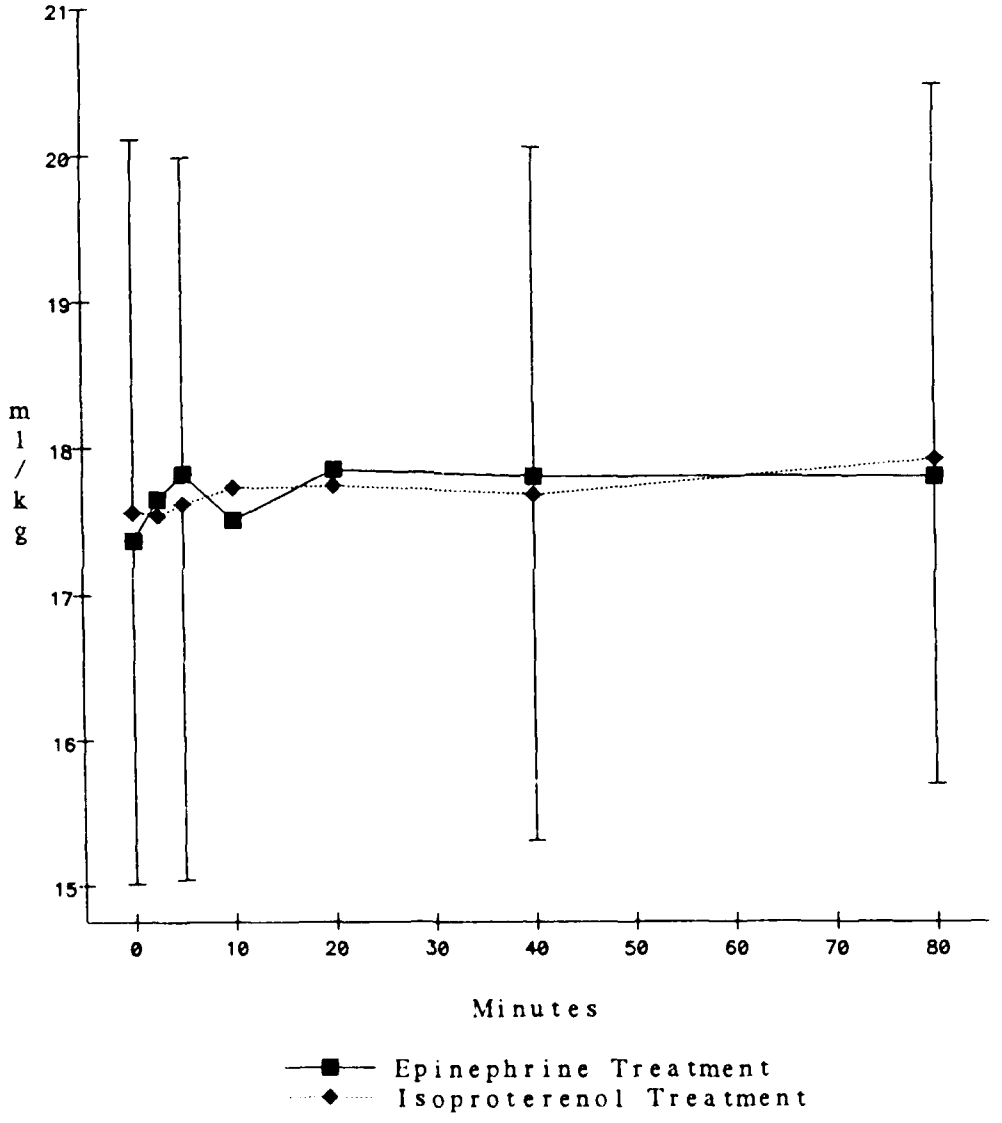
RED CELL VOLUME: ISOPROTERENOL TREATMENT



Effect of isoproterenol upon the red cell volume of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 8

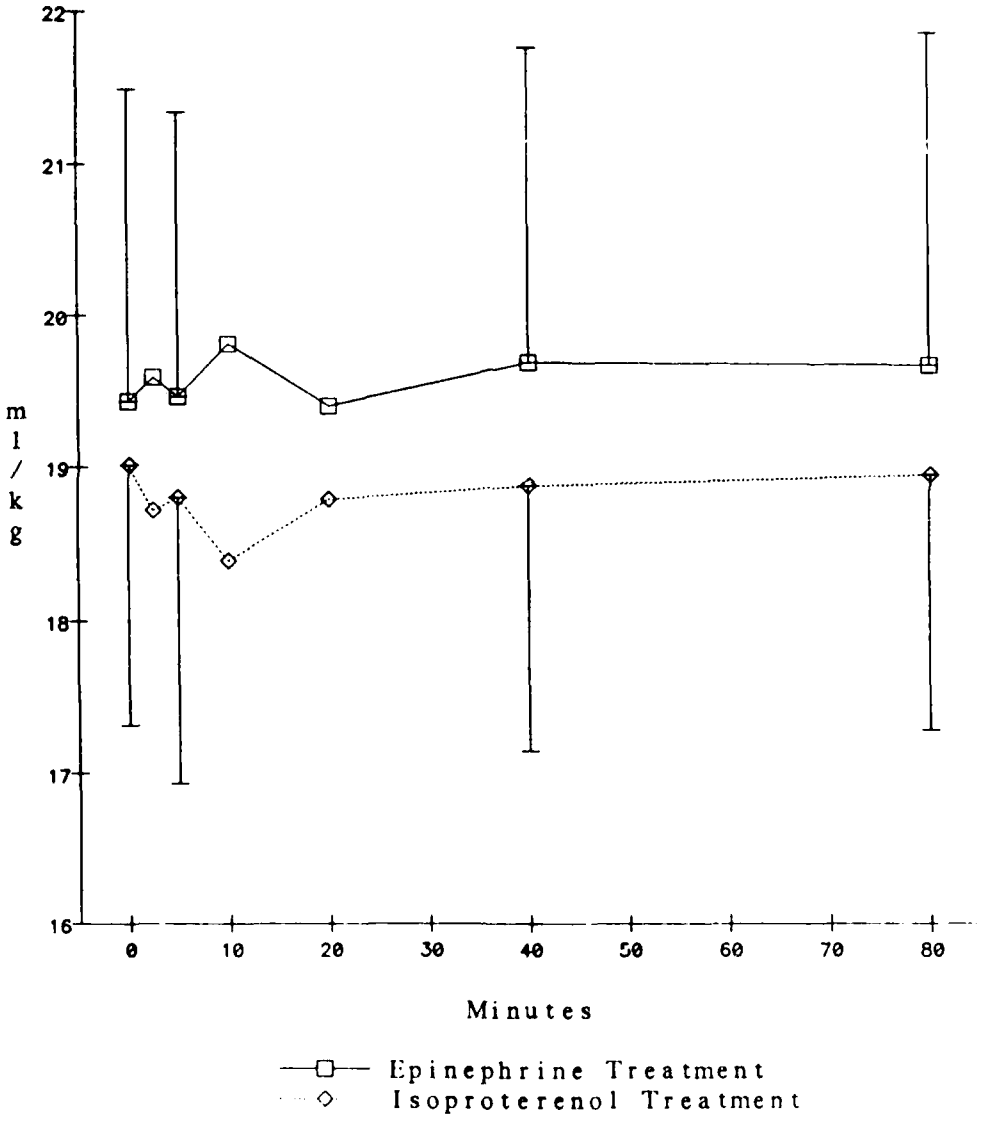
RED CELL VOLUME: SPLEEN INTACT



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the red cell volume of intact, anesthetized guinea pigs (mean ± standard deviation).

FIGURE 9

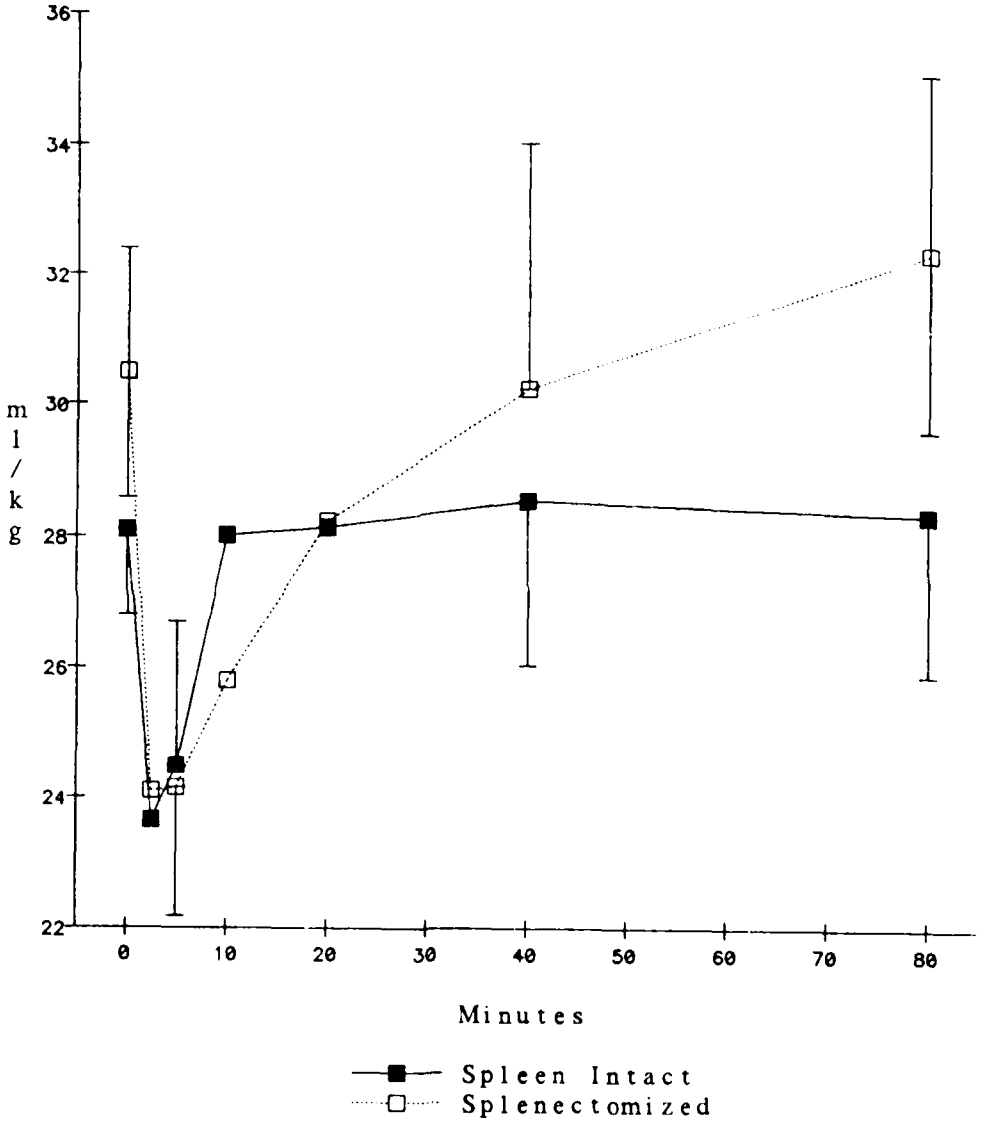
RED CELL VOLUME: SPLENECTOMIZED ANIMALS



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the red cell volume of splenectomized, anesthetized guinea pigs (mean ± standard deviation).

FIGURE 10

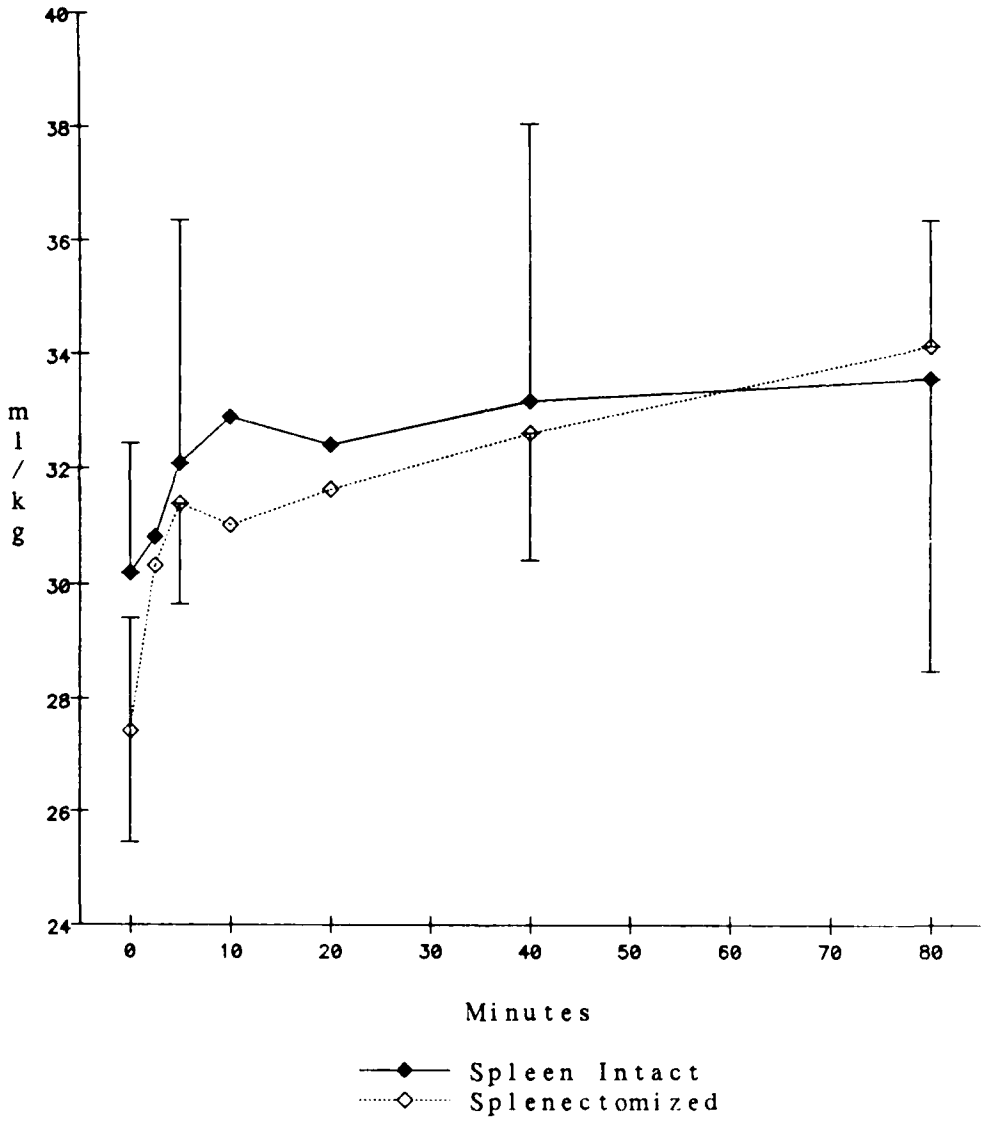
PLASMA VOLUME: EPINEPHRINE TREATMENT



Effect of epinephrine upon the plasma volume of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

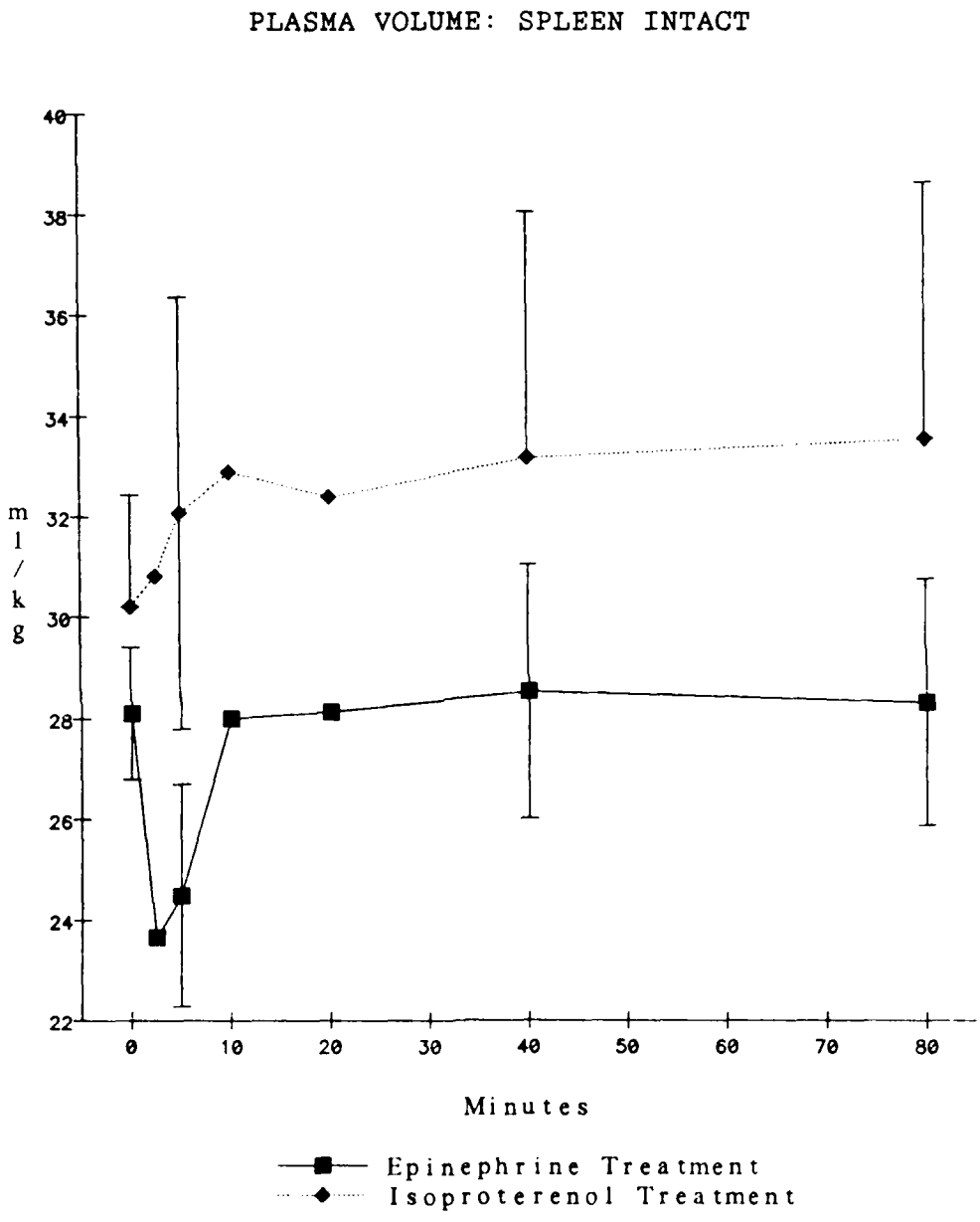
FIGURE 11

PLASMA VOLUME: ISOPROTERENOL TREATMENT



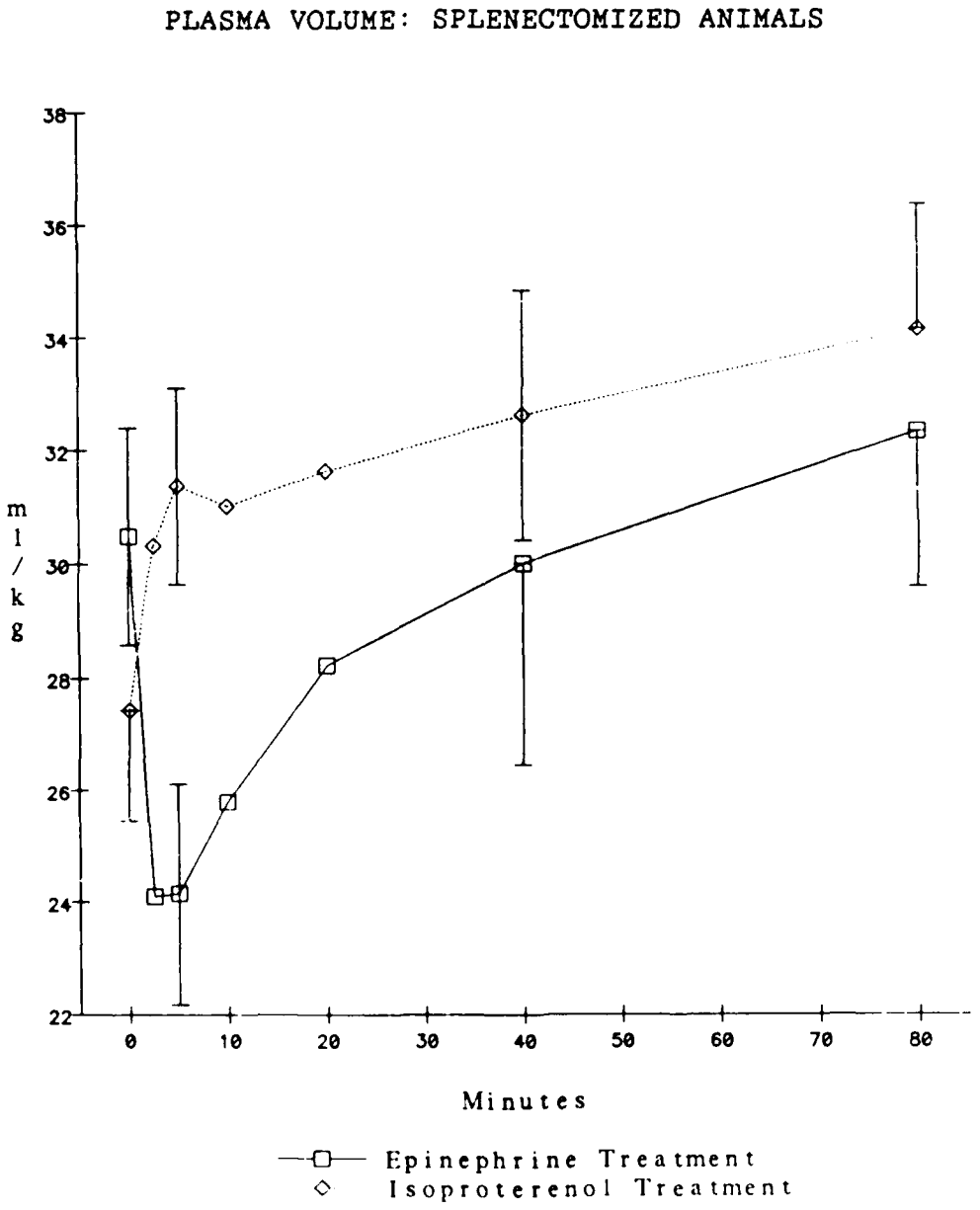
Effect of isoproterenol upon the plasma volume of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 12



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the plasma volume of intact, anesthetized guinea pigs (mean ± standard deviation).

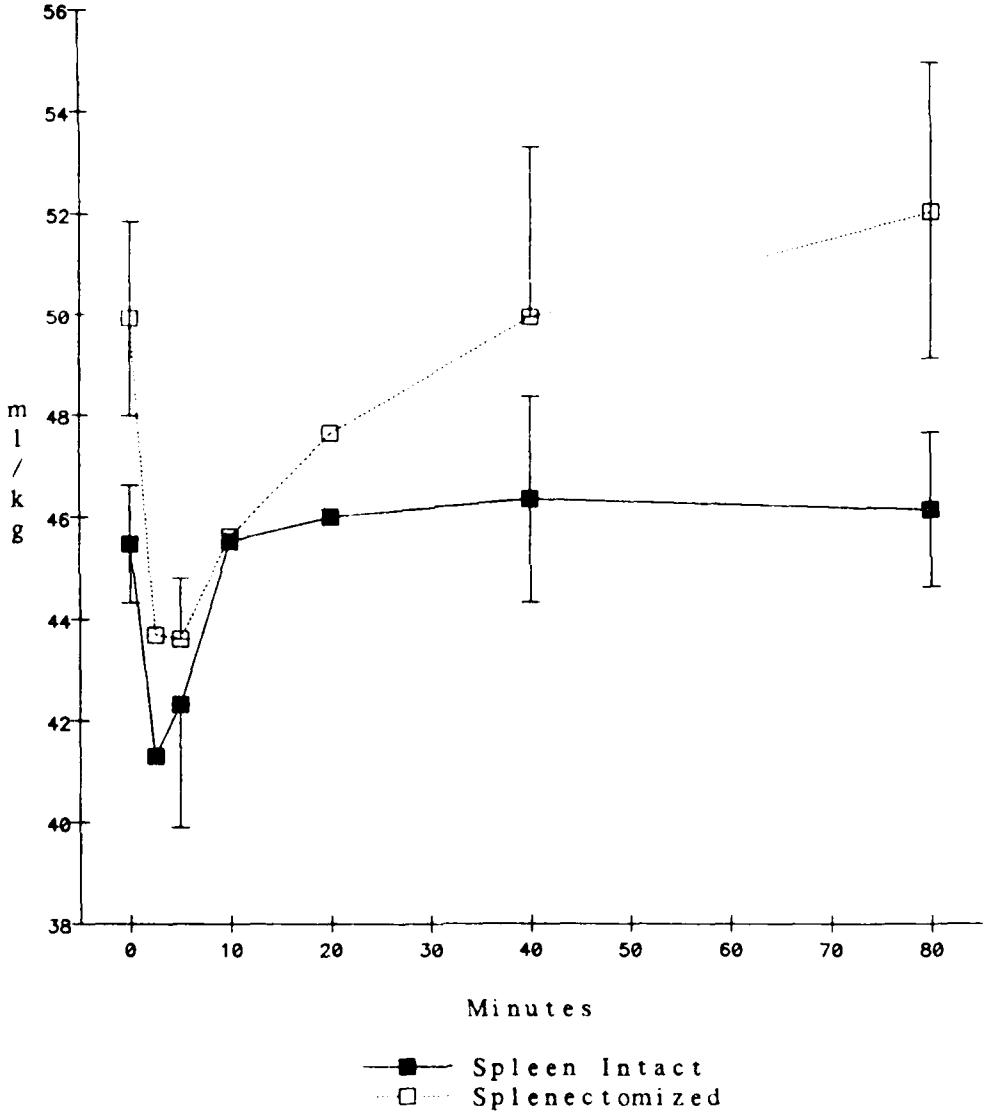
FIGURE 13



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the plasma volume of splenectomized, anesthetized guinea pigs (mean ± standard deviation).

FIGURE 14

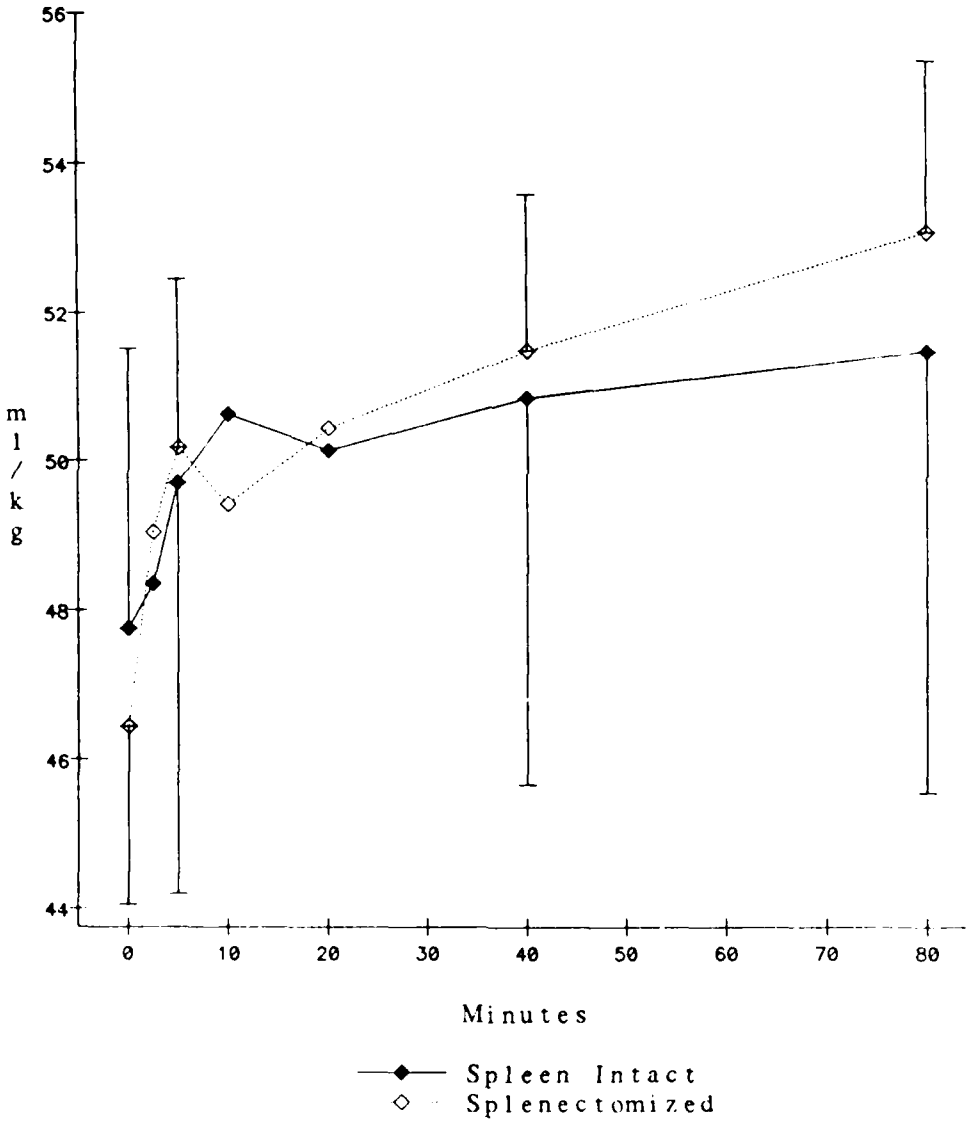
WHOLE BLOOD VOLUME: EPINEPHRINE TREATMENT



Effect of epinephrine upon the whole blood volume of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 15

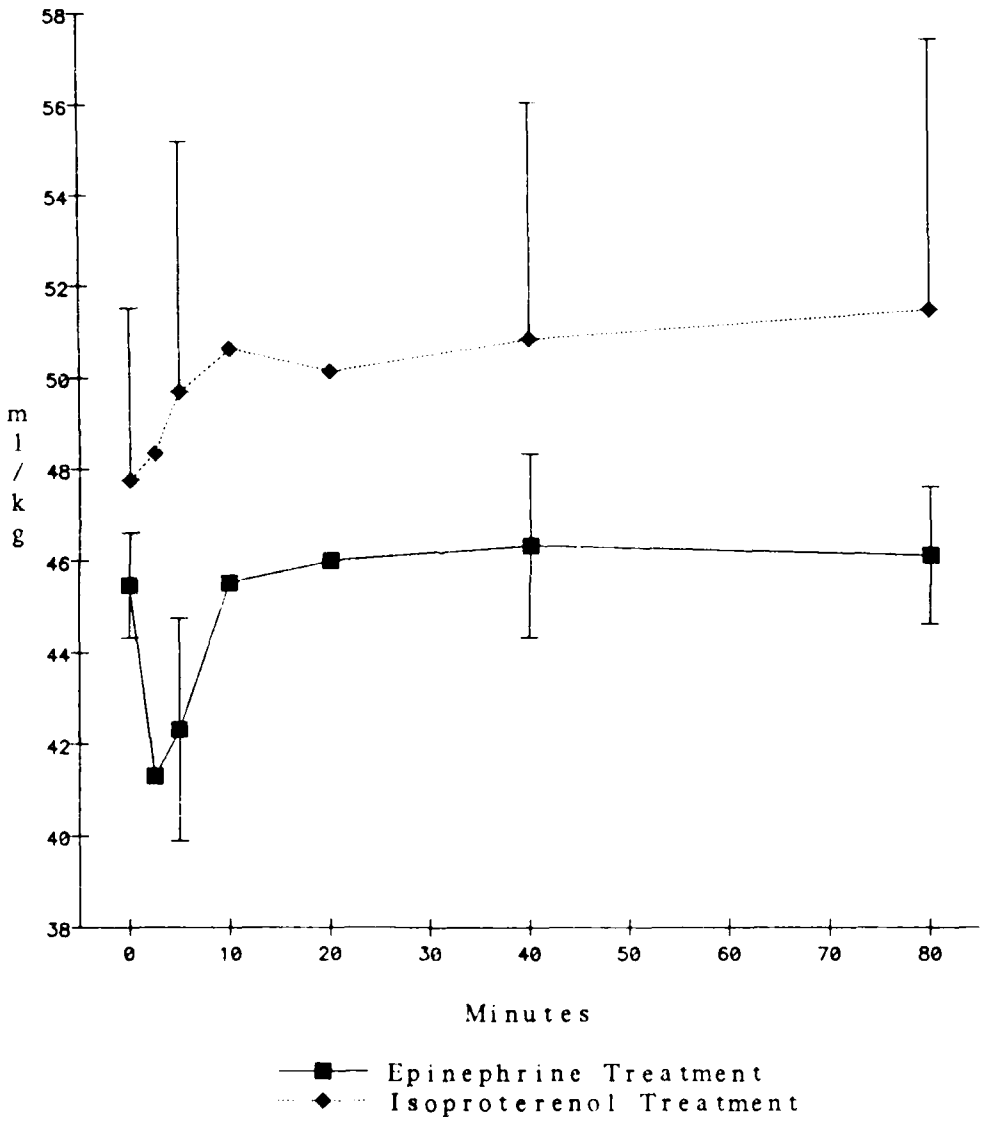
WHOLE BLOOD VOLUME: ISOPROTERENOL TREATMENT



Effect of isoproterenol upon the whole blood volume of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 16

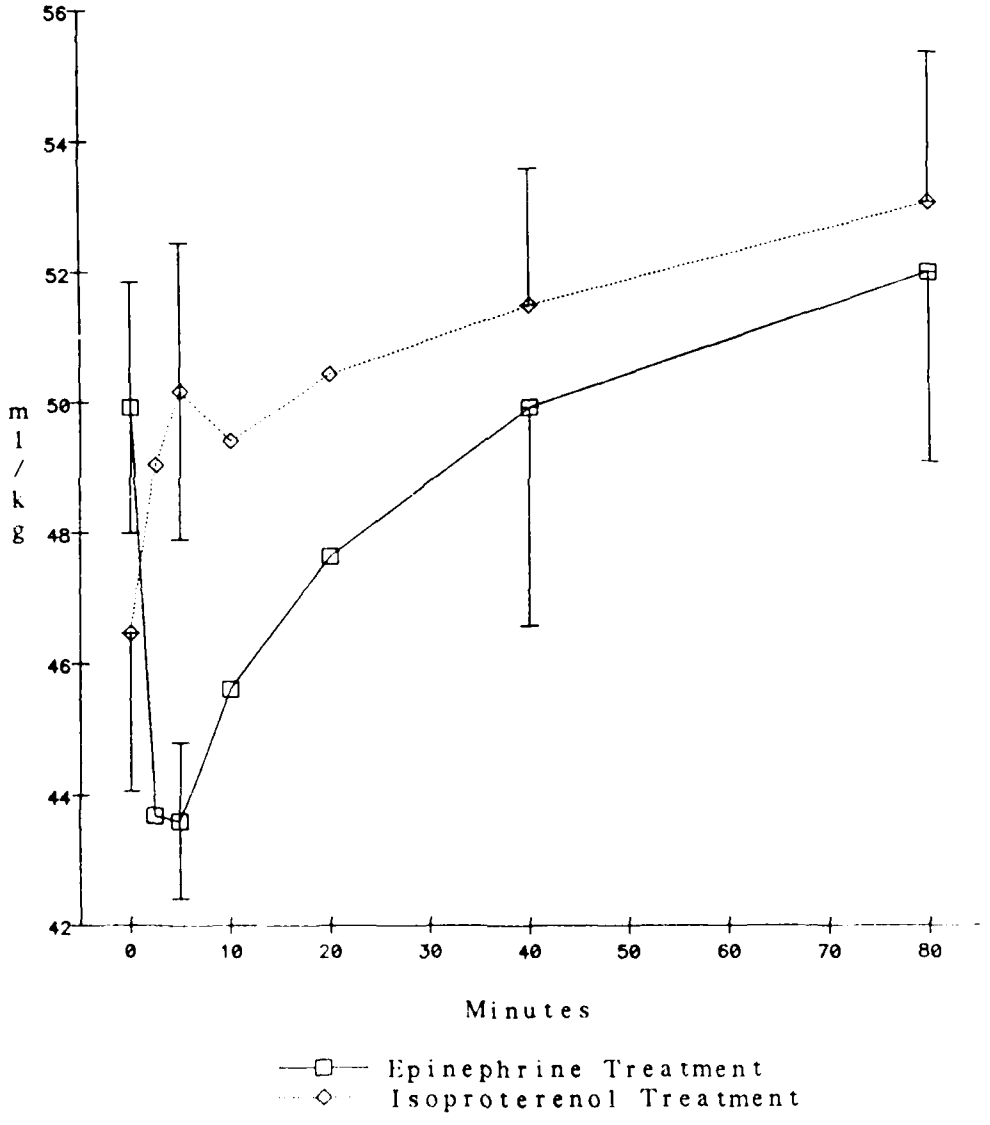
WHOLE BLOOD VOLUME: SPLEEN INTACT



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the whole blood volume of intact, anesthetized guinea pigs (mean ± standard deviation).

FIGURE 17

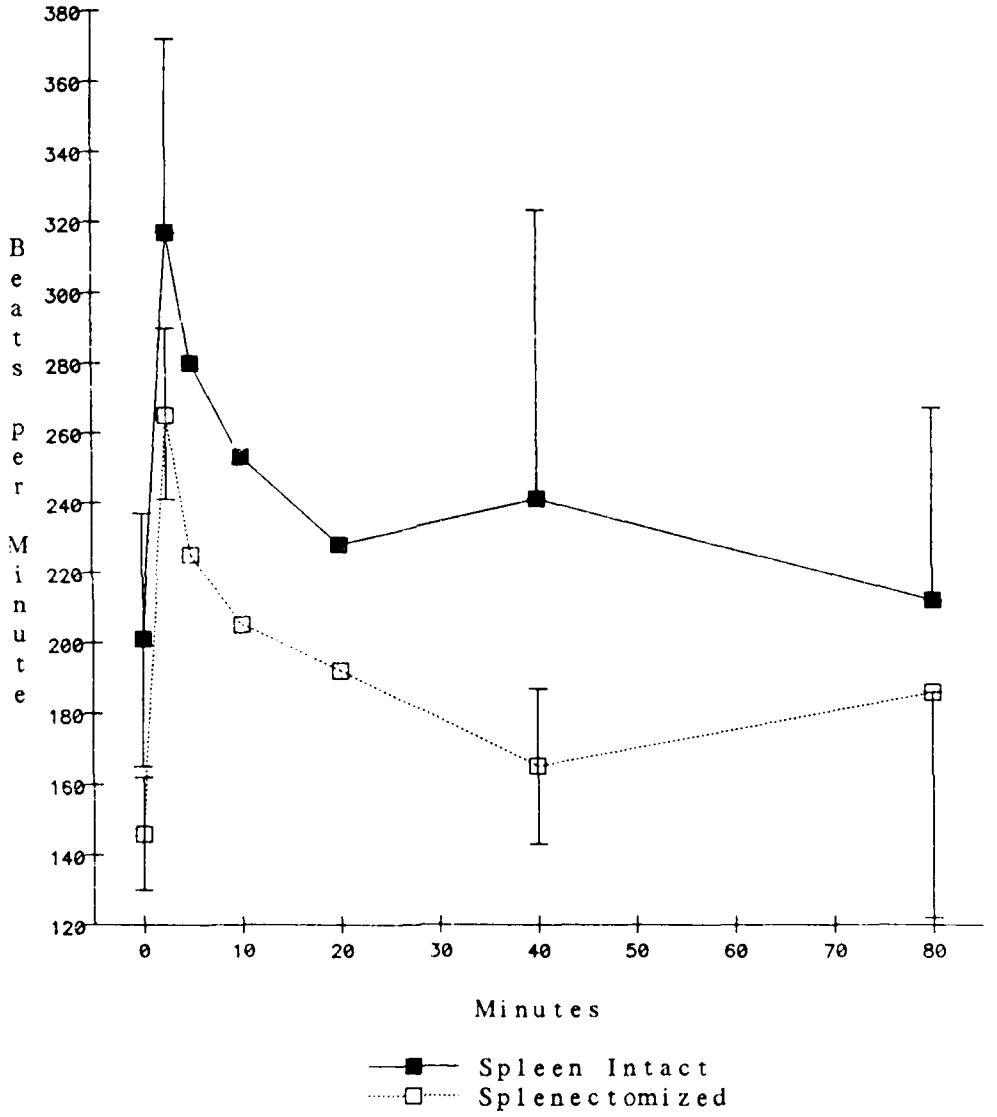
WHOLE BLOOD VOLUME: SPLENECTOMIZED ANIMALS



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the whole blood volume of splenectomized, anesthetized guinea pigs (mean ± standard deviation).

FIGURE 18

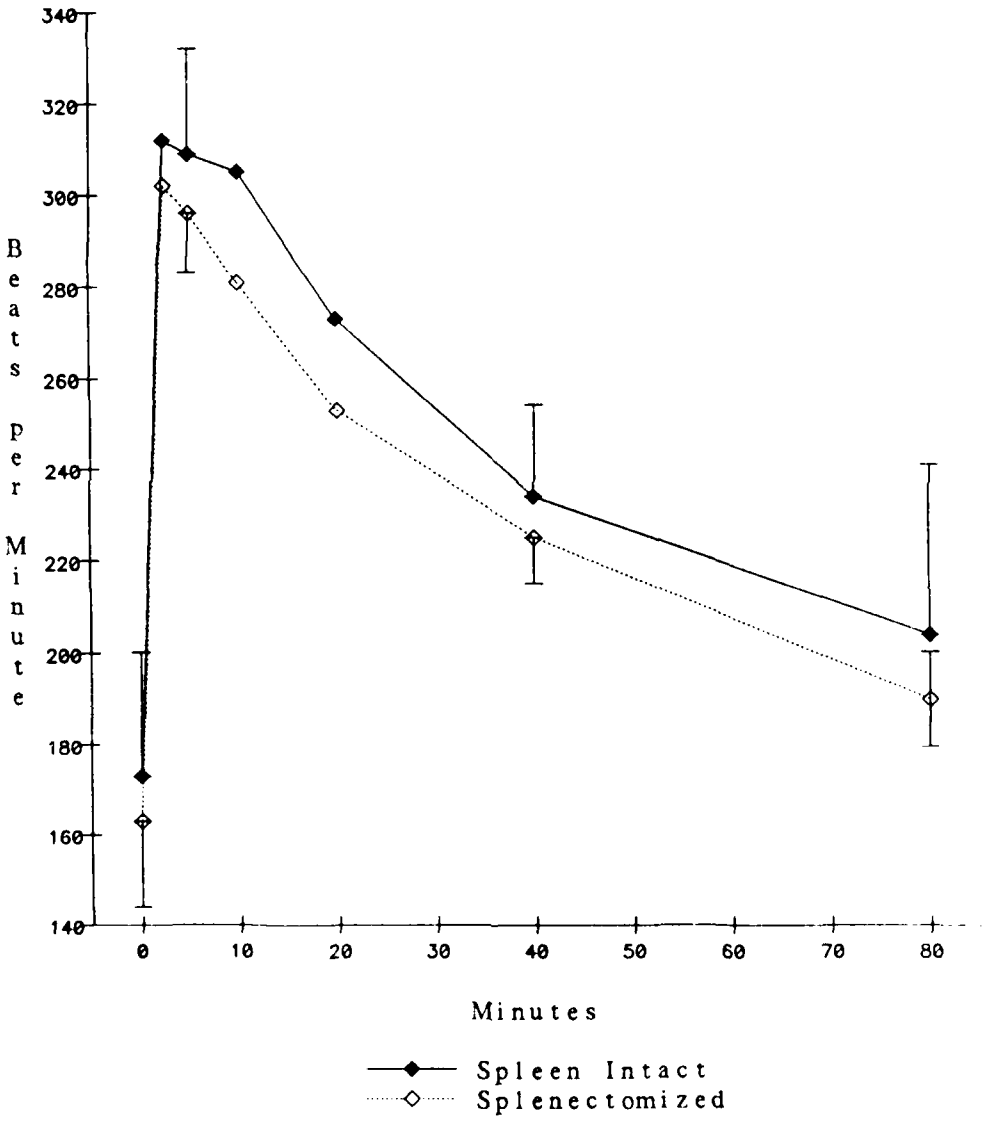
HEART RATE: EPINEPHRINE TREATMENT



Effect of epinephrine upon the heart rate of intact (n=3) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 19

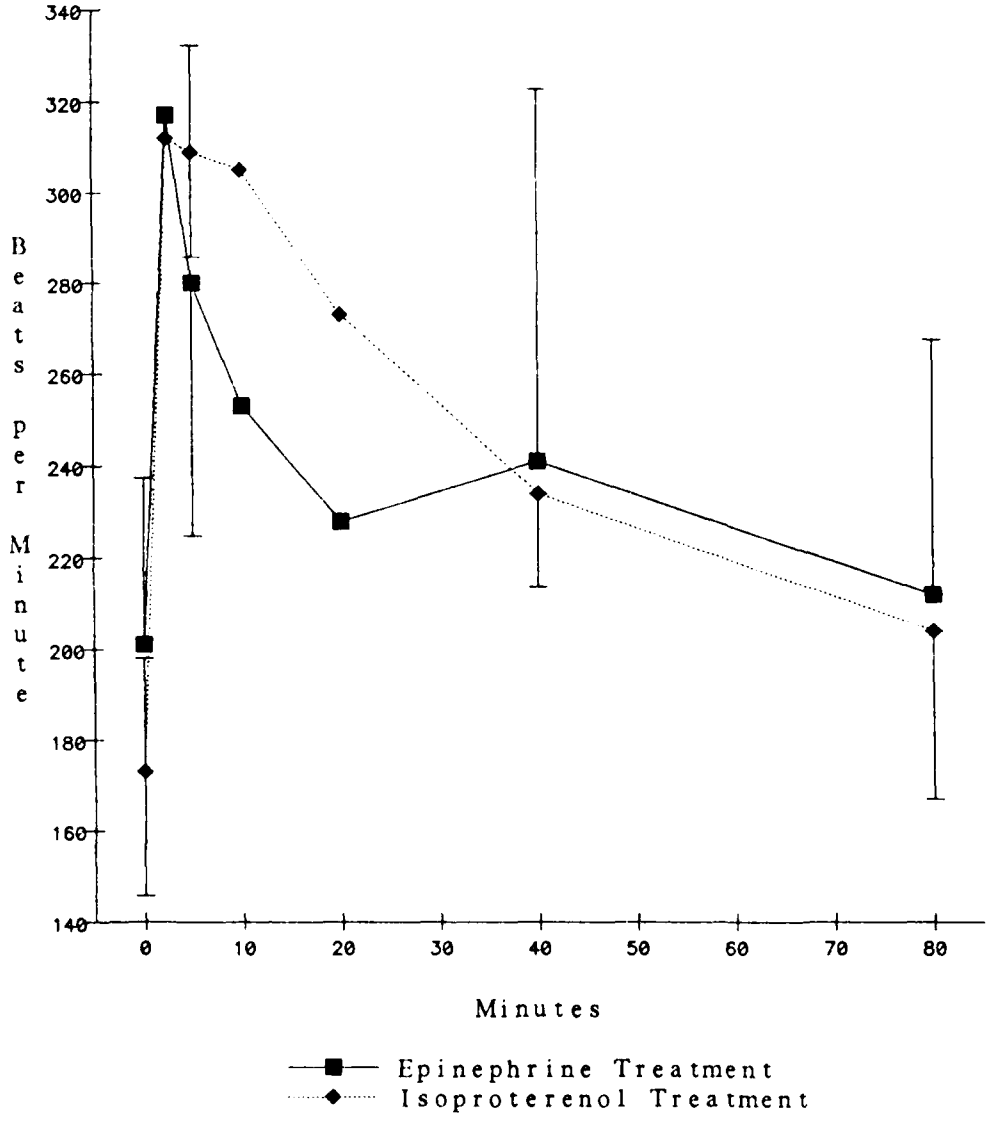
HEART RATE: ISOPROTERENOL TREATMENT



Effect of isoproterenol upon the heart rate of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 20

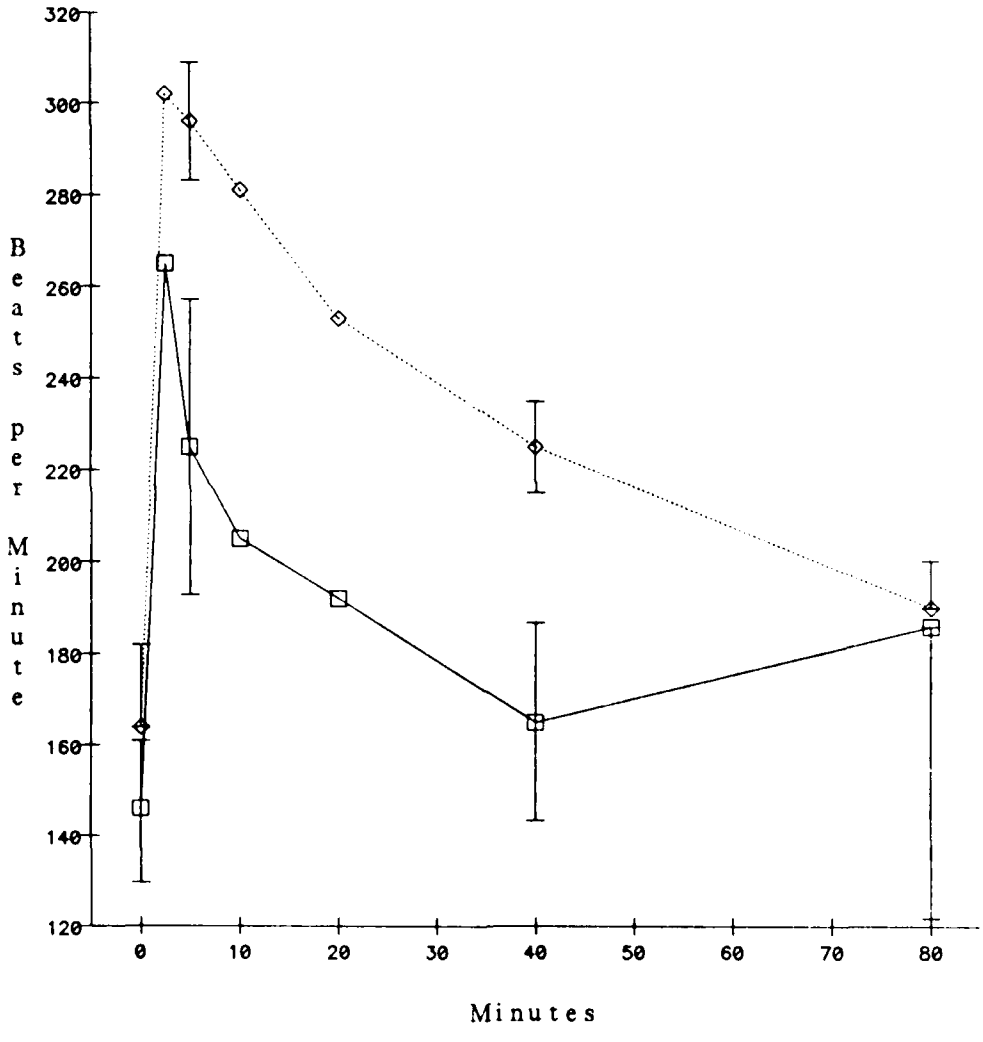
HEART RATE: SPLEEN INTACT



Effect of epinephrine (n=3) and isoproterenol (n=5) upon the heart rate of intact, anesthetized guinea pigs (mean ± standard deviation).

FIGURE 21

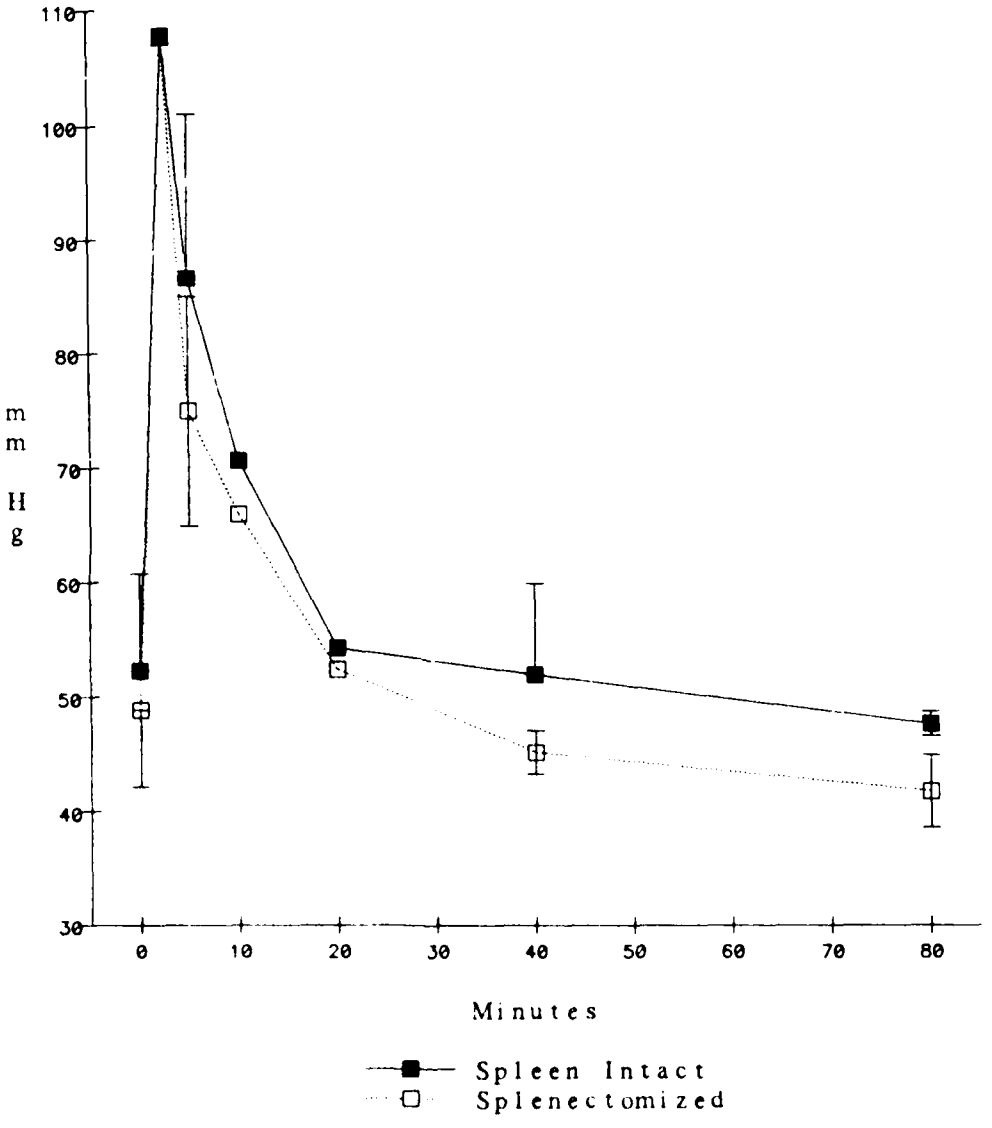
HEART RATE: SPLENECTOMIZED ANIMALS



Effect of epinephrine (n-5) and isoproterenol (n-5) upon the heart rate of splenectomized, anesthetized guinea pigs (mean ± standard deviation).

FIGURE 22

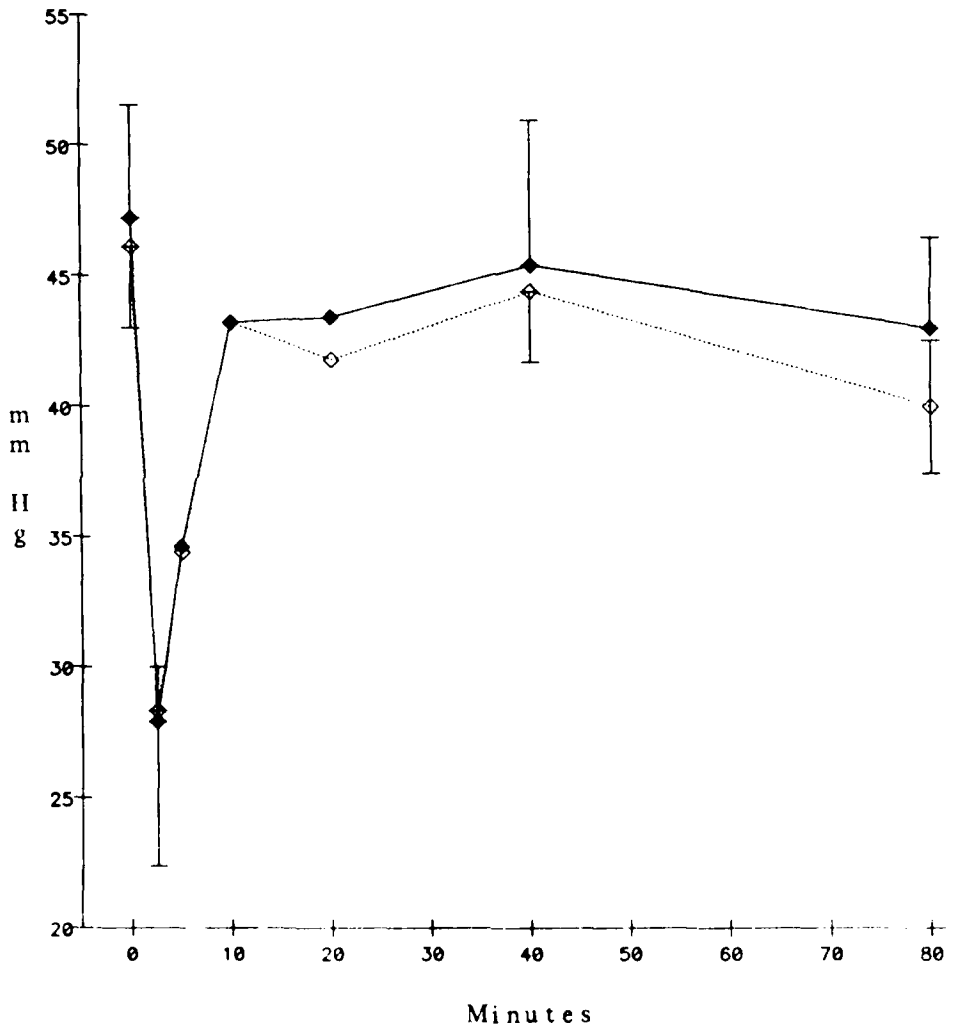
MEAN ARTERIAL PRESSURE: EPINEPHRINE TREATMENT



Effect of epinephrine upon the mean arterial pressure of intact (n=3) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 23

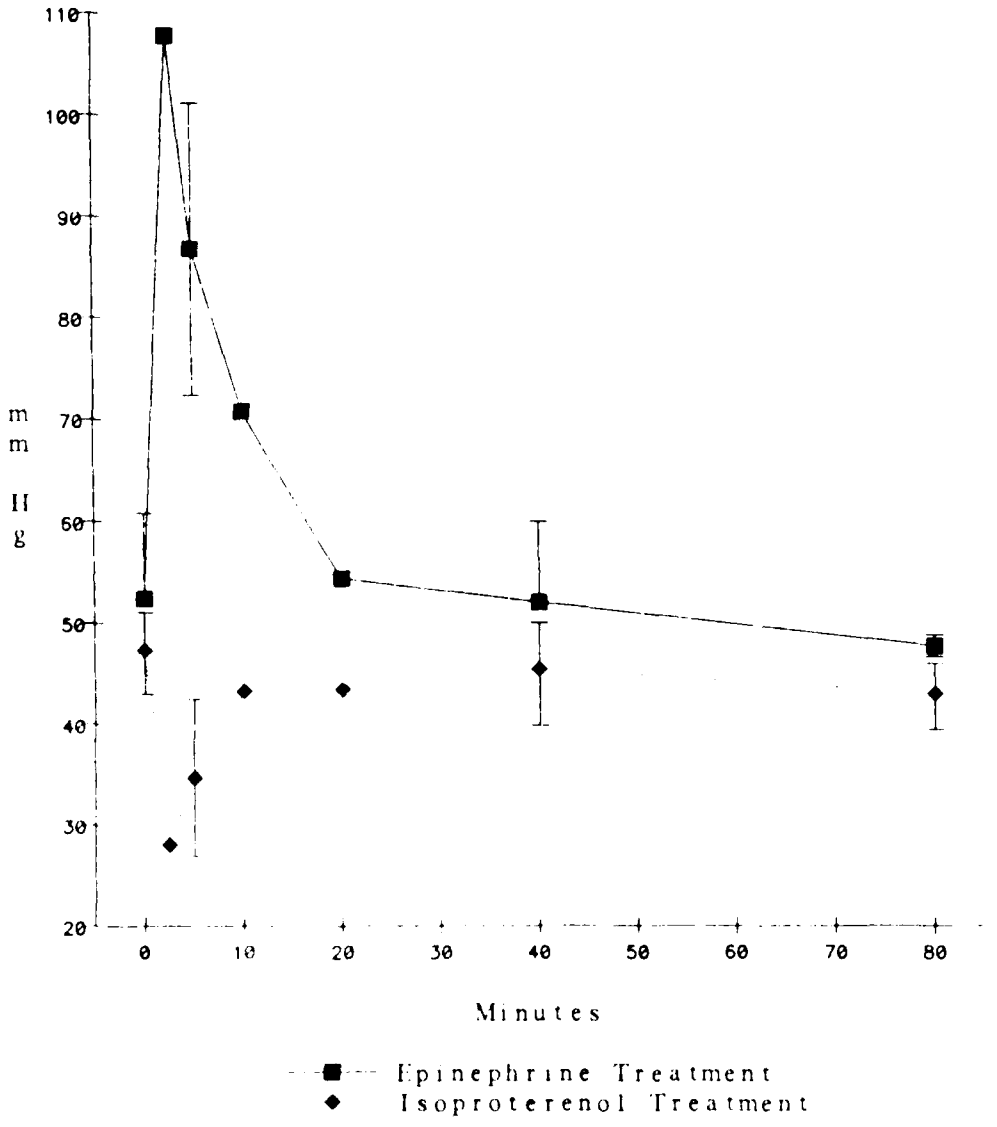
MEAN ARTERIAL PRESSURE: ISOPROTERENOL TREATMENT



Effect of isoproterenol upon the mean arterial pressure of intact (n=5) and splenectomized (n=5) anesthetized guinea pigs (mean ± standard deviation).

FIGURE 24

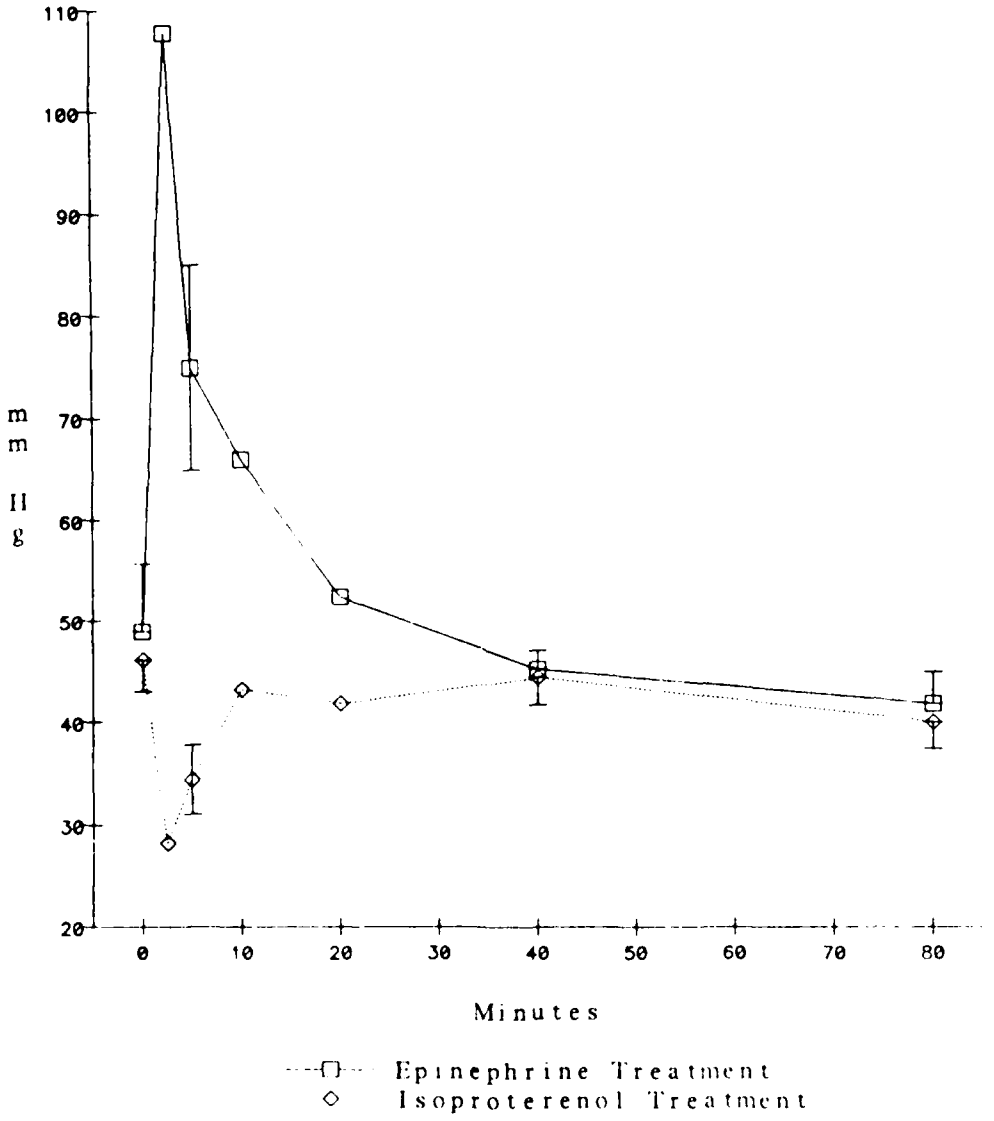
MEAN ARTERIAL PRESSURE: SPLEEN INTACT



Effect of epinephrine (n=3) and isoproterenol (n=5) upon the mean arterial pressure of intact, anesthetized guinea pigs (mean ± standard deviation).

FIGURE 25

MEAN ARTERIAL PRESSURE: SPLENECTOMIZED ANIMALS



Effect of epinephrine (n=5) and isoproterenol (n=5) upon the mean arterial pressure of splenectomized, anesthetized guinea pigs (mean ± standard deviation).

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