PROBLEMS IN AERIAL APPLICATION: I. – V.

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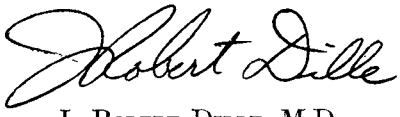

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PROBLEMS IN AERIAL APPLICATION

I. Acute Effects of the Insecticide Endrin on Renal Function and Hemodynamics

I. Introduction.

Renal hemodynamic changes in the dog during the early phase of acute intoxication by endrin,* a chlorinated hydrocarbon insecticide, have been reported by this laboratory.[1] The sympatho-adrenal systems have been assigned a prominent role in renal vascular response, but no attempt has been made to define a priority of function. The present study was therefore designed to evaluate the relative contribution of the neural and humoral components of the renal vascular response to endrin.

II. Methods.

Acute experiments were performed on five intact and six adrenalectomized adult mongrel dogs, 16 to 20 kg in weight (Figure 1). Each animal served as its own control, and effects of various maneuvers were compared to a control period of 10 to 20 minutes prior to administration of endrin. All dogs were anesthetized with sodium pentobarbital** (30 mg/kg) administered intravenously. Convulsions resulting from administration of endrin were controlled by succinylcholine chloride*** (0.5 mg/kg), and respiration was maintained with a Starling respirator using room air. Control samples were taken after a minimum equilibration period of 30 minutes. Previous studies have shown that vascular changes due to succinylcholine chloride and sodium pentobarbital are minimal.[2, 3]

Endrin was administered by infusion of a lethal dose (10 mg/kg) in alcohol (25 mg/ml) into the blood reservoir. A priming solution of p-aminohippurate (PAH) and creatinine was added to the blood reservoir, and blood levels were maintained by continuous infusion with a Harvard infusion-withdrawal pump. Urine was not collected since renal plasma flow (RPF) and glomerular filtration rate (GFR) were determined directly from the arterial-venous differencs of the clearance reagents. Renal blood flow was measured directly with a graduated cylinder and stop watch. Blood was returned to the femoral vein from a reservoir via a Sigmamotor pump. Systemic arterial pressure (SAP) was measured from the cannulated femoral artery with a Statham pressure transducer connected to a Sanborn direct-writing recorder.

A. *Renal denervation experiments.* Five experiments were performed in which the kidneys were exposed by incision through the flank region and the renal vein cannulated for direct-flow measurements. Care was taken to avoid severing or damaging renal nerves. Endrin was infused as described above and, following a steady-state response of renal blood flow, phenol was applied to the renal pedicle. Flows were followed to determine the effects of the chemical denervation. Surgical denervation was then performed. Phentolamine† (2.5 mg) was injected into the renal artery after flow had stabilized, and consecutive 30-second flows were taken for about 5 minutes. Phenoxybenzamine†† (10 mg/kg in 100 ml of saline), another adrenergic blocking agent, was added to the reservoir and infused intravenously. The experiment was terminated after monitoring systemic pressure and arterial renal-blood-flow changes for a maximum of 30 minutes.

B. *Renal denervation experiments on adrenalectomized animals.* Six animals were adrenalectomized surgically, then prepared and treated as above.

* Endrin — 1,2,3,4,10,10-hexachloro-6,7,-epoxy-1,4,4a,5,6,7,8,8a-octa - hydro - 1,4, - endo-endo-5,8,-dimethanonapthalene, obtained from Nutritional Biochemical Corp., 21010 Miles Ave., Cleveland, Ohio.

** Nembutal, obtained from Abbott Laboratories, N. Chicago, Ill.

*** Anectine, obtained from Burroughs Wellcome Co. Tuckahoe, N.Y.

† Regitine—obtained from Ciba Pharmaceutical Co., Summit, N.J.

†† Dibenzyline—supplied through the courtesy of Smith, Kline, and French.

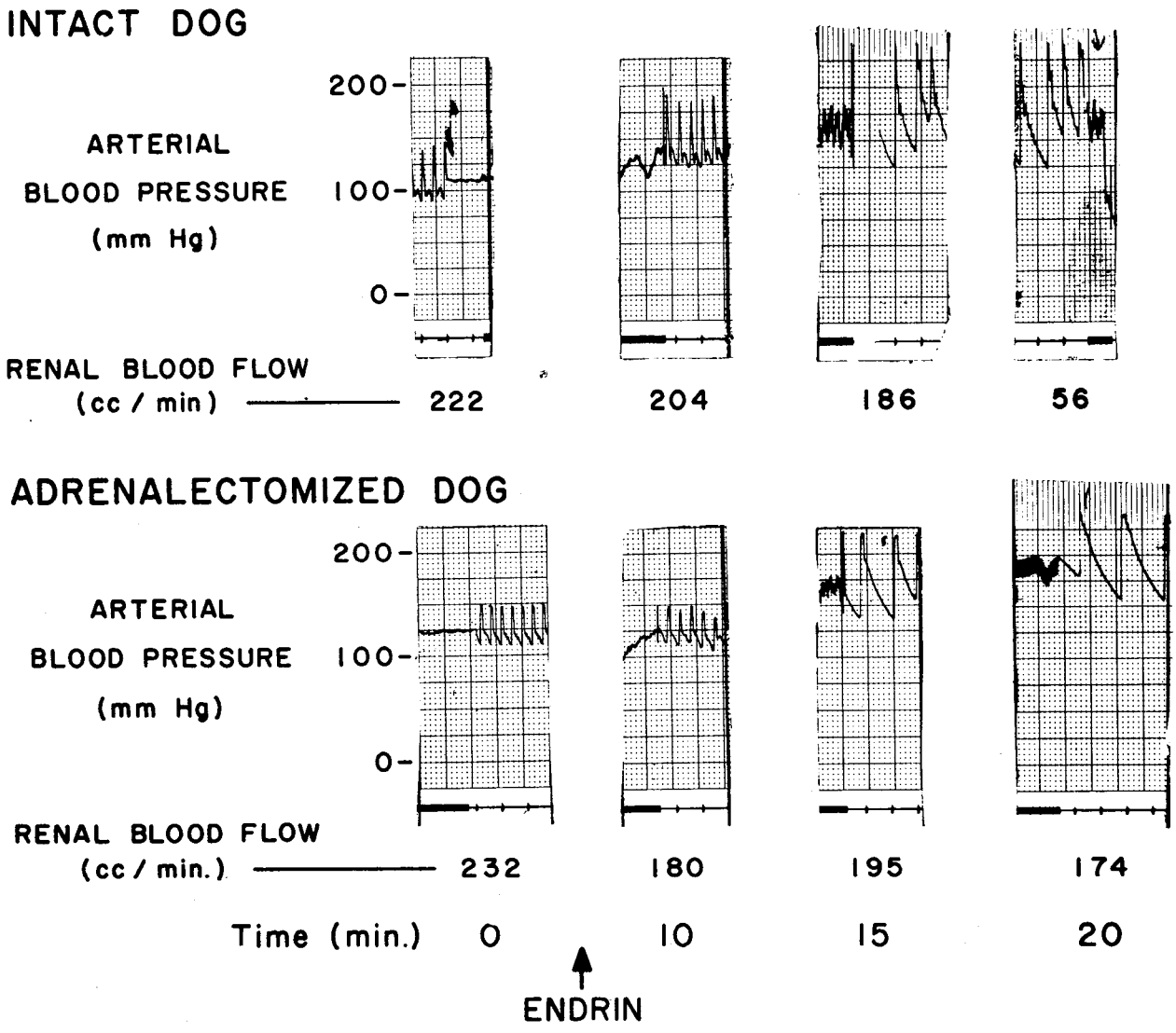

FIGURE 1. Development of bradycardia and hypertension after endrin in intact and adrenalectomized dogs.

III. Results.

A. *Renal denervation experiments (five dogs).* These animals showed a marked increase in renal resistance with decreased renal blood flow, systemic hypertension (Figure 2B), and bradycardia (Figure 3) within 10 to 20 minutes after infusion of endrin. Denervation of the renal pedicle produced a variable effect on renal blood flow. In some instances, a mild increase in flow occurred. Injection of phentolamine into the renal artery of these animals significantly lowered renal vascular resistance and restored blood flows through the kidneys to a level nearly equal to control values. GFR and filtration fraction showed decreases at about 30 minutes after endrin, but subsequently recovered (Figure 3).

B. *Renal denervation in adrenalectomized animals (six dogs).* The mean increase in systemic arterial pressure and development of bradycardia were similar in adrenalectomized animals and in intact-animal experiments (above). There was a variable effect on renal blood flow in contrast to results obtained from animals with intact adrenals (Figure 2A). The mean effects of denervation and phentolamine were less than those observed in intact animals, although infusion of phenoxybenzamine resulted in a marked increase in blood flow. Filtration fraction re-

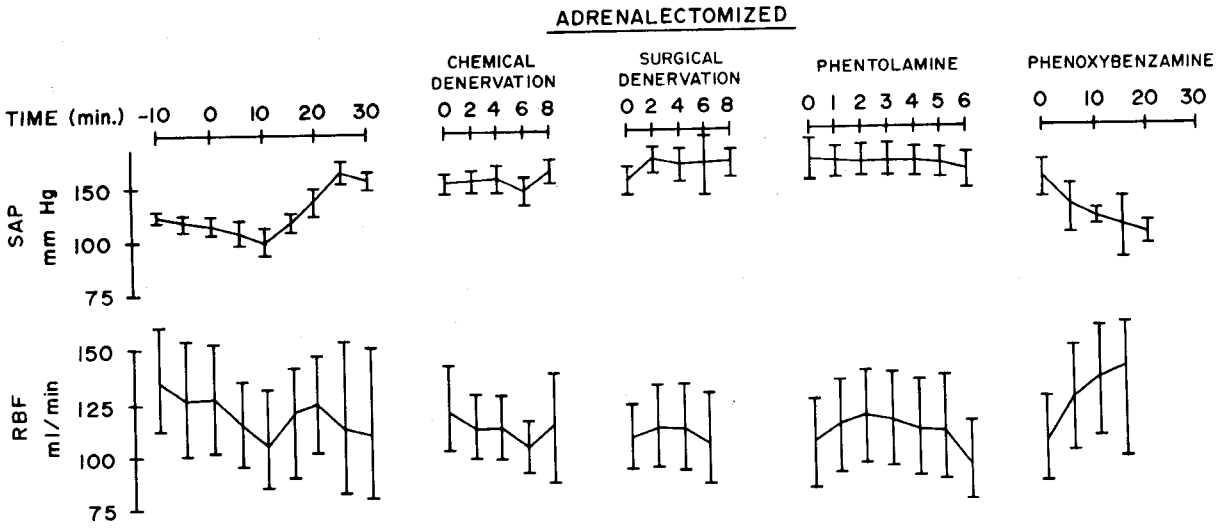

FIGURE 2A. Effects of denervation and adrenergic-blocking agents on adrenalectomized dogs administered endrin. Mean changes and standard errors for systemic arterial pressure (SAP) and renal blood flow (RBF) for each procedure on six adrenalectomized dogs, p values for SAP changes are 0.01 for the period of endrin response and 0.1 or greater for responses to other maneuvers. p values for RBF changes are 0.1 for each maneuver. p values were calculated for the final point plotted for each maneuver as compared to zero time for that maneuver. Zero time in each instance indicates that measured renal blood flow had been stable for 1 to 4 minutes.

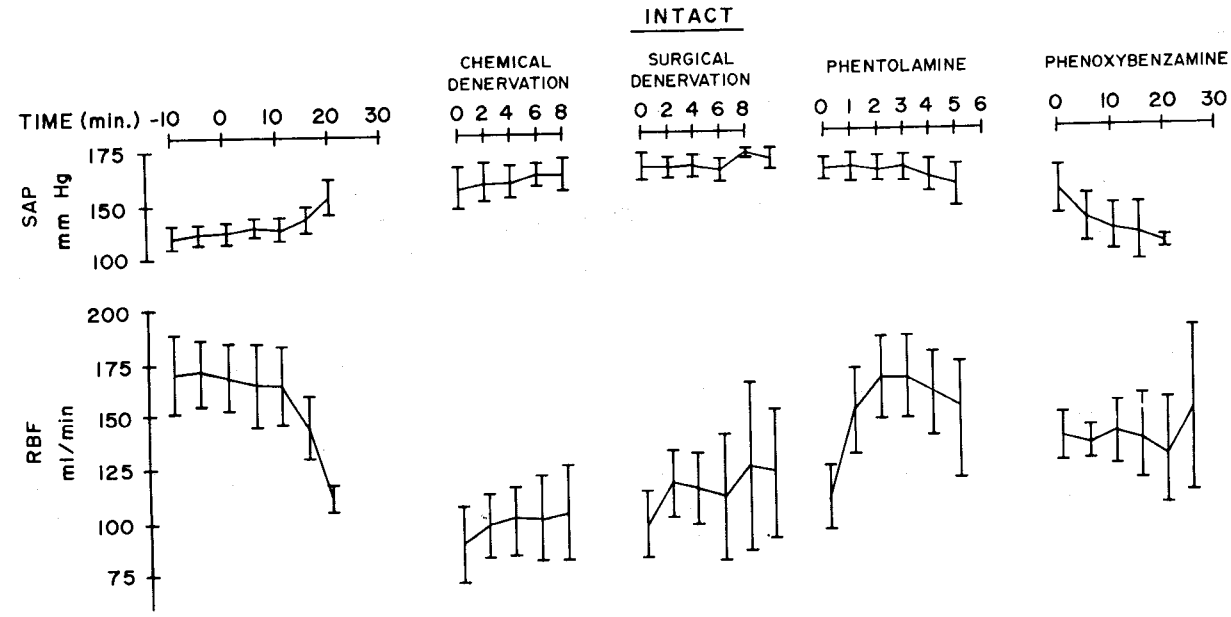

FIGURE 2B. Effects of denervation and adrenergic-blocking agents on intact dogs administered endrin. Mean changes and standard errors for five intact dogs are as described for Figure 2A. p values for SAP are 0.1 or larger for all maneuvers except the response to phenoxybenzamine where p value is 0.05. p values for RBF are 0.05 for response to endrin and phentolamine and 0.1 or larger for responses to other maneuvers. p values were calculated as in Figure 2A.

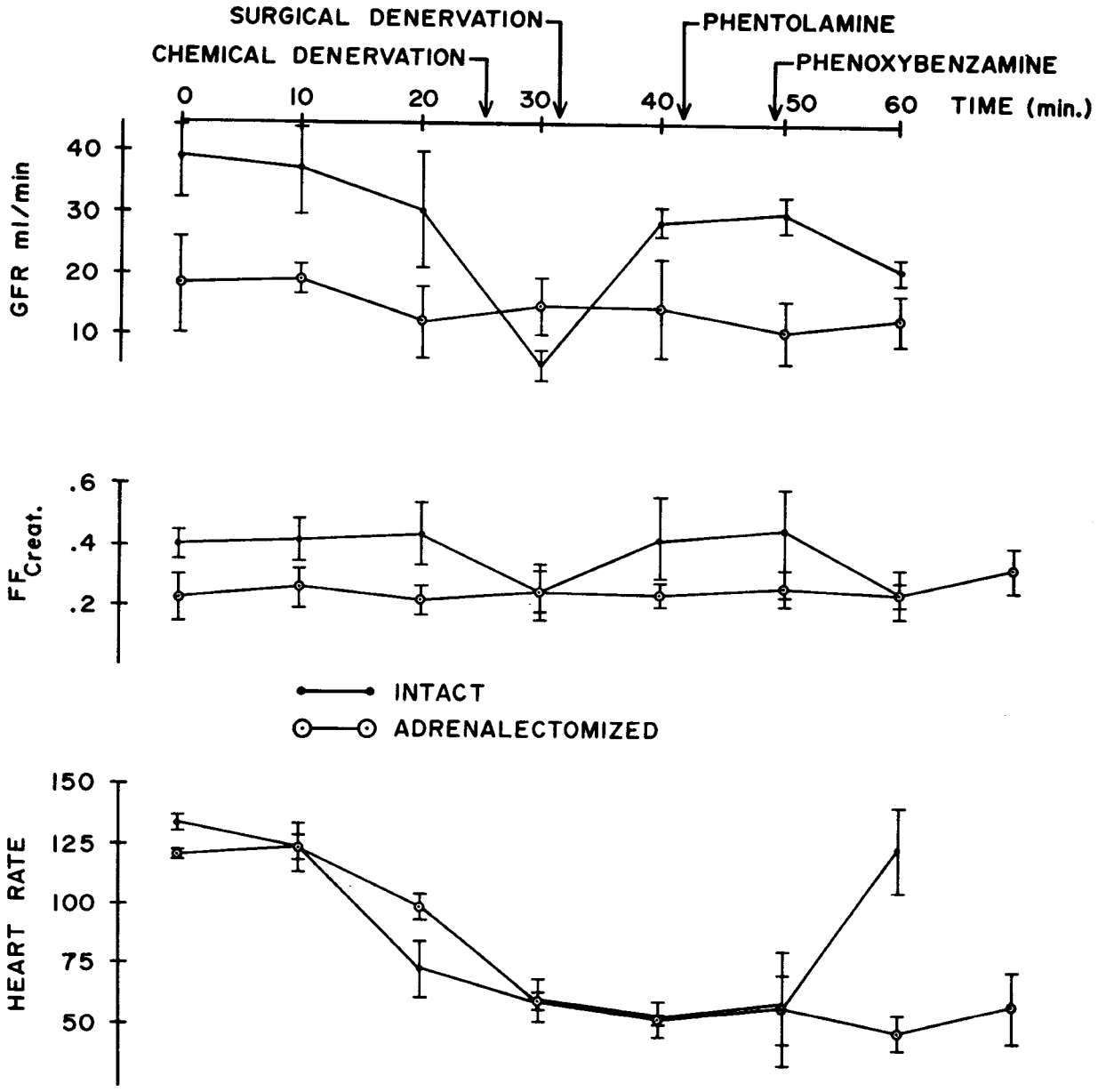

FIGURE 3. Comparison of the mean changes and standard errors for glomerular filtration rate (GFR), filtration fraction (FF_{Creat}) from clearance of creatinine, and heart rate in intact and adrenalectomized animals administered endrin.

p values for GFR are 0.1 for all points except the 30-minute sample in intact animals, which is 0.05. p values for FF_{Creat} are 0.1 or less for all points plotted. p values for heart rate are 0.1 for the 10-minute point in both groups and the 60-miute point for intact dogs. All other points have a p value of 0.01 in both groups.

Points at the top of the figure indicate the mean time at which the procedures indicated in Figures 2A and 2B were done.

mained constant, and GFR showed only a slight depression at an earlier time (Figure 3). Figure 1 shows the record of development of hypertension and bradycardia after endrin in typical intact and adrenalectomized animals.

IV. Discussion.

Factors that may be involved in changes of renal function after endrin poisoning are the effects of endrin on the adrenal glands, the central nervous system, and on the kidney directly.

Intact dogs in these studies developed decreased glomerular filtration rates and renal blood flow with systemic hypertension and bradycardia during the first hour after exposure and demonstrated a tendency to return toward control values with time (Figure 3).

It appears that endrin has no direct effect on the kidney vasculature,[1] since previous work has demonstrated that endrin stimulated the sympatho-adrenal system, and an adrenergic blocking agent caused return of renal blood flows toward control values. Animals that were adrenalectomized did not develop the marked increase in renal resistance seen in intact animals. Lack of response in adrenalectomized dogs (Figure 2A) and the sharp increase in renal blood flow when phentolamine was administered to intact animals (Figure 2B) are evidence that changes in renal vascular resistance are primarily due to circulating catecholamines.

Changes in GFR in intact animals coincident with development of hypertension and decreased renal blood flow could also be accounted for by circulating agents that cause afferent arteriolar constriction.

The autoregulatory response of increased renal resistance to systemic hypertension has been shown to be only a fraction of the total change seen in endrin poisoning.[1] In the adrenalectomized animals, this autoregulatory response becomes more significant as systemic hypertension and bradycardia develop to the same extent in adrenalectomized animals as in intact (Figure 1) but with a much smaller increase in renal vascular resistance (Figures 2A and 2B).

Effects of the central nervous system on the kidney may be derived (a) directly from neural control of the renal vascular bed or (b) indirectly from central-nervous-system-potentiated systemic hypertension and adrenal-gland discharge. Direct neural control has a minor and variable role as indicated by changes in blood flow during the denervation experiments (Figure 2A and 2B). Systemic hypertension causes an autoregulatory response that has previously been shown to play a minor role in changes in renal

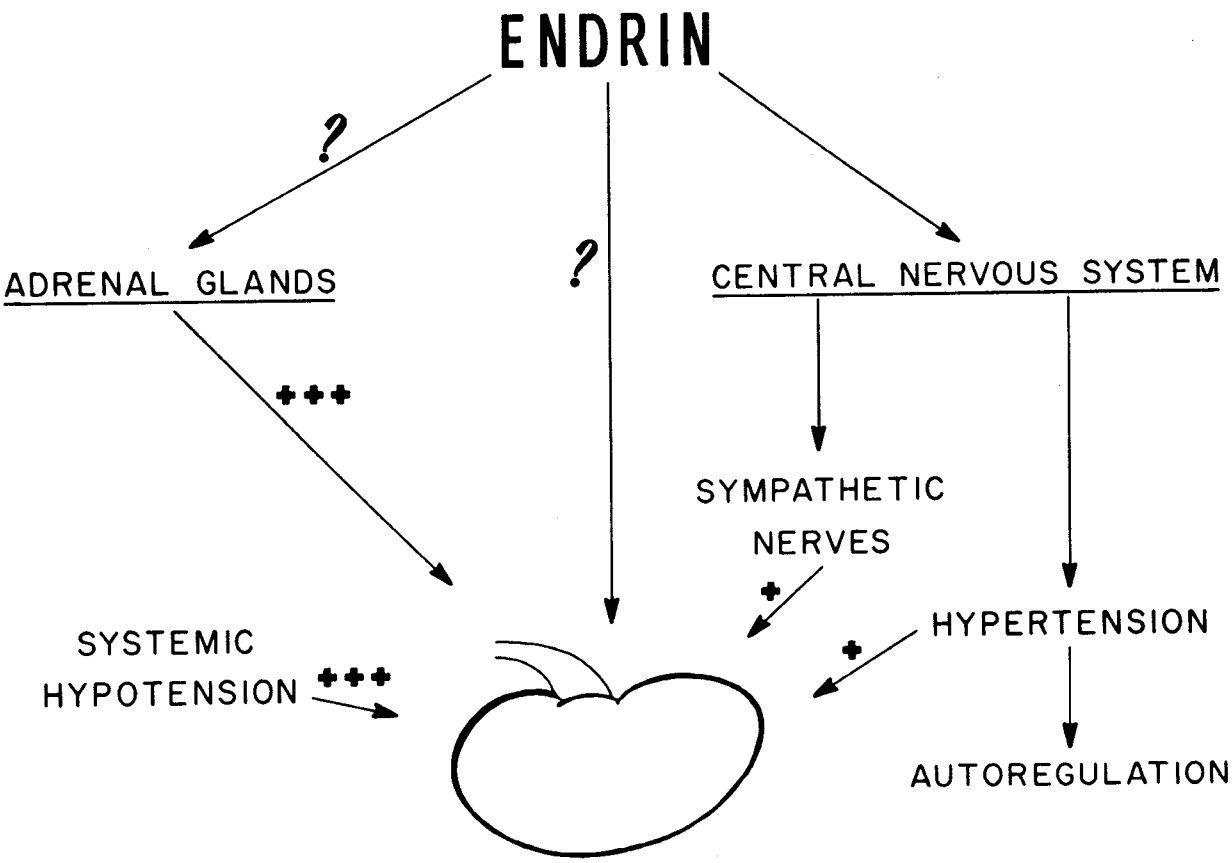

FIGURE 4. A schema summarizing the effects of endrin on the kidney.

vascular resistance.[1] This evidence indicates that the major influence causing changes in the renal vascular bed in response to acute endrin poisoning was from effects of circulating catecholamines originating from the adrenal glands.

The schema of Figure 4 presents a summary of ways in which endrin may affect the kidney hemodynamically and functionally, directly through central nervous system potentiated release of catecholamines and sympathetic nerve discharge, or indirectly through systemic hypertension and autoregulation in acute poisoning and terminal systemic hypotension in chronic poisoning.[1]

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3. Smith, H. W.: *The Kidney.* Oxford University Press N.Y., N.Y., 1955, p. 445.

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PROBLEMS IN AERIAL APPLICATION:

II. Peripheral Vascular Effects of the Insecticide Endrin*

I. Introduction.

A clearer understanding of the mechanism of action of insecticides is needed in order to deal more comprehensively with insecticide poisoning. This is especially true in the case of crop-dusting pilots, who are repeatedly exposed to insecticides. Acute exposure to chlorinated hydrocarbon insecticides may be fatal in animals or man, whether absorption is by ingestion, inhalation or dermally.[1, 8] This group of insecticides includes a large number of compounds, ranging in toxicity from DDT, which is only moderately toxic to mammals, to endrin, a highly toxic poison in animals and man. Until recently, little was known of the cardiovascular effects of endrin poisoning. Reports from this laboratory have shown that lethal intravenous infusion of endrin causes severe cardiovascular alterations in the dog, including early hypertension followed by hypotension, bradycardia, increased cerebrospinal fluid and cerebral venous pressure,[2] and increased renal vascular resistance.[10] Hyperactivity of the sympathetic and parasympathetic nervous systems also has been indicated.[2] The chlorinated-hydrocarbon insecticides aldrin and dieldrin also cause deleterious vascular and neurological effects in animals.[3, 4] The present investigation was undertaken to delineate the peripheral vascular effects of acute and lethal endrin poisoning in the dog.

II. Methods.

Mongrel dogs of both sexes were intravenously anesthetized with sodium pentobarbital, 30 mg/kg. Pressures were obtained with Statham pressure transducers and recorded using a Sanborn direct-writing recorder. Heparin sodium, 3 mg/kg body weight, was used as anticoagulant. Ten experiments were completed as follows. Pressures were obtained from four sites along the vascular system of a dog forelimb using a modification of the Haddy technique to segment the limb vascular bed;[5] limb-brachial-artery orifice pressure (LAP) was taken as aortic blood pressure; small artery pressure (SAP) was obtained from a PE 50 cannula placed in a metacarpal artery and advanced retrogradely under the foot pad; small vein pressure (SVP) was measured through PE 50 tubing secured in a digital vein with its tip pointing upstream; and large-vein orifice pressure (LVP) was maintained at atmospheric pressure. The forelimb was surgically separated from the body except for the bone, artery, and, in half of the series, the major nerves. The limb was perfused at natural flow through the intact brachial artery at the dog's systemic arterial blood pressure. Venous blood flowed by gravity from the cut brachial and cephalic veins into a plastic reservoir and was returned to the animal through a cannulated femoral vein by a Sigmamotor pump. The extracorporeal system consisted of plastic tygon tubing and was primed with 100 cc of high-molecular-weight dextran.** Limb-blood-flow rates (BF) were obtained with a graduated cylinder and stopwatch. Resistances were calculated by dividing the pressure difference across a particular vascular segment by the total leg blood flow (limb resistances: total=LAP-LVP/BF; arterial=LAP-SAP/BF; small vessel=SAP-SVP/BF; venous=SVP-LVP/BF). Aortic blood pressure of the animal was recorded from a cannulated femoral artery. Hematocrits and pH's were determined from femoral vein blood, Electrical activity was recorded from a flank muscle of the dog (biceps femoris) and from a muscle group of the forelimb being studied. Stainless-steel needles, placed longitudinally in the muscles 4 to 8 cm apart, were utilized. Lethal amounts of endrin insecticide, 10 mg/kg of body weight, were infused intravenously at 0.5 to 1.0 ml/min. The ethanol solution contained approximately 25 mg/ml of endrin.

* With the assistance of R. E. Hopla and C. C. Gill.

** Obtained from Cutter Laboratories, Berkeley, California.

Five, innervated, isolated, dog-limb preparations were prepared as described above except that the bone was severed to allow continuous weighing of the limb with a strain-gauge weighing device. Changes of limb weight of 0.1 gram could be determined accurately.[11] Animals were pretreated with the muscle relaxant succinylcholine chloride (Anectine, 0.5 mg/kg body weight) to prevent convulsions that interferred with limb weight measurements. Additional succinylcholine was given as needed. Respiration was accomplished by means of a Harvard constant-volume respirator. Endrin insecticide was given, and all parameters were followed for 30 minutes, at which time the limb nerves were severed. The adrenergic blocking agent, phentolamine (Regitine, 5 mg), was then injected into the brachial artery.

Ten control experiments were completed in which the animals received only the alcohol carrier used in the endrin-infusion series. Five limbs were innervated and five were denervated.

III. Results.

A. *General.* Severe tonic-clonic convulsions appeared in all animals approximately 10 minutes after the onset of endrin infusion, except in the succinylcholine-treated group. Electrical activity in the dog flank muscle of both groups reached a mean peak frequency of 223 per minute 20 to 30 minutes after endrin. Bradycardia, copious, mucoid salivation, and hyperexcitability to noise also occurred.

B. *Innervated forelimb experiments.* Mean data, with standard errors, before and after infusion of endrin are presented in Figure 1. Total limb vascular resistance increased from 2.5 to 4.6 resistance units (mm Hg/cc/min) 10 minutes after endrin and rose to 5.2 units by 30 minutes. Most of the early increase occurred in the small vessel segment (small artery to small vein), while the arterial segment contributed the major resistance to blood flow during the later phase. Limb blood flow decreased progressively from approximately 20 to 6 cc/min per 100 gm. Mean electrical activity rose from near zero to peak frequency of 284 and 435 per minute in the dog flank muscle and forelimb muscle, respectively. Systemic arterial blood pressure showed an early increase but decreased from 20 minutes post-endrin until the end of the experiment. These changes were accompanied by a marked fall in blood pH from 7.21 to 6.67 and increased hematocrit. Respiration was spontaneous and essentially the same frquency as the convulsions.

C. *Denervated forelimb experiments.* Figure 2 shows mean data, with standard errors, before and after endrin infusion. Limb-vascular-resistance increases were similar to the innervated group (Figure 1) for the first 10 to 15 minutes after endrin. Beyond this point, limb resistance increased steeply to high levels, most of this resistance increase occurring in the small vessel segment. Limb blood flow decreased from 28 to 2 cc/min per 100 gm. Electrical activity increased from a mean frequency of near 0 to 160 per minute in the dog flank muscle but did not change appreciably in the denervated forelimb. Systemic arterial blood pressure increased initially and fell subsequently as in the preceding group. Blood pH fell from 7.25 to 6.69 and hematocrit increased.

D. *Innervated, weighed forelimb experiments.* Figure 3 gives mean values, with standard errors, showing the effect of endrin on leg weight and other parameters. Arterial blood pressure began to increase at about the 15th minute following the start of endrin infusion and continued upward for the remainder of the experiment. Blood flow in the isolated limb fell from 16 to 4 cc/min per 100 gms of leg and total limb resistance increased approximately five fold, most of which was due to an increased resistance in the small vessel segment. Venous resistance also increased approximately five times over control. Concomitant with these changes was a steady loss of leg weight (-5.5 gm) and increase in total body hematocrit. Blood pH fell from 7.32 to 7.14, a value appreciably higher than in the preceding groups in which respiration was spontaneous.

Figure 4 represents mean data showing the effect of denervation and adrenergic blockade on limb resistances after endrin. Sectioning the nerves to the isolated limb at the peak post-endrin response resulted in an immediate fall in total resistance of five resistance units, and subsequent injection of phentolamine dropped total resistance another five units. Most of the resistance drop was in the small vessel segment. Limb nerve section in control experiments decreased total limb vascular resistance approximately 100%, while phentolamine injected into the same limbs did not appreciably affect limb resistances.

Control experiments, in which only alcohol was given, were completed for all of the preceding groups and showed no appreciable changes from baseline levels.

IV. Discussion.

Results from this investigation show that forelimb vascular resistance increases markedly within a few minutes after intravenous administration of lethal amounts of endrin insecticide. Most of the total resistance increase is usually in the small vessel segment, of which the arterioles constitute the major resistance to blood flow.

A. *Mechanisms of vascular resistance changes.* It is apparent that sympathetic innervation of limb vessels is not necessary for the large resistance increases following endrin administration. The resistance patterns of the innervated and denervated groups (Figures 1 and 2) are similar for the first 10 to 15 minutes after endrin; however, the experiments in which nerve section and phentolamine injection were completed after endrin indicate that the nerves can account for a considerable portion of the total resistance increase. Earlier experiments show that in the kidney the increased vascular resistance that normally occurs following endrin infusion is due primarily to circulating catecholamine-like agents but that the nerves also play a role.[10] The observation that resistance increases considerably more in the denervated limbs than the innervated is noteworthy. It might be expected that resistance in the innervated limbs would increase as much as, or more than, in the denervated limbs; however, the opposite occurs. Recall that most of the early increase in limb resistance in both groups is in the small vessel segment. However, resistance in this segment begins to fall in the innervated limb while it continues to rise in the denervated ones at 10 to 15 minutes post-endrin. The difference in these groups is probably due to the high level of muscle activity in the innervated limb and subsequent high local concentration of vasodilator metabolites that antagonize the effects of sympathetic nerve activity and circulating constrictor agents. It has been shown elsewhere that a slight local excess of metabolic breakdown products causes an active decrease in limb resistance, most of which occurs in the small vessel segment.[6]

B. *Mechanism of vascular fluid shifts.* The disproportionately large increase in pre-capillary resistance compared to the increase of post-capillary resistance and fall in limb blood flow should result in a decreased capillary pressure and consequent loss of interstitial fluid. This is indicated in the last group of dogs, in which limb weight falls progressively after endrin. Most of the early weight loss can be explained as a result of decreasing blood flow and, probably, capillary reabsorption. The later fall in weight when limb flow stabilizes appears due primarily to capillary reabsorption of interstitial fluid; however, resistance continues to increase so a further decrease in vascular volume cannot be excluded. Since the hematocrit increases, it seems unlikely that hemoconcentration results from loss of vascular fluid in peripheral skeletal muscle or skin.

The development of severe hypertension after endrin in the nonconvulsing group compared to the hypotension in the convulsing groups is not readily explainable. Succinylcholine chloride has no cardiovascular effects in the concentrations used.[2] The extremely low pH's in the convulsing, spontaneously breathing dogs might play a role in their failure to develop hypertension.[7, 9]

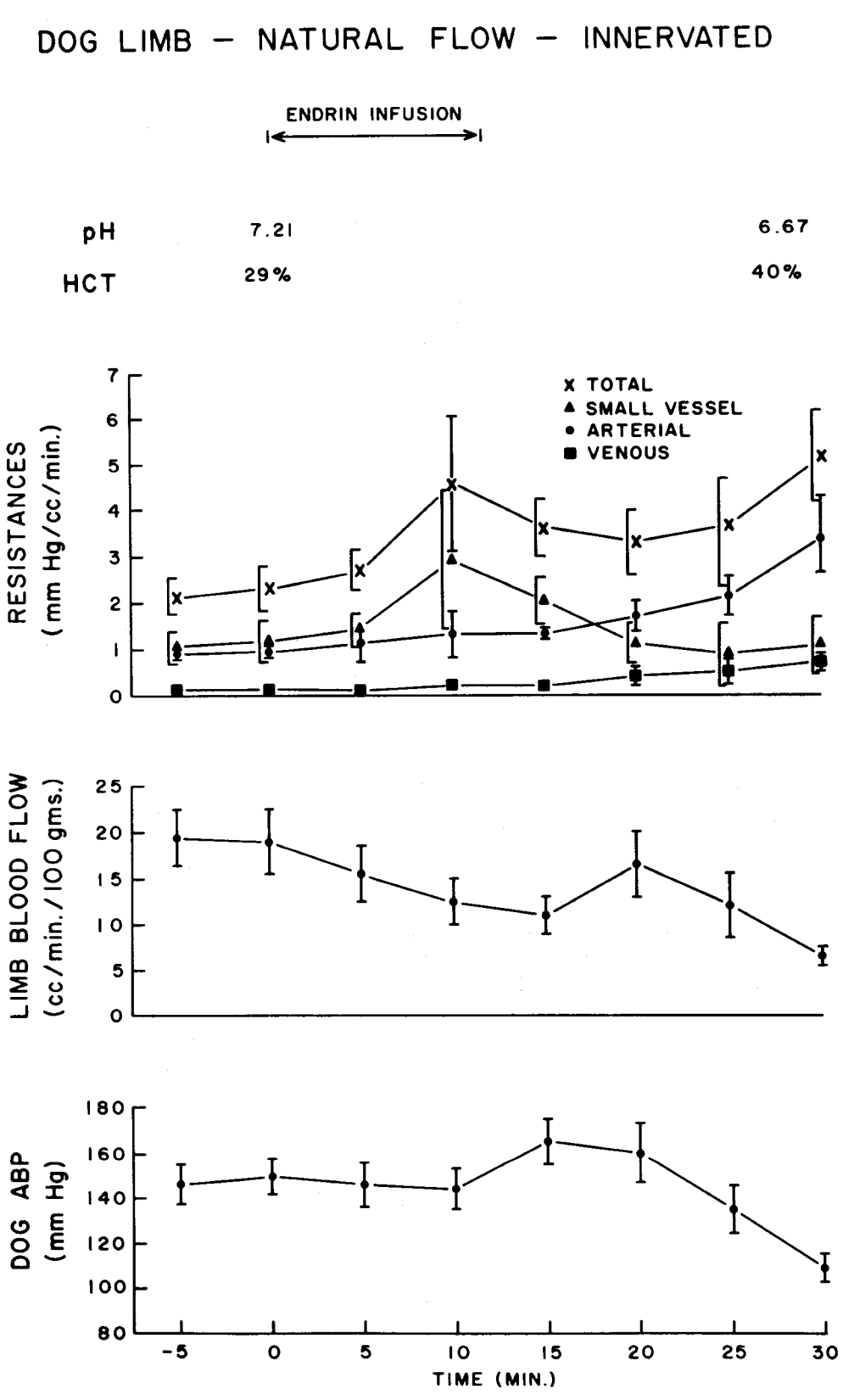

FIGURE 1. Isolated, innervated limb preparation. Mean values ± standard errors from five dog experiments. The effects of intravenous infusion of lethal amounts of endrin are shown. Endrin infusion began at zero time. The bar at top of graph represents the average infusion time. Respiration was spontaneous.

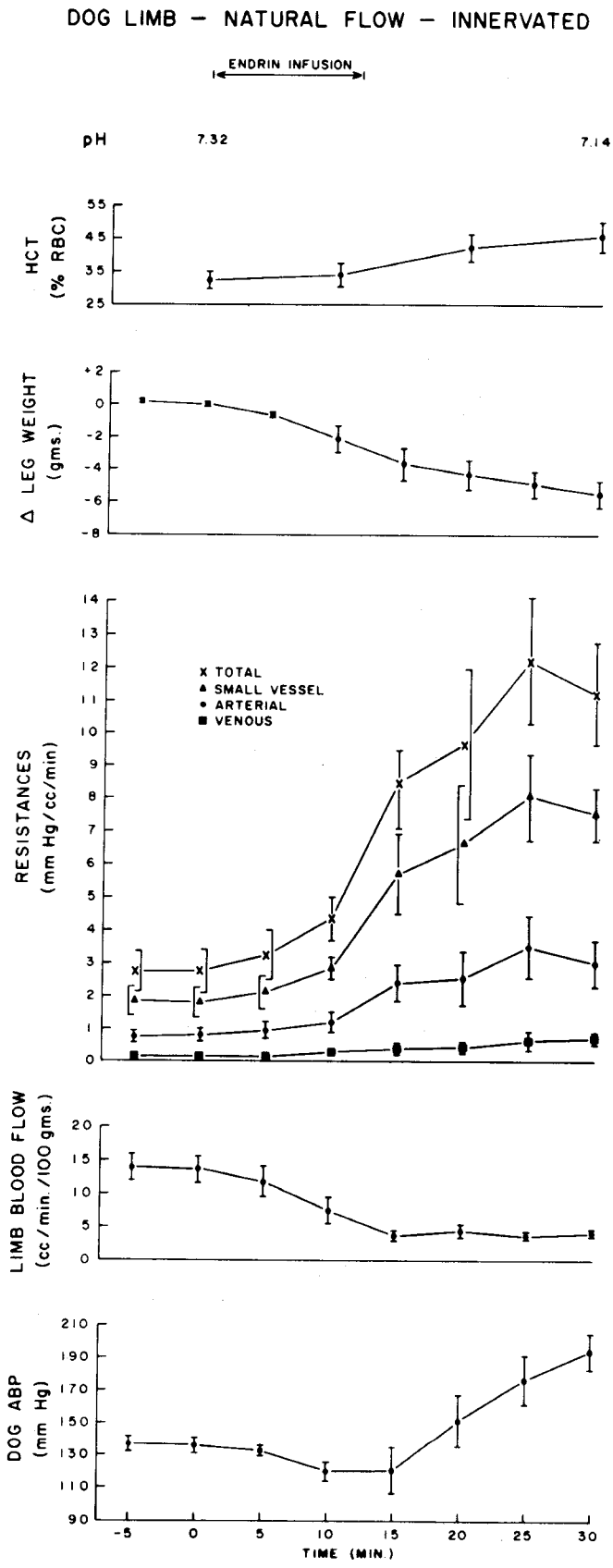

FIGURE 2. Isolated, denervated limb preparation. Mean values ± standard errors from five dog experiments. The effects of intravenous infusion of lethal amounts of endrin are shown. Endrin infusion began at zero time. The bar at top of graph represents the average infusion time. Respiration was spontaneous.

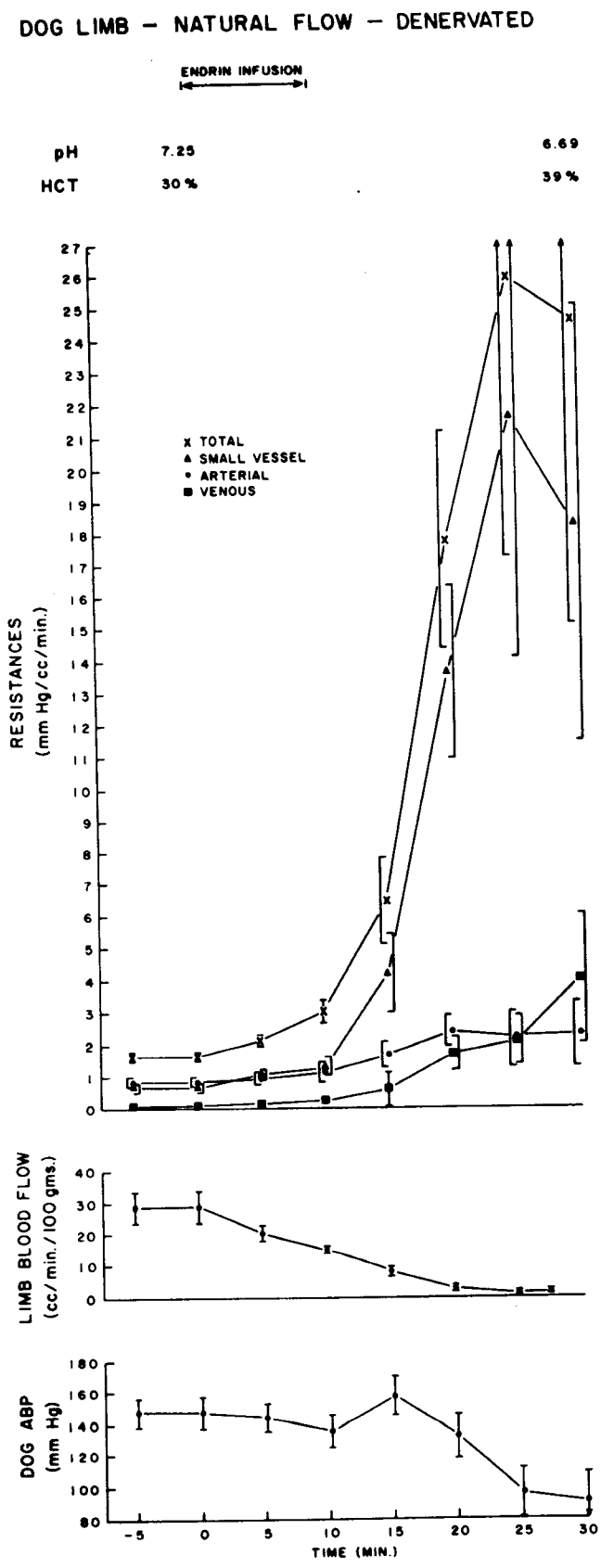

FIGURE 3. Isolated, innervated limb preparation with continual limb weight measurements. Mean values ± standard errors from five dog experiments. The effects of intravenous infusion of lethal amounts of endrin are shown. Endrin infusion began at zero time. The bar at top of graph represents the average infusion time. Animals were pretreated with the muscle relaxant succinylcholine chloride. Respiration was accomplished with a constant volume respirator.

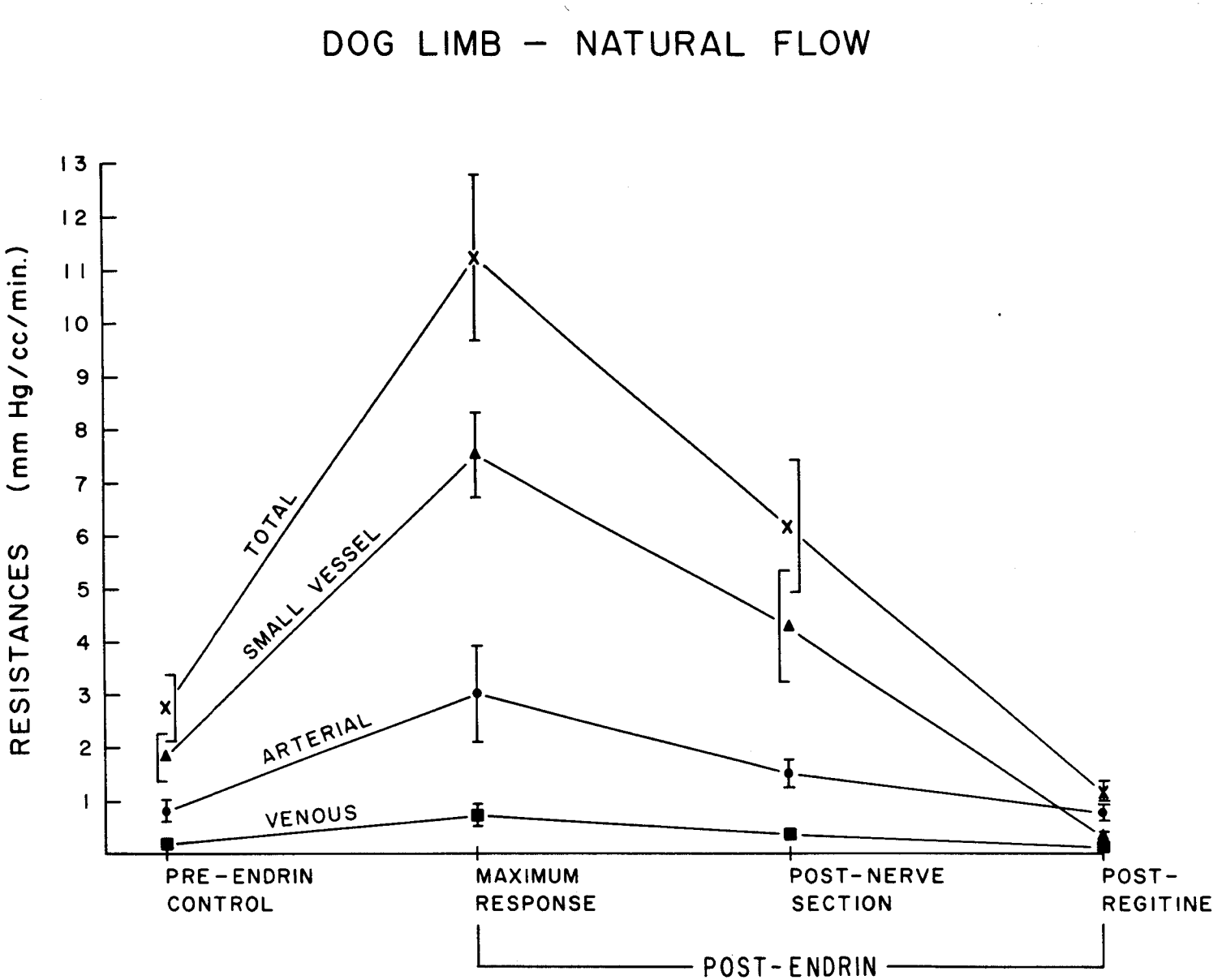

FIGURE 4. Isolated, innervated, limb preparation. Mean values ± standard errors from five dog experiments. The maximum resistance increase after endrin, and the effects of denervation and adrenergic blockade (phentolamine) on limb resistances are shown. Animals were pretreated with the muscle relaxant succinylcholine chloride.

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PROBLEMS IN AERIAL APPLICATION:

III. Mechanisms of Hemoconcentration During Acute Endrin Insecticide Poisoning

I. Methods.

A. *General.* In 45 experiments, mongrel dogs of both sexes were anesthetized intravenously with sodium pentobarbital, 30 mg/kg. Heparin sodium, 3 mg/kg body weight, was used as anticoagulant. Superlethal amounts of endrin insecticide, 10 mg/kg body weight, were infused intravenously at 0.5 to 1.0 ml/min. The endrin was dissolved in 95% ethanol (approximately 30 mg/ml). The effect of the alcohol carrier has been previously tested and has no systemic effect on the measured parameters at the rates used. Pressures were obtained with Statham pressure transducers and recorded on a Sanborn direct-writing recorder. In all of the following groups, mean systemic arterial blood pressure and heart rates were obtained from a cannulated femoral artery. Blood pH was determined before and after endrin with a Beckman expanded-scale pH meter. Hematocrits were measured before and usually at 10-minute intervals after endrin using a microcapillary centrifuge. Gross visual and histological examination of the lungs for edema was usually made at the end of each experiment.

B. *Splenectomized and eviscerated animals.* To determine whether hemoconcentration after endrin is associated with splenic release of erythrocyte rich blood or if hepatosplanchnic pooling is involved, the spleen was removed through a midline incision in 10 dogs, while total evisceration was completed in 10 others. Endrin was then infused into these dogs and was also given to 10 sham-operated animals.

C. *Dog perfused-lung preparation.* In each of five experiments, a table dog was used to supply blood to perfuse an isolated lung. The pulmonary artery was connected to the femoral artery of the table dog with tygon tubing, and constant-flow perfusion of the lung was established with Sigmamotor pump. The lung was removed from the donor-animal's body and placed on a plastic tray to allow continuous weighing with a strain-gauge weighing device.[2] Blood from the cut pulmonary vein flowed by gravity into a reservoir and was returned to a cannulated femoral vein of the table dog by a Sigmamotor pump. Pulmonary-artery perfusion pressure was monitored from the inflow tubing. Pulmonary blood flow was measured with a cylinder and stopwatch. Endrin was infused into the pulmonary artery of the isolated lung and passed to the table dog in the venous blood. Any direct effect of endrin on the lung vasculature should therefore become evident before affecting the table dog.

D. *Venous-return preparation.* In contrast to the other dogs, these animals received succinylcholine chloride to prevent convulsions. The basic preparation has been described in detail elsewhere.[3] Blood was led from the cannulated superior and inferior vena cavae through tygon tubing into a reservoir, situated in a warming water bath, and returned to the cannulated right atrium with a Sigmamotor pump. The azygos vein was ligated. Total venous return (cardiac inflow or pulmonary blood flow) to the heart was measured with a cylinder and stopwatch. The reservoir level was maintained constant with the return pump. Small-bore catheters were secured in the pulmonary artery and left atrium through needles that were subsequently withdrawn, and pressures were recorded. Right-atrial pressure was recorded from the inflow tubing. Total pulmonary vascular resistance (pulmonary artery—left atrial pressure/pulmonary blood flow) was calculated at 10-minute intervals after endrin. All of the above parameters were followed for 60 minutes after endrin administration. An earlier report from this laboratory has shown the venous-return preparation to be comparably stable over this period of time and usually longer.[4]

E. *Mean corpuscular volume.* In five splenectomized dogs, blood pH, duplicate hematocrit, and duplicate red blood cell counts were determined

before and 15 and 30 minutes after endrin. Mean corpuscular volume was calculated by dividing the percentage of red blood cells per 1,000 ml of whole blood by the red cell count (erythrocytes per cubic millimeter of blood).

II. Results.

General effects of endrin seen in all groups include severe tonic-clonic convulsions and hyperexcitability to noise (except in succinylcholine-treated animals), bradycardia, copious mucoid salivation, decreased blood pH, and increased hematocrit as previously reported.[1] Histological examination of lung tissue in all groups after endrin showed little evidence of pulmonary edema; however, by gross examination, some lungs appeared edematous.

Mean values, with standard errors, of percent change in hematocrit from pre-endrin levels and systemic arterial blood pressures from 10 splenectomized, 10 eviscerated, and 10 sham-operated dogs receiving lethal amounts of endrin are graphed in Figure 1. Arterial blood pressure of the sham-operated group failed to fall until about 20 minutes after endrin but decreased steadily beyond this point. Blood pH decreased from 7.29 to 6.85 and 6.88 by 30 to 60 minutes, respectively, after endrin. Arterial blood pressure of the splenectomized and eviscerated groups began to fall immediately after endrin and continued downward to levels significantly lower ($P<0.01$) than the sham-operated group by 60 minutes post-endrin. Blood pH in the splenectomized dogs decreased from 7.29 to 6.66 and 6.71 at 30 and 60 minutes, respectively, after endrin; blood pH fell from 7.24 to 6.57 and 6.49, respectively, at 30 and 60 minutes post-endrin in the eviscerated group. The hematocrit increases of the splenectomized and eviscerated groups are not significantly different (8% increase each) and attained a value about half as high as the sham-operated group (14% increase) by 30 minutes after endrin. This relationship was maintained as the hematocrits remained relatively constant (8%, 7% and 15% increase respectively) from 30 to 60 minutes after endrin.

Table 1 shows the effect of endrin on mean corpuscular volume, hematocrit, red-cell count, pH, and arterial blood pressure in five splenectomized dogs. Arterial blood pressure fell, severe acidosis developed, hematocrit increased, erythrocyte concentration increased slightly, and the calculated mean corpuscular volume increased in four of five experiments.

Figure 2 gives mean data, with standard errors, showing the effect of endrin on the table dog and

TABLE 1. Effect of endrin on mean corpuscular volume in splenectomized dogs.

Exp. No.	Time after endrin (min)	ABP (mm Hg)	pH	Hct (%)	RCC x10^6 (cu mm)	MCV (cu μ)
1	0	175	7.31	45.0	7.58	59.4
	15	160	7.26	49.0	8.38	60.3
	30	130	6.52	56.0	8.62	65.0
2	0	150	7.30	37.0	5.62	65.9
	15	145	7.30	37.5	6.50	57.6
	30	55	6.40	37.5	6.63	56.6
3	0	135	7.34	31.0	6.01	51.6
	15	150	7.18	33.5	5.51	60.8
	30	50	6.39	37.5	5.86	64.0
4	0	165	7.32	45.5	6.55	69.5
	15	175	7.32	47.0	6.83	68.7
	30	120	6.56	50.5	6.83	73.9
5	0	145	7.32	45.0	7.11	63.3
	15	145	7.32	46.0	7.45	61.8
	30	100	6.59	50.0	7.55	66.3

Average endrin infusion time = 9 min; ABP = mean systemic arterial blood pressure Hct = blood hematocrit; RCC = erythrocyte count; MCV = mean corpuscular volume.

isolated lung. As systemic arterial blood pressure of the table dog decreased, pulmonary artery pressure of the isolated lung increased from 16 to 23 mm Hg. This rise indicates an increase in total pulmonary vascular resistance since flow is constant. Lung weight underwent a slight increase until 15 minutes after endrin and then decreased by 3.2 gm as pulmonary artery pressure achieved a peak increase.

Figure 3 shows the effect on total pulmonary resistance, hematocrit, and systemic arterial blood pressure in the venous return preparation. Systemic arterial blood pressure did not change significantly by 20 minutes after endrin, while pulmonary vascular resistance increased from 0.012 to 0.037. Hematocrit increased during this period from 44% to 48% and stabilized at this value. From 20 to 40 minutes after endrin, systemic arterial pressure increased markedly and fell to a value still above control by 60 minutes. During this period, pulmonary vascular resistance remained at an elevated value. The development of extreme hypertension after endrin in these succinylcholine-treated animals (nonconvulsing) was a consistent finding and is in marked contrast to the early hypotension in the animals in which convulsions are not prevented.[1] This striking difference is not readily explainable but may be related to the more severe acidosis that developed in the convulsing dogs or to the convulsions.

Figure 4 gives mean values with standard errors showing the effect of endrin on several parameters in the same venous return animals of Figure 3. Cardiac inflow (pulmonary blood flow) increased from 70 to 83 cc/min per kg of dog by 30 minutes after endrin as previously observed (Reins, D. and L. B. Hinshaw, unpublished observations). After 40 minutes, pulmonary blood flow began to decrease and leveled at 59 cc/min per kg by the experiment's end. Left atrial, pulmonary-artery, and right atrial pressure increased markedly by 20 minutes post-endrin.

III. Discussion.

The hematocrit can increase due to an increase in erythrocyte size and/or an increase in erythrocyte concentration. The concentration of red blood cells can increase through (a) loss of plasma water as a result of filtration; (b) addition of erythrocytes to the circulating blood volume; or (c) a combination of both. Results show that approximately half of the rise in hematocrit after endrin occurring in intact sham-operated dogs is prevented by removal of the spleen, indicating that expulsion of cell rich blood from spleen storage[5] is responsible for an appreciable portion of the hematocrit elevation.

The hematocrit increase seen in splenectomized dogs appears to involve other mechanisms: An effective means for concentrating red blood cells is by extravasation of vascular fluid, but this does not seem to occur in the hepatosplanchnic region as evidenced by this study and in another report showing no evidence for loss of vascular water in skeletal muscle (Emerson, T. E., Jr. and Hinshaw, L. B., submitted for publication). The pulmonary vascular bed is a possible site for loss of vascular water and cannot be excluded as being involved in the post-endrin hematocrit increase. Even though lung weight fell in the isolated, perfused lungs, this could be due to pulmonary vascular constriction following release of catecholamine-like agents into the blood after endrin[6] with displacement of intravascular volume. It is also possible that in these experiments a predominant pre-capillary constriction occurred, and effective capillary filtration decreased. However, this condition may exist only in the lung isolated from the heart, in which pulmonary vein orifice pressure is always zero. In the venous return experiments, left atrial pressure increased to a maximum mean value of 24 mm Hg, which presumably increased pulmonary capillary pressure by an almost equal amount. It has been demonstrated by others that pulmonary edema results when pulmonary venous pressure approaches or exceeds plasma-colloid-osmotic pressure, which averaged 22.5 mm Hg in their study.[7] Assuming these data as a guide, pulmonary venous pressure in our experiments must have approached or exceeded colloid-osmotic pressure, and some loss of pulmonary vascular water probably occurred.

An earlier report from this laboratory[1] demonstrated a marked increase in cerebral venous and cerebrospinal fluid pressure after endrin, which suggests the possibility of vascular fluid loss in the brain area. Another potential area for loss of plasma water is through the kidney secondary to an increased urine flow rate if endrin has an early diuretic effect. However, this possibility has not been adequately explored.

The development of severe acidemia should lead to an increased hematocrit subsequent to an in-

creased erythrocyte size due to the Hamburger (bicarbonate) shift.[8] Although mean corpuscular volume (MCV) increases in most animals, it is difficult to accept the directional change with great assurance. The inherent error in enumeration of red cells, which is necessary to calculate MCV, is on the order of 11% when duplicate samples are used and can be higher. However, the tendency for MCV to increase suggests that some of the unaccounted rise in hematocrit in splenectomized dogs is attributable to an increase in red cell size. The number of red blood cells per cubic millimeter of blood also increased in four of five experiments, which suggest some loss of plasma fluid from the vascular compartment as discussed in preceding paragraphs.

The fact that systemic arterial blood pressure and cardiac output and hence cardiac work were below control when left atrial pressure was elevated by 20 minutes post-endrin demonstrates a failing heart. A possible cardiac action of endrin merits further investigation.

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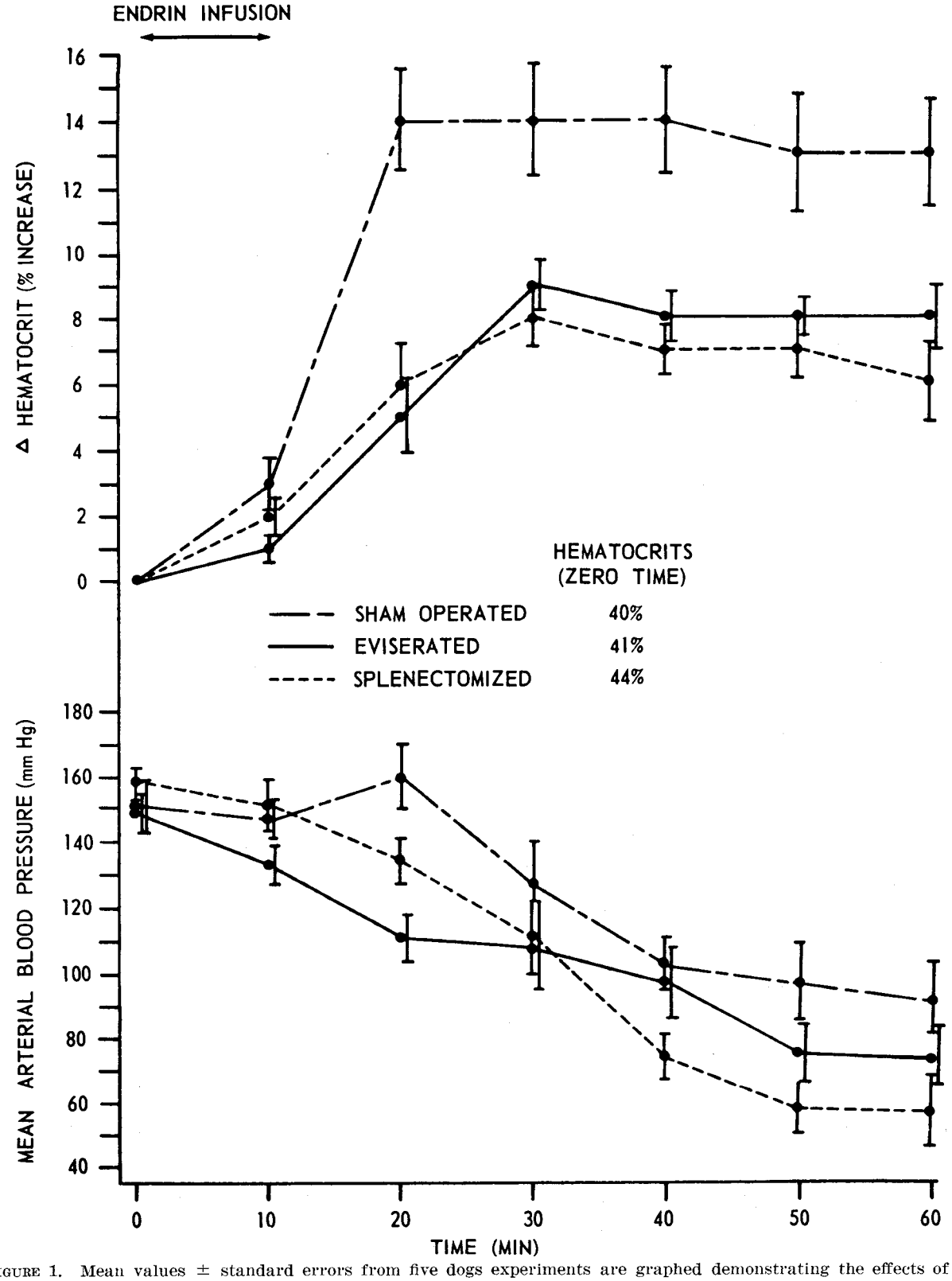

FIGURE 1. Mean values ± standard errors from five dogs experiments are graphed demonstrating the effects of endrin insecticide. △ hematocrit = increase in hematocrit from control pre-endrin level. Endrin infusion began at zero time.

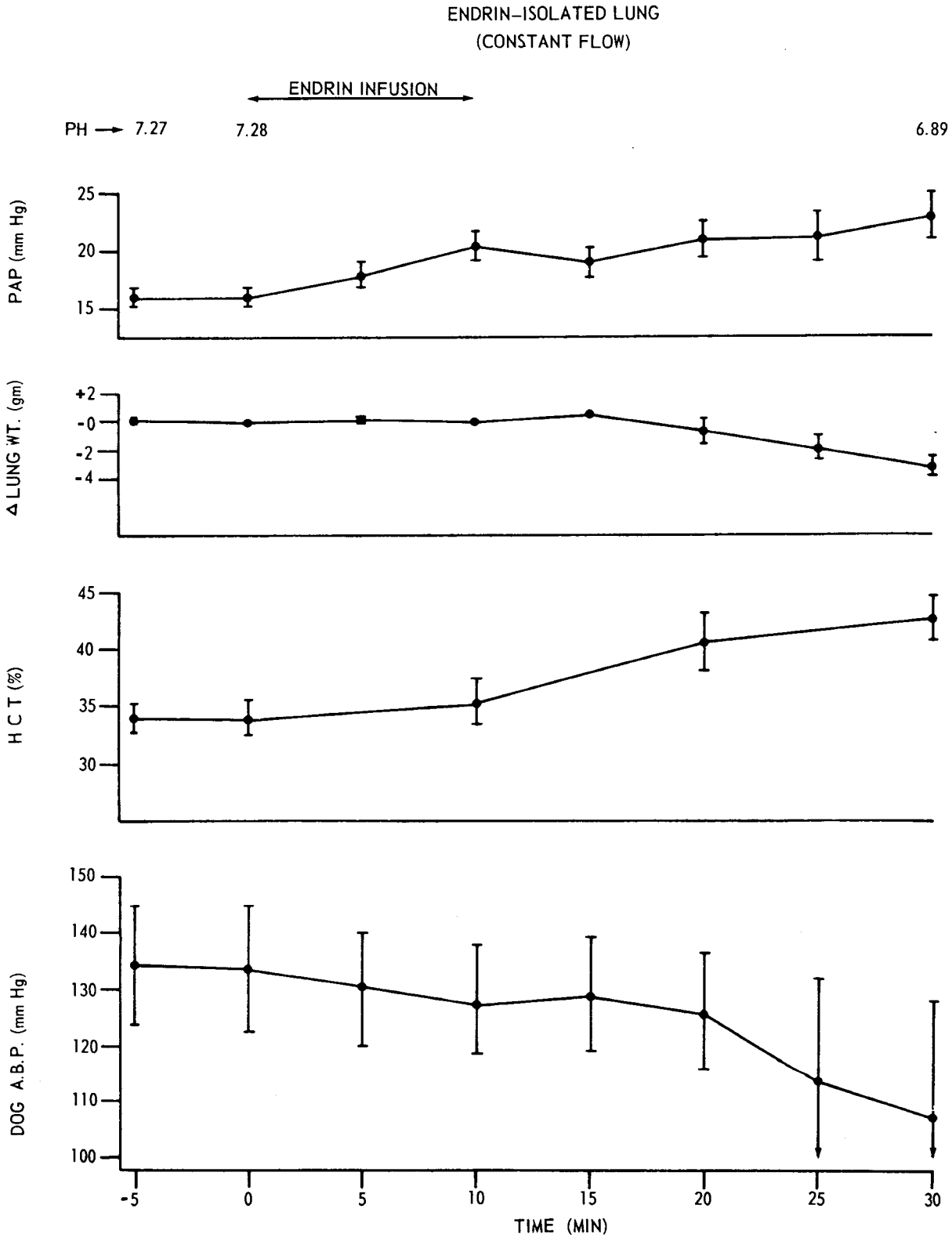

FIGURE 2. Mean values ∓ standard errors from five dog pump-perfused isolated lung experiments in which endrin was infused are shown. Dog ABP = table dogs mean systemic arterial blood pressure; Hct = blood hematocrit; △ lung wt = change in isolated lung weight from control; PAP = pulmonary artery pressure. Pulmonary blood flow was constant. Endrin infusion began at zero time.

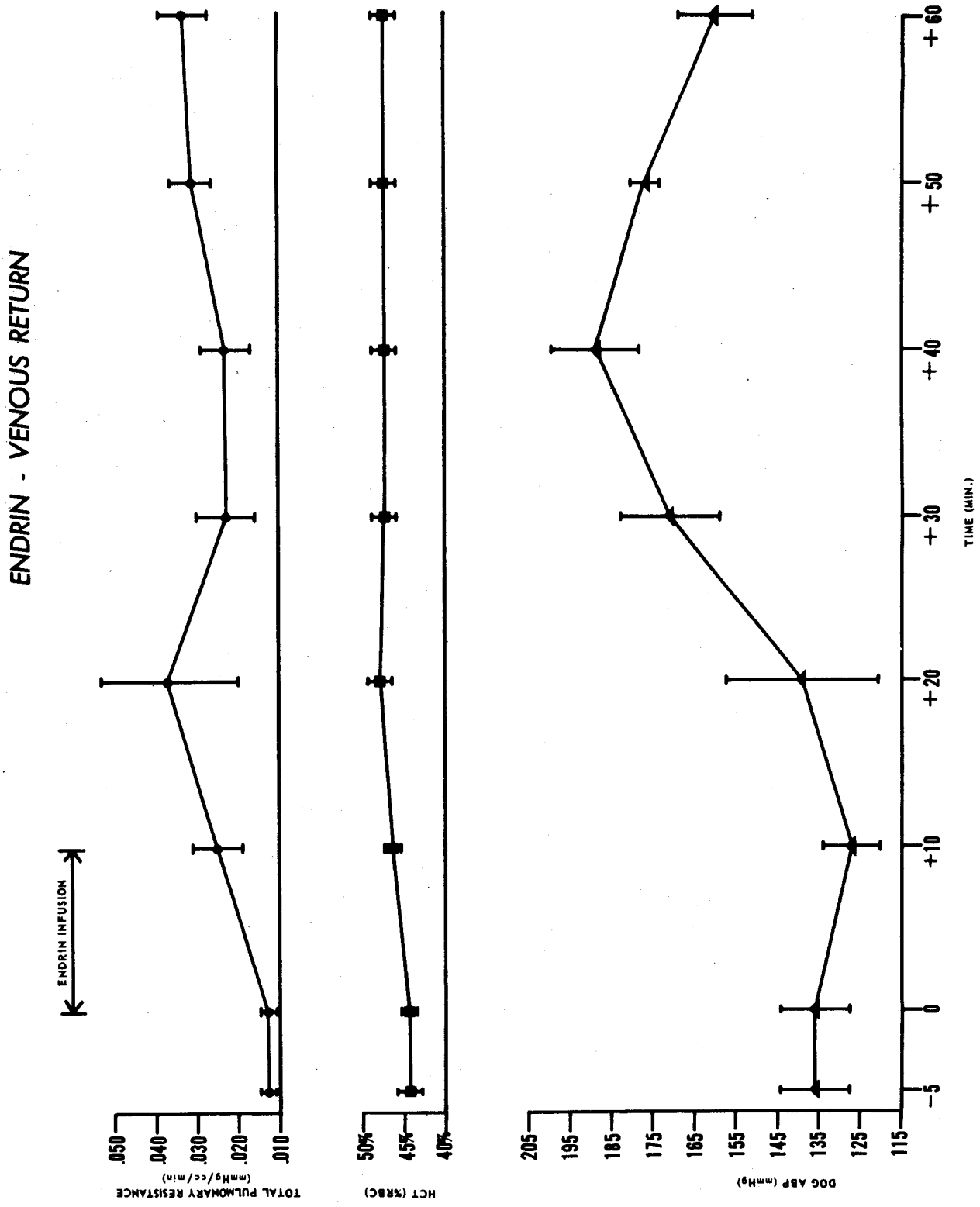

FIGURE 3. Mean values ∓ standard errors from five venous return experiments showing the effect of intravenous endrin infusion on systemic arterial blood pressure (ABP), hematocrit (HCT), and pulmonary vascular resistance. Dogs were pretreated with the muscle relaxant succinylcholine chloride. Endrin began at zero time.

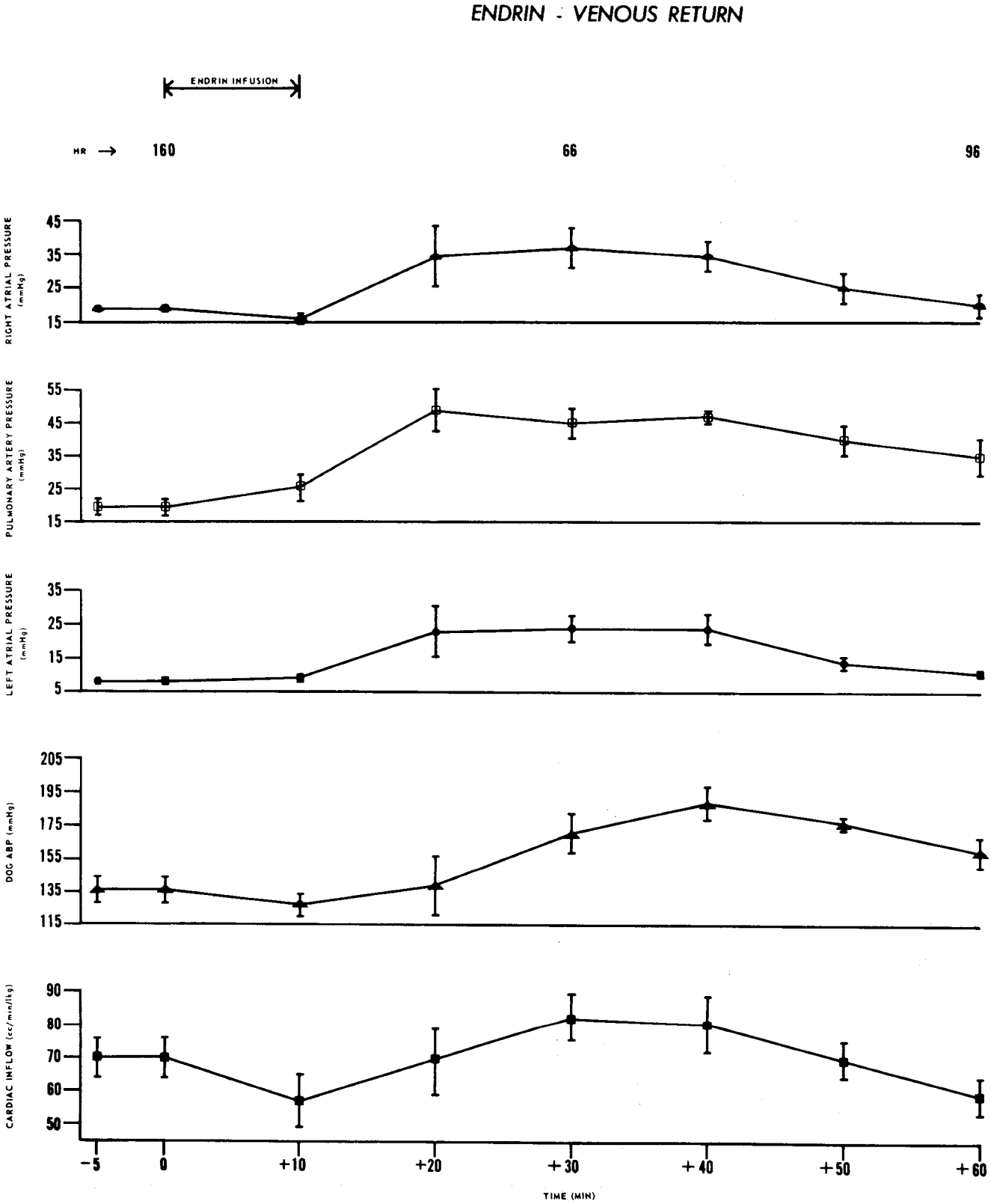

FIGURE 4. Mean values ∓ standard errors from five venous return experiments showing the effect of intravenous endrin infusion on several parameters. Dog ABP = dog's mean systemic arterial blood pressure. Dogs were pretreated with the muscle relaxant succinylcholine chloride. Endrin infusion began at zero time.

PROBLEMS IN AERIAL APPLICATION: levels Release

IV. Effect of Endrin on Venus Return and Catecholamine Release

I. Introduction.

Hypertension followed by hypotension and death have been reported in dogs poisoned with endrin.[1–3] The purpose of the present study was to examine changes in cardiac output (venous return), total peripheral resistance, and plasma and adrenal catecholamine levels as related to the development of hypertension in dogs acutely poisoned with endrin. Studies were performed in totally perfused dogs in which conditions of pressure and flow could be continuously monitored and evaluated.

II. Methods.

Experiments were performed on 50 adult mongrel dogs of both sexes. Six dogs from the intact group and five from the eviscerated series served as control animals and were treated as experimentals except that they received no endrin. Convulsions ordinarily resulting from endrin infusion were prevented by immobilizing animals with succinylcholine chloride as previously described.[3] Intact dogs were prepared as described by Weil, et al.,[4] in order to measure total venous return. Evisceration was done in one series of experiments.[5] Endrin or a solvent blank was infused into the reservoir after a minimum equilibration period of 10 minutes.

Polyethylene catheters in the femoral artery and portal vein were connected to Statham pressure transducers. Systemic arterial pressure (SAP) and heart rate were recorded on a Sanborn direct-writing recorder. Portal vein pressure was also recorded from intact animals but showed no changes.

Total venous return was measured directly with a graduated cylinder and stop watch. Stroke volume and total peripheral resistance were calculated from measured parameters. Blood samples for determination of pH, hematocrit, and catecholamines were taken immediately preceding infusion of endrin and 30 and 60 minutes after infusion. Blood samples were also taken from both artery and vein at these times for O_2 and CO_2 determinations by the method of Van Slyke and Neil.[6] At the end of the experiment, adrenal glands and tissue from the left ventricle of the heart were quickly removed and frozen with dry ice. Estimations of epinephrine (E) and norepinephrine (NE) were made by the method of Anton and Sayre,[7] using the Aminco-Bowman spectrophotofluorometer. Recovery was estimated with each set of analyses by carrying known amounts of epinephrine and norepinephrine through the entire assay procedure. Mean recovery values were 85.26% (SE$\pm$4.35) for E and 73.85% (SE $\pm$2.64 for NE.

A toxicity study was also made with 24 dogs anesthetized with sodium pentobarbital. Endrin was given by intravenous injection, and survival times were noted. Data from six intact control dogs indicate that the volume of solvent (ethyl alcohol) used produced no effect on measured parameters.

Previous studies[1–3,8] have shown that a maximum response in the parameters of interest in this study was achieved by using an endrin dose of 10 mg/kg. Where applicable, statistical significance was determined by applying the "t" test to the mean of control and experimental values at a given point in time.

III. Results.

Results from the various measured and calculated parameters are shown in Figures 1 and 2 (intact dog) and 3 (eviscerated dogs) and Tables 1 and 2. Control and experimental dogs were treated identically until infusion of endrin was begun. Data obtained at these points were pooled and used as pretreatment values in the figures.

A. *Intact dogs (10 experimental, 6 control dogs).* These dogs responded to endrin with a decreased heart rate and increased SAP. Venous return increased from a mean of 55 ml/min/kg in the control period to 89 ml/min/kg ($P<0.01$) during the 30 minutes. Total peripheral resistance

(TPR) increased slightly in control dogs and decreased slightly in experimental dogs ($P<0.2$) at the end of 45 minutes (Figure 1). Hematocrit and pH remained relatively constant.

During the first 30 minutes, oxygen uptake remained constant for experimental animals, then decreased from 4.5 to 2.9 ml/min/kg at 60 minutes ($P<0.2$). Oxygen uptake for controls demonstrated an over-all decrease as compared to zero time.

E plasma level increased sixfold at 30 minutes to 4.7-fold at 60 minutes (Figure 4A), whereas NE increased tenfold after 60 minutes (Figure 4B). No significant changes in plasma levels of E or NE occurred in control dogs during the 60 minutes of observation (Figures 4A and 4B). Concentration of epinephrine diminished in adrenal glands. No change in catecholamine concentrations occurred in cardiac tissue (Table 1).

TABLE 1. Tissue levels of epinephrine and norepinephrine.

	Epinephrine		Norepinephrine	
	Controls	Treated	Controls	Treated
Adrenals	(4)* 868.6	(5) 618.7†	(4) 138.9	(5) 160.4
μg/pr ±SE	±53.4	±92.5	±30.5	±25.0
Heart	(4) 0.158	(5) 0.151	(4) 0.516	(5) 0.500
μg/gm ±SE	±0.053	±0.095	±0.059	±0.085

* Number of animals given in parentheses.
† Differs from controls at $P<0.1$ level.

B. *Eviscerated dogs (five control dogs, five experimental dogs).* Heart rate decreased in both control and experimental dogs of this group. The decrease in experimental dogs was greater than that of the controls ($P<0.05$ at 30 minutes). SAP of experimental dogs increased above that of control dogs after 20 minutes and remained elevated for the duration of the experiment (Figure 3). Stroke volume increased from 6 to 9 ml in experimental animals at 20, 25, and 30 minutes ($P<0.1$, Figure 3). Venous return decreased in both control and experimental dogs. Flow from experimental animals differed maximally from control animals at 30 minutes ($P<0.2$). TPR/kg in the experimental dogs increased only slightly above that of the control values ($P<0.2$ at 60 minutes). pH level of plasma from experimental animals decreased until the pH was one pH unit below that of controls ($P<0.1$) at 60 minutes. Hematocrit increased only slightly in this group of dogs.

C. *Toxicity study (24 dogs).* Results from this study are presented in Table 2 and show the LD_{100} to be 5 mg/kg.

TABLE 2. Toxicity of endrin given intravenously to dogs.

Dose (mg/kg)	No. of animals	Mortality (%)	Mean survival time (hr) ±SE
10	5	100	2.11 ±0.57
5	6	100	2.75 ±0.26
3	8	75	20.19 ±8.13
1	5	0	

IV. Discussion.

Increased systemic arterial pressure has been reported as an early effect of a lethal dose of endrin administered to dogs immobilized with succinylcholine chloride,[1,3] but the mechanism of this effect has not been previously elucidated. Studies with the chlorinated hydrocarbon DDT, a related insecticide, have indicated that increased levels of catecholamines are a factor in the lethal cardiovascular effects of this compound.[9,10] In this study with endrin, elevation of both epinephrine and norepinephrine levels and probable parasympathetic activation,[1,3] make it difficult to evaluate the exact role of the catecholamines in the cardiovascular effects of this compound. The high levels of epinephrine and norepinephrine found are probably of sufficient magnitude to cause an increased SAP through increased circulating blood volume. The high levels of epinephrine are, apparently, not producing the changes in heart rate that might be expected because of an overriding effect of central nervous system stimulation or carotid and cardiac reflexes.

Pressure in a closed system is directly proportional to flow and resistance of the system. Any change in either of these latter parameters will effect a change in pressure. In the present study, venous return increased with corresponding elevation of cardiac output while TPR was not appreciably altered ($P<0.2$ at point of greatest divergence from controls). Evisceration of dogs greatly diminished the increase in venous return after endrin, indicating that the primary source of increased circulating blood volume was visceral vasculature. The possibility of extravascular dilution was not considered probable since the hematocrit tended to increase even in the eviscerated dogs when the splenic stores of RBC were removed, as has been reported in splenectomized dogs during acute endrin intoxication.[8]

Isolated liver studies have demonstrated that injected catecholamines cause the organ to discharge stored blood,[11] and splenic contraction has also been shown to be caused by increased levels of epinephrine and norepinephrine.[12] The level of these substances in the circulating plasma as

found in the present studies is presumed sufficient to cause this action. An increased metabolic activity might also be expected to cause emptying from visceral pools, but a decrease in oxygen uptake after endrin indicates a lessened activity. Depletion of epinephrine content of the adrenal of dogs treated with endrin indicates the source of the high plasma levels of this hormone. The level of norepinephrine in the adrenal did not change, and epinephrine and norepinephrine levels in the myocardium remained unaltered.

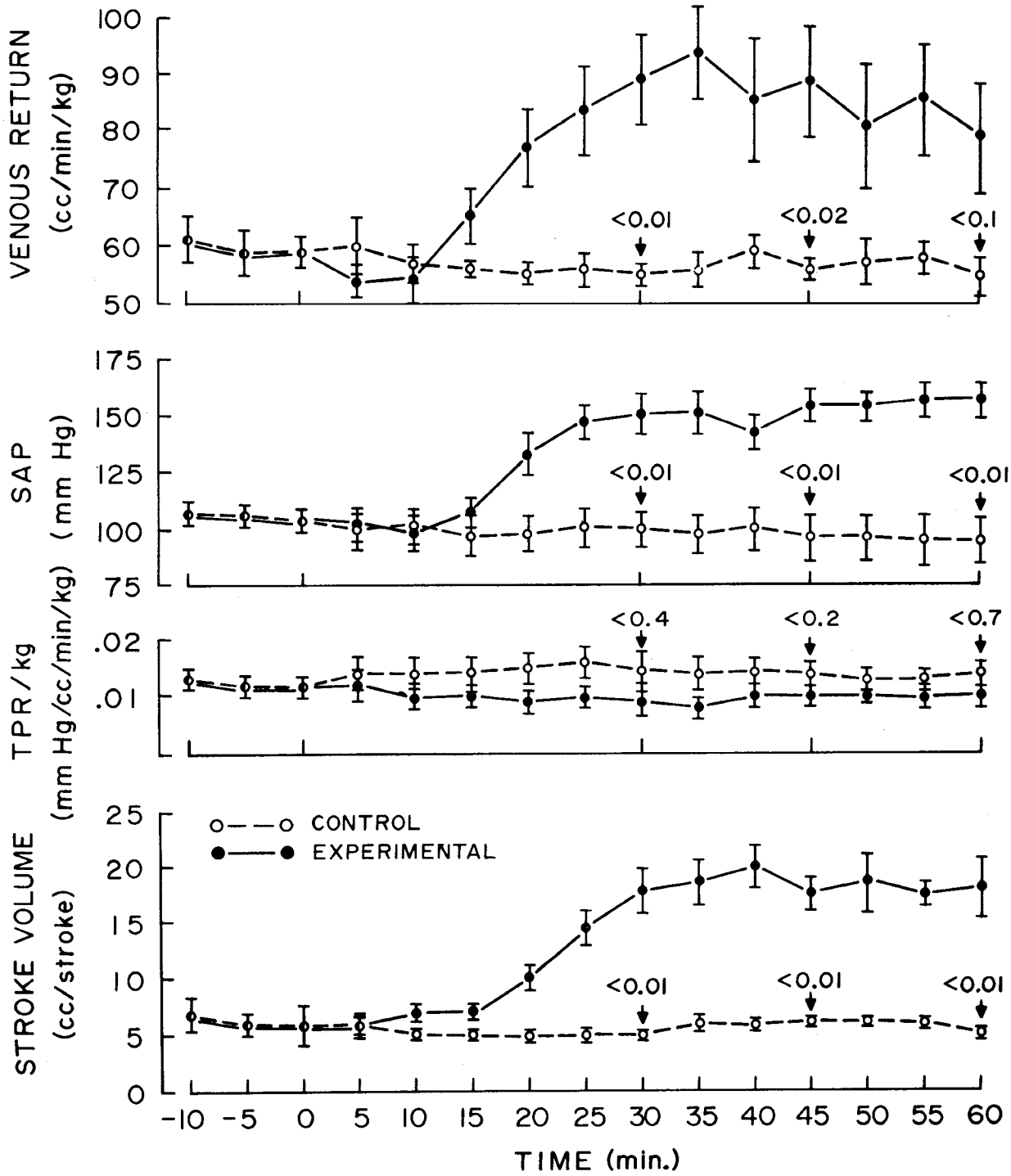

FIGURE 1. Comparsion of values from intact control and experimental dogs. P values at 30, 45, and 60 minutes are presented directly under points for control dogs.

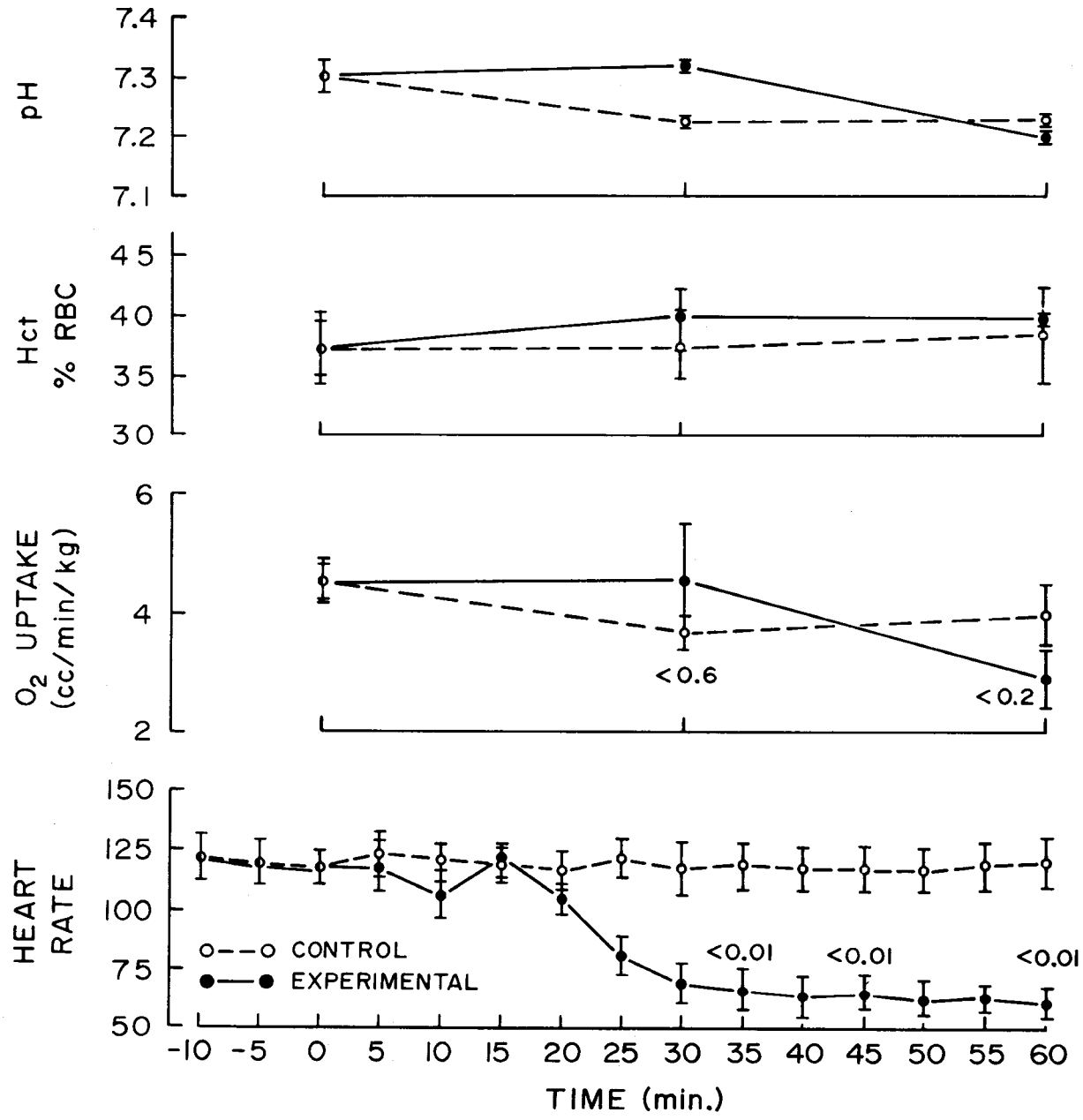

FIGURE 2. Heart rate, oxygen uptake, hematocrit, and pH from intact control and dogs and intact dogs infused with endrin during the venous return study.

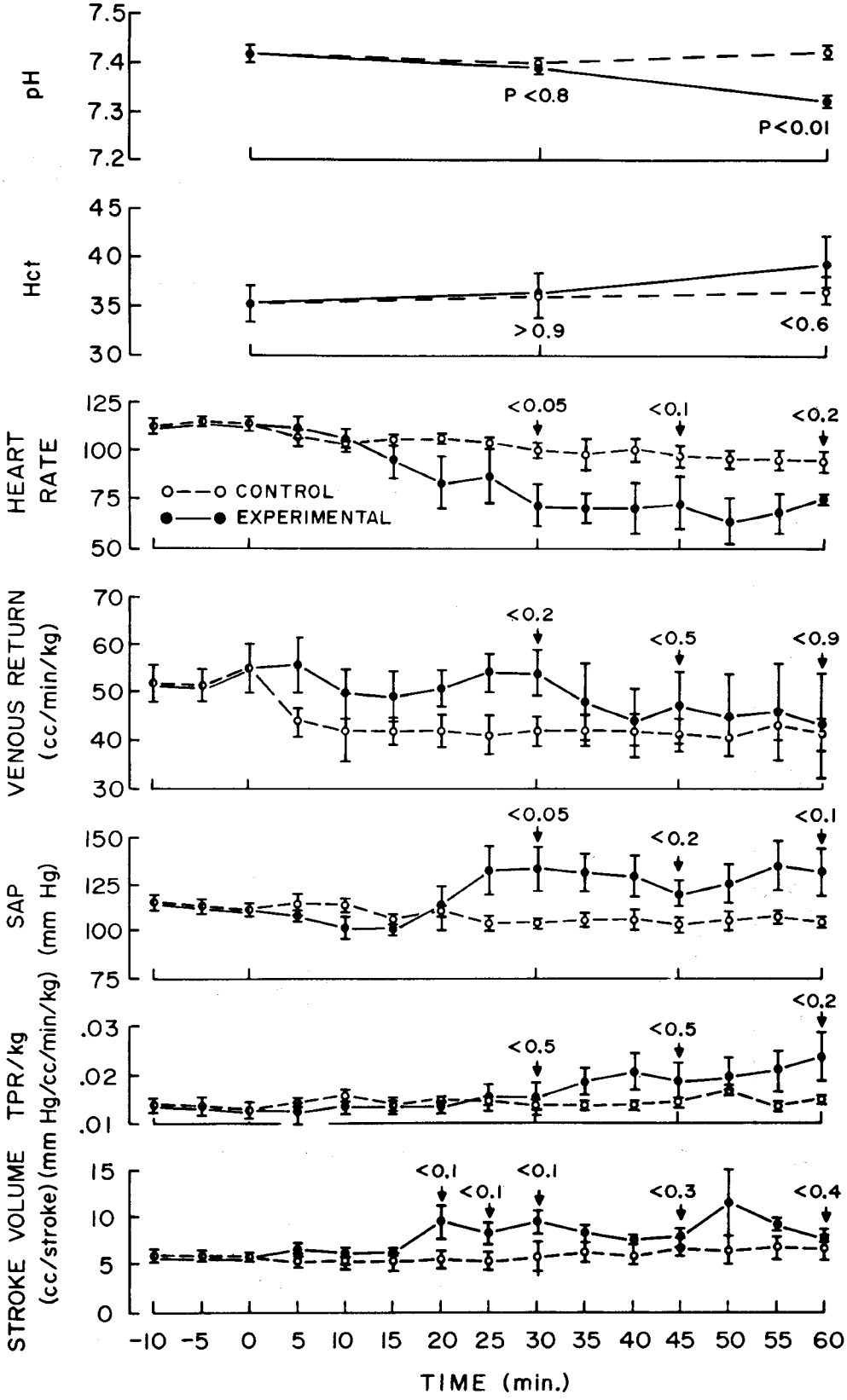

FIGURE 3. Data obtained from eviscerated control dogs in venous return studies compared to that from eviscerated dog venous return after endrin infusion.

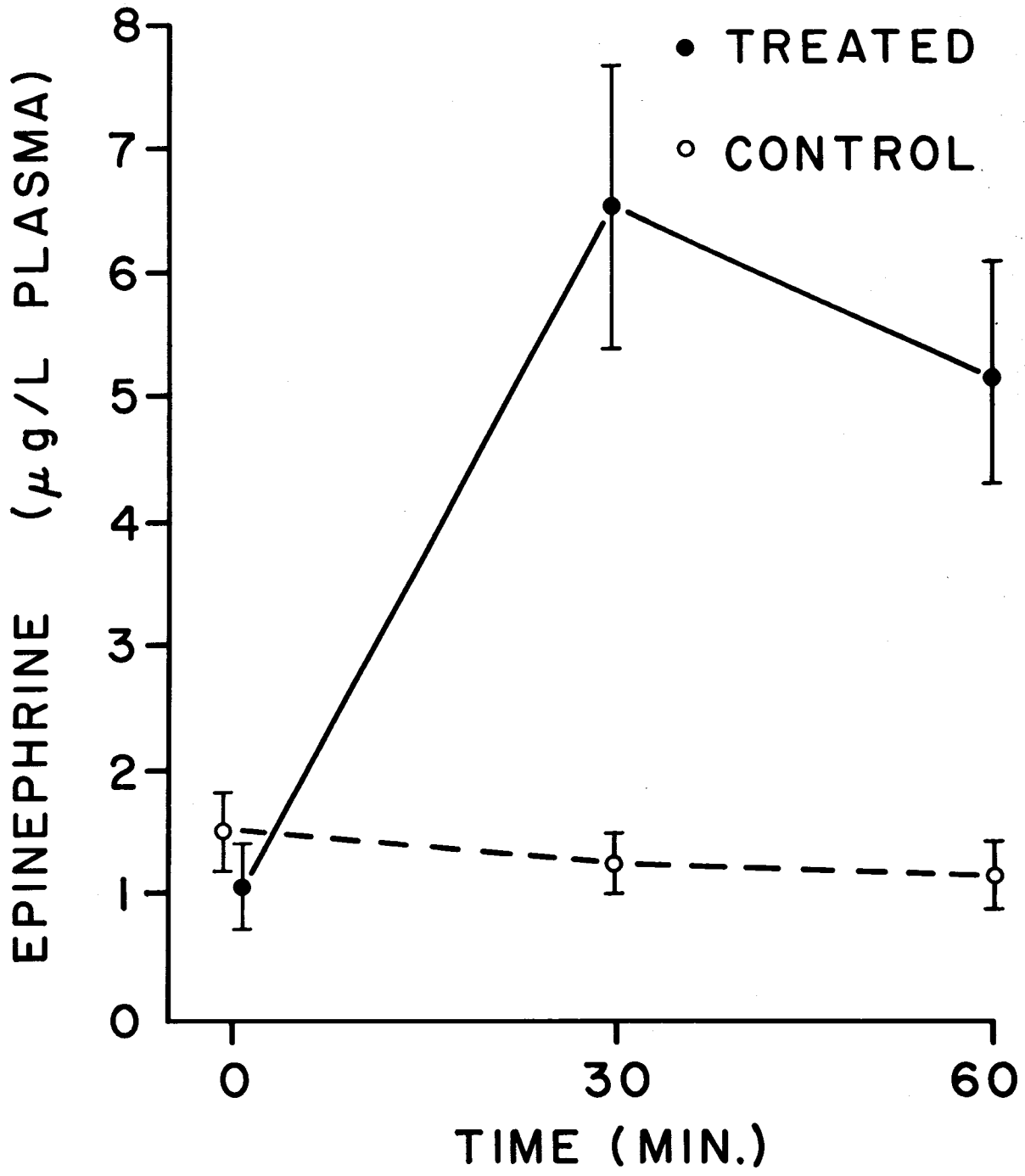

FIGURE 4A. Changes in epinephrine levels in plasma of experimental and control dogs.

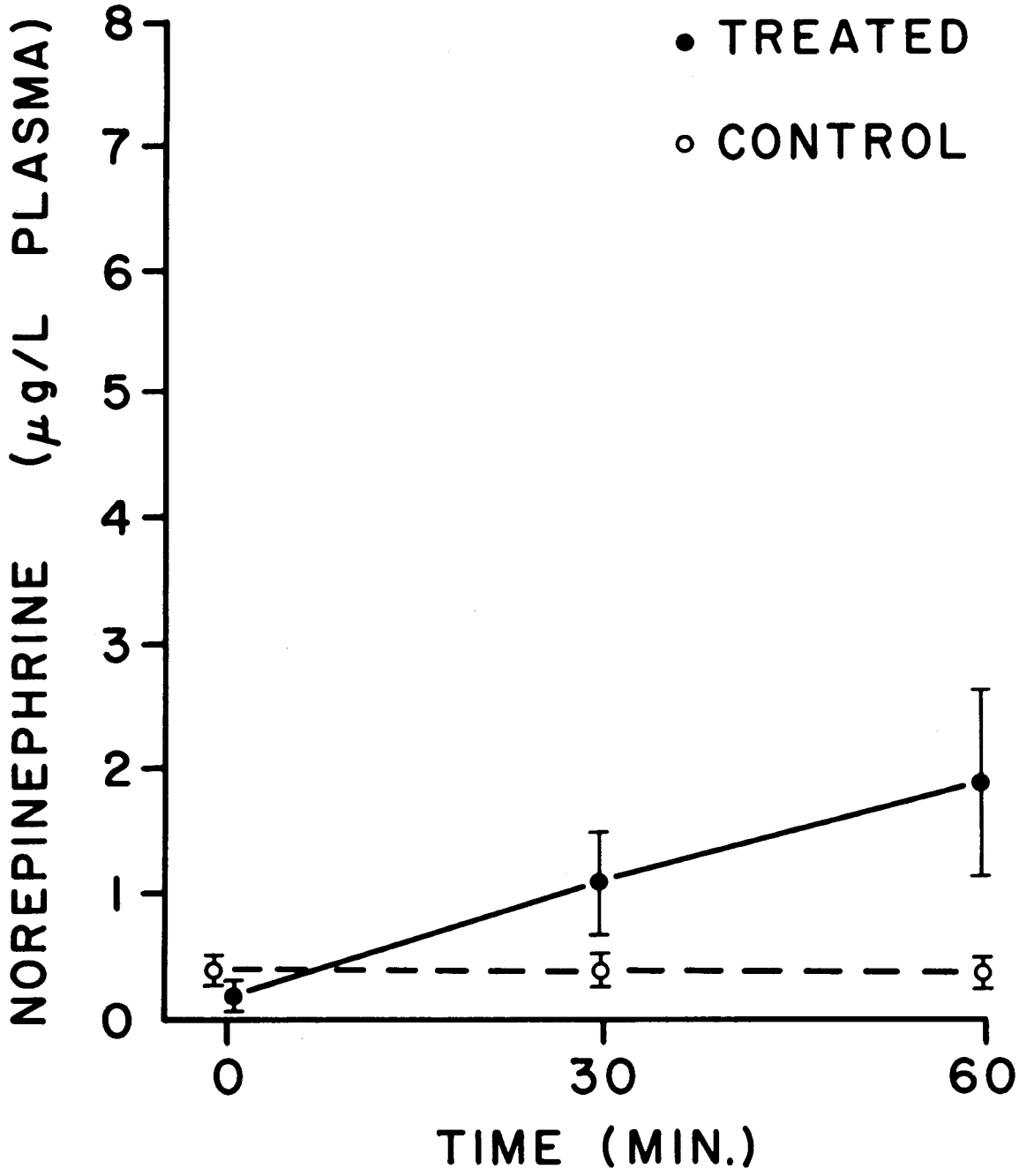

FIGURE 4B. Changes in norepinephrine levels in plasma of experimental and control dogs.

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PROBLEMS IN AERIAL APPLICATION:

V. Effects of the Insecticide Endrin on the Cardiovascular System

I. Introduction.

Acute and chronic effects of the chlorinated hydrocarbon insecticide endrin in dogs have been previously reported.[1, 2] Evidence was provided for an initial action of endrin on the central nervous system with a subsequent sympathoadrenal discharge.[2] Systemic hypertension with marked bradycardia is an early manifestation of endrin poisoning.[1, 2] These previous studies involved either multiple injections of sodium pentobarbital or succinylcholine chloride for the purpose of controlling convulsions during the post-endrin period. The purpose of the present investigation was to elaborate further on the findings of the earlier studies. By avoiding the repeated use of barbiturates or muscle relaxants, possible effects of these agents in modifying the cardiovascular responses to endrin were eliminated. The effects of endrin on the heart, pulmonary bed, and systemic circulation were evaluated, and the role of the adrenal glands in the early vascular response to endrin was determined.

II. Methods.

Several cardiovascular experiments with the chlorinated hydrocarbon insecticide endrin (1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4, endo, endo-5, 8-dimethanoaphthalene, obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio) were performed on adult mongrel dogs intravenously anesthetized with sodium pentobarbital (30 mg/kg in body weight).

Previous studies with endrin employed superlethal doses of endrin.[1, 2] Initial survival experiments on dogs have been performed to determine the dose of endrin to be employed. Results of these are shown in Table 1. A dose of 3 mg/kg was selected to be injected intravenously in all experiments (endrin dissolved in 95% ethanol, 20 mg/ml).

The basic experimental preparation was one in which total venous return was continuously measured, and cardiac inflow was adjusted to equal venous return.[3] Animals were inspired by means of a Starling constant volume respirator, and the chest was opened by a midsternum splitting incision. The right atrium was cannulated following heparinization (3 mg/kg) and ligation of the azygos vein. The superior and inferior venae cavae were separately cannulated and drained into an external reservoir placed in a water bath to maintain normal blood temperature. Blood was returned from the reservoir to the right atrium by a Sigmamotor pump manually adjusted to maintain the reservoir level constant (venous return equal to cardiac inflow). Current unpublished work in this laboratory has shown insignificant volumes of blood to be pooled in the pulmonary circuit after endrin, the weight of the perfused lung decreasing after endrin. Cardiac inflow, as measured when the blood-reservoir level remained constant, was assumed equal to cardiac output in the present study. All cardiac output data, therefore, were derived from flow rate into the right atrium. Total peripheral resistance was calculated by dividing mean systemic arterial pressure by cardiac output. Pulmonary vascular resistance was calculated by dividing the pressure drop between pulmonary artery and left atrium by pulmonary blood flow. Control experiments, in which endrin was not given, were performed in all instances. Left and right atrial pressures were measured in both the venous return experiments and in the isolated heart-lung preparation, previously reported from this laboratory,[4] and aortic pressure was measured.

A metabolic evaluation was performed in which oxygen uptake and carbon dioxide production were determined after endrin by the Van Slyke manometric procedure and in separate experiments following administration of 100% oxygen

during the entire post endrin period. Samples were collected in oiled syringes from the femoral artery cannula and from the pooled venous outflow. Oxygen uptake was then calculated from the A-V difference using the measured outflow (cardiac inflow) at that time.

As previously reported,[1] pH was found to be decreased by endrin. It was thought possible that changes in pH performed a role in causing convulsions seen in endrin poisoning, so the organic amine buffer THAM [Tris(hydroxymethyl)-amino-methane, obtained from Abbott Laboratories, North Chicago, Illinois] was infused as an isotonic solution (36.34 gm/*l*) at a rate that would maintain blood pH constant. The average volume of THAM required by the end of the 60-minute period was 55 cc/kg (SD ±4.3).

Heart rate, pH, and hematocrit were measured in all experiments. Plasma total catecholamine concentrations were assayed by a semiautomated fluorometric procedure.[5] Blood samples for catecholamine analyses were withdrawn at specified intervals during a 1-hour period after endrin in intact animals and in bilaterally adrenalectomized dogs prepared as previously reported.[6]

Blood pressures were obtained by means of vessel cannulation in connection with Statham pressure transducers and registered on a Sanborn multichannel direct-writing recorder. All cardiovascular experiments were of an acute nature, terminating 1 hour after endrin administration.

III. Results.

A. *Systemic Vascular Responses.*—Figure 1 (all figures and tables are in the Appendix) shows the changes in mean systemic arterial pressure, cardiac inflow, and total peripheral resistance within 1 hour after intravenous administration of 3 mg/kg of endrin. Six control animals, not given endrin, showed relatively stable values during this period. Differences in measured or calculated parameters were evaluated for significance at 15-minute intervals after injection of endrin. In seven experimental animals, significant changes in venous return (cardiac inflow) and total peripheral resistance were observed within 30 minutes after endrin injection. A marked rise in cardiac inflow was observed to coincide with a steady drop in resistance. Mean systemic arterial pressure remained fairly constant. Convulsions beginning within 8 to 15 minutes after injection of endrin were observed, and extensive salivation was seen in all experiments. There was no consistent correlation between the onset of convulsions and the onset of hemodynamic alterations in all experiments.

B. *Cardiopulmonary Responses.*—Figure 2 presents findings on the effect of endrin on the pulmonary artery pressure and pulmonary vascular resistance. Although pulmonary artery pressure increases significantly, pulmonary resistance is relatively unchanged because of the large increase in pulmonary blood flow (Figure 1). Mean left atrial pressure increased markedly within 15 minutes after endrin injection as shown in Figure 3. Right-atrial pressure remained low throughout most of the experimental period.

Cardiac standstill occurred in some experiments in which endrin was given, and the experiments were necessarily terminated. The most critical period for development of cardiac standstill was observed within 25 to 30 minutes after injection of endrin and affected about 20% of the dogs used. To determine if endrin has a direct action on the heart, a series of experiments was performed on isolated heart-lung preparations, in which the cardiopulmonary circulation was totally separated from the remainder of the dog. Details of the experimental preparation have been previously described.[4] Ten experiments were performed at an estimated endrin dosage of 10 mg/kg, based on the weight of heart-lung dog. Results from these experiments are seen in Table 2 and indicate that left-heart failure has occurred. Left-atrial pressures rose to high values after endrin injection, while right-atrial pressures remained low. The more sustained elevated left-atrial pressures seen in the heart-lung preparation may have been due to the higher dose. Experiments on the totally isolated heart support those on the intact organ inasmuch as effects are similar though occurring at a different time interval.

C. *Metabolic Responses.*—Experiments were performed to determine the effect of endrin on oxygen uptake and carbon dioxide production, and results are presented in Figure 4. It is seen that control animals show little change in these parameters, whereas injection of endrin results in marked increases in both oxygen uptake and carbon dioxide production. Oxygen was continuously delivered into the Starling respirator from zero time to the termination of experiments in the separate study depicted in Figure 4. Results

show that both oxygen uptake and carbon dioxide production are increased with oxygen administration. Mean RQ values, however, remain relatively unchanged in all experiments. All dogs given endrin convulsed strongly. There was no apparent difference in the degree of convulsions seen in animals in each of the experiments shown in Figure 4. Table 3 presents the mean arterial- and venous-blood concentrations of these gases, and it is seen that arterial and venous concentrations of oxygen are very low within 30 minutes after endrin, venous concentrations decreasing to 2 vol %. With oxygen administration, only venous blood shows a drop in oxygen content after endrin. The arterial concentration of carbon dioxide after endrin decreases at 60 minutes, while the venous concentration is seen to vary. It was incidentally found that arterial blood was capable of taking up more oxygen if it was equilibrated in a tonometer with room air.

Acidosis was observed after endrin with pH values regularly below 7.0 at 60 minutes post-endrin. These data are seen in Figure 5. Infusion of the organic amine buffer THAM maintained the pH constant, but oxygen administration had no effect (Figure 5). pH was well maintained with THAM, but changes in hemodynamic parameters were in the same direction (Figure 6). Neither oxygen administration nor THAM infusion altered changes in mean systemic arterial pressure, although cardiac flow (venous return) increased with THAM infusion (Figure 6). Large volumes (approximately 500 cc/experiment) of an isosmotic solution of THAM were required to maintain pH normal. This was considered instrumental in lowering blood viscosity and increasing circulating blood volume, which would probably effect an increase in the cardiac inflow (see effect of THAM on circulating hematocrit, below).

D. *Heart Rate, Hematocrit, and pH Changes.*—Figures 5, 7, and 8 present data on hematocrit, pH, and heart-rate changes. There is some evidence of bradycardia after endrin, which is mitigated by both oxygen administration and THAM infusion. Hematocrit changes are insignificant in all instances (Figures 7 and 8) but appear to fall with THAM infusion (Figure 8), presumably because of the volumes of fluid required to maintain pH constant. Administration of oxygen had no effect on pH changes as normally observed in animals given endrin alone (Figure 5).

E. *Adrenal Response.*—Previous work suggested participation of the adrenal medulla in the vascular response to endrin.[2] Experiments were therefore included in the present study to determine plasma total catecholamine concentrations in 28 dogs given endrin. Findings are presented in Figure 9. Control experiments (animals not given endrin) showed statistically insignificant changes ($P > 0.10$) in mean plasma catecholamine concentrations. Animals given endrin alone exhibited large mean increases in catecholamine concentration from less than 4 $\mu g/l$ of plasma to over 55 $\mu g/l$ ($P > 0.01$) by the termination of the experiments. Increases in concentration were significantly depressed, however, when pH was maintained constant by use of THAM values approximating only 10 $\mu g/l$ by 60 minutes after endrin ($P < 0.05$, compared with animals given endrin without THAM). Increases in catecholamine concentration were somewhat less (43 $\mu g/l$) when oxygen was administered with endrin, but this difference was not significantly different from animals given endrin alone ($P > 0.10$). A series of acutely operated bilaterally adrenalectomized dogs was given endrin. Values of catecholamine were lowest at zero time and increased significantly to 2.6 $\mu g/l$ by 60 minutes post endrin ($P < 0.01$), Hemodynamic effects of endrin in adrenalectomized animals are shown in Table 4. Responses are not significantly different from those obtained in dogs with intact adrenal glands.

IV. Discussion.

The summary schema shown in Figure 10 helps to evaluate results obtained in these experiments. Endrin injected intravenously at a dosage causing death in approximately 75% of the animals appears to have at least two actions: one on the central nervous system and one on the left ventricle. It is not understood why endrin has a damaging action on the left ventricle while the right ventricle appears to be unaffected. It does not seem feasible that the lung would release an agent in response to endrin that would have a depressant action in the left-ventricular musculature. Results from isolated heart studies do not suggest that increased cardiac work caused the left ventricle to fail, since both cardiac output and aortic pressure remain constant after endrin.

The presence of pulmonary hypertension would apply a greater stress to the right ventricle, but, even in the face of this, right atrial pressure remained low. The apparent adverse effect of endrin on the heart raises considerations regarding possible target sites of action of this class of insecticides. Gowdey and coworkers [7] assert that effects of chlorinated hydrocarbon insecticides are exerted through stimulation of central mechanisms, not peripherally. Recent work in this laboratory has shown that endrin has no direct vascular action on the isolated perfused liver: neither hepatic-artery nor portal-vein pressure were altered by endrin administration.

A notable feature in the present study was the marked rise in venous return after endrin, and the question is raised as to the mechanism of the increase. Endrin does not have a direct excitant action on the heart causing its output to increase, thus elevating venous return. The increased volume of blood returned to the right heart after endrin originates primarily in the hepatosplanchnic bed, since parellel experiments performed in this laboratory [8] show no increase of venous return in animals with total abdominal evisceration. The release of stored blood in the intact dog results in a continued maintenance of a high cardiac output. Since the released blood remains in the active circulation, venous return is continually maintained at a higher level. The increase in venous return is not induced by circulating catecholamines, since adrenalectomy does not modify the response. The slight though significant elevation in plasma total catecholamines after endrin in adrenalectomized dogs suggests the active participation of the sympathetic nervous system. Releasing reservoir stores of blood could result from a massive sympathetic discharge that would augment the cardiac output by increasing venous return. Systemic peripheral resistance markedly falls with endrin administration, which could be due to the marked rise in blod flow. Read and coworkers,[9] using an open-chest dog preparation similar to that employed in the present study, have shown total peripheral resistance to be inversely related to blood flow on a passive basis. The drop in resistance in the present study could, therefore, be passively induced, resulting in part from a rise in cardiac output. Although total vascular resistance represents net changes in resistance, some vascular beds may be dilating and some constricting. Reins and coworkers [2] have shown marked renal vasconstriction, and therefore elevated resistance in the renal bed in dogs administered endrin.

Results from this study offer support for an active participation of the sympathoadrenal system as suggested earlier.[2] The effect, however, of greatly increased levels of catecholamines on cardiac output as observed in the present study is surprisingly negligible. The humoral action of endrin is apparently potentiated by acidosis, since maintenance of blood pH in the normal range significantly decreases the amounts of catecholamines released after endrin. It does not appear that hypoxia is a stimulus for catecholamine release, since administration of 100% oxygen does not significantly reduce the elevation of circulating catecholamines over experiments in which endrin alone is administered. The role of released catecholamines in this form of stress is unknown, since their circulation in large concentrations appears to have little effect on any of the measured parameters. Central-nervous stimulation apparently accounts for the cardiovascular effect of endrin as well as parasympathetic participation in evoking salivation and bradycardia. These latter effects have been described by Emerson and coworkers [1] after injection of endrin and by Gowdey and coworkers [10] following the administration of aldren. Prominent central-nervous effects of the chlorinated hydrocarbon insecticides have been documented by Gowdey and coworkers,[10] Treon and coworkers,[11] Arena,[12] Bell,[13] and Conley.[14]

Consistent findings in endrin poisoning are acidosis and hypoxia. Marked convulsions seen in the present study may have interfered with the normal respiratory exchanges of blood gases, since animals with endrin exhibit cyanosis. Alleviation of acidosis and hypoxia as performed in the present study had no effect on lessening the severity of the convulsions. The convulsions apparently result from a direct action of endrin on the central nervous system.

V. Summary.

Effects of the chlorinated-hydrocarbon insecticide endrin on the cardiovascular system of the anesthetized dog have been studied. Results indicate that endrin may display both central and peripheral actions. Hemodynamic alterations appear to result from central-nervous stimulation and an apparent toxic action on the left ventricle.

A marked and progressive increase in venous return (cardiac output) occurs within 30 minutes following endrin administration. Total peripheral resistance falls significantly and remains low. Endrin appears to exert a toxic action on the left ventricle; left heart failure, demonstrated by elevated left atrial pressure regularly occurred. Endrin produced a rise in pulmonary artery pressure, but no changes in pulmonary vascular resistance or right atrial pressure were observed. The effect of the insecticide in increasing cardiac output primarily through indirect pathways was opposed by the tendency for heart failure to occur. Animals given endrin exhibited large increases in blood catecholamine concentration that were significantly depressed when pH was maintained constant with a blood buffer. Bilateral adrenalectomy significantly decreased catecholamine concentrations that, however, were significantly elevated over preendrin values. Cardiovascular alterations were not significantly correlated with blood concentrations of catecholomines after endrin.

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APPENDIX

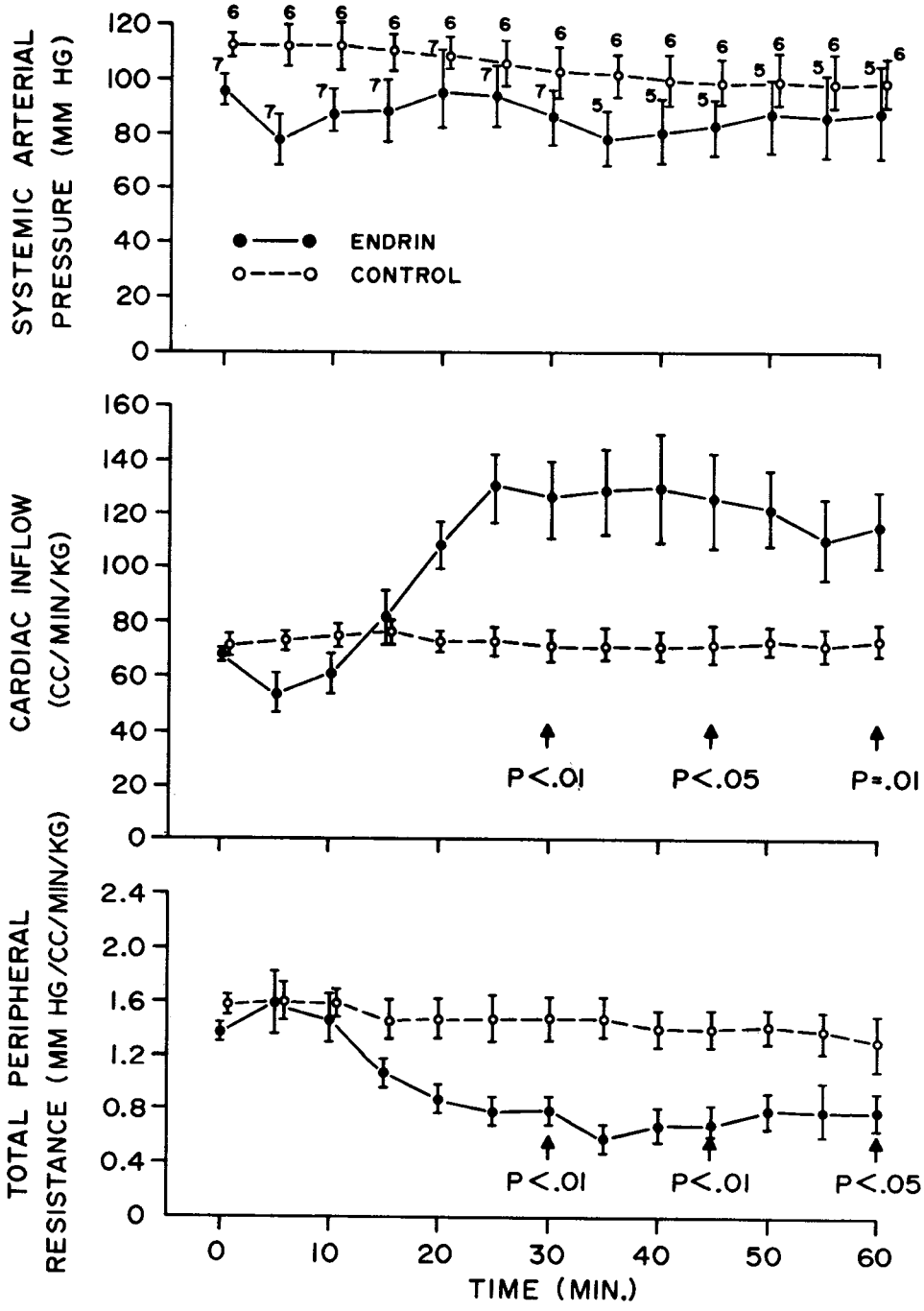

FIGURE 1. Effect of endrin on systemic hemodynamics in the dog (M ±SE) (numbers of experiments are indicated in upper frame).

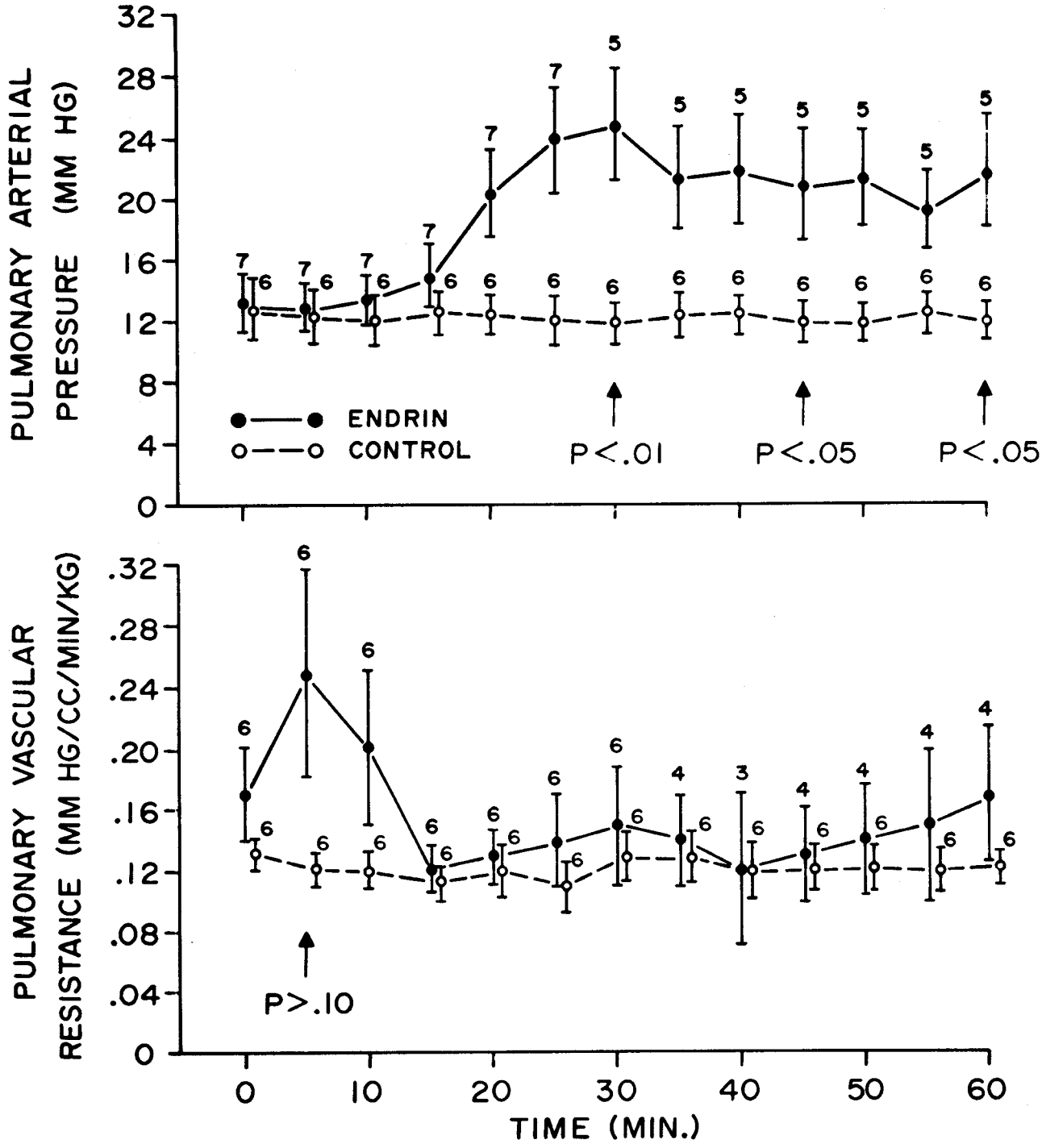

FIGURE 2. Effect of endrin on pulmonary hemodynamics in the dog (M ±SE) (numbers of experiments are indicated in upper frame).

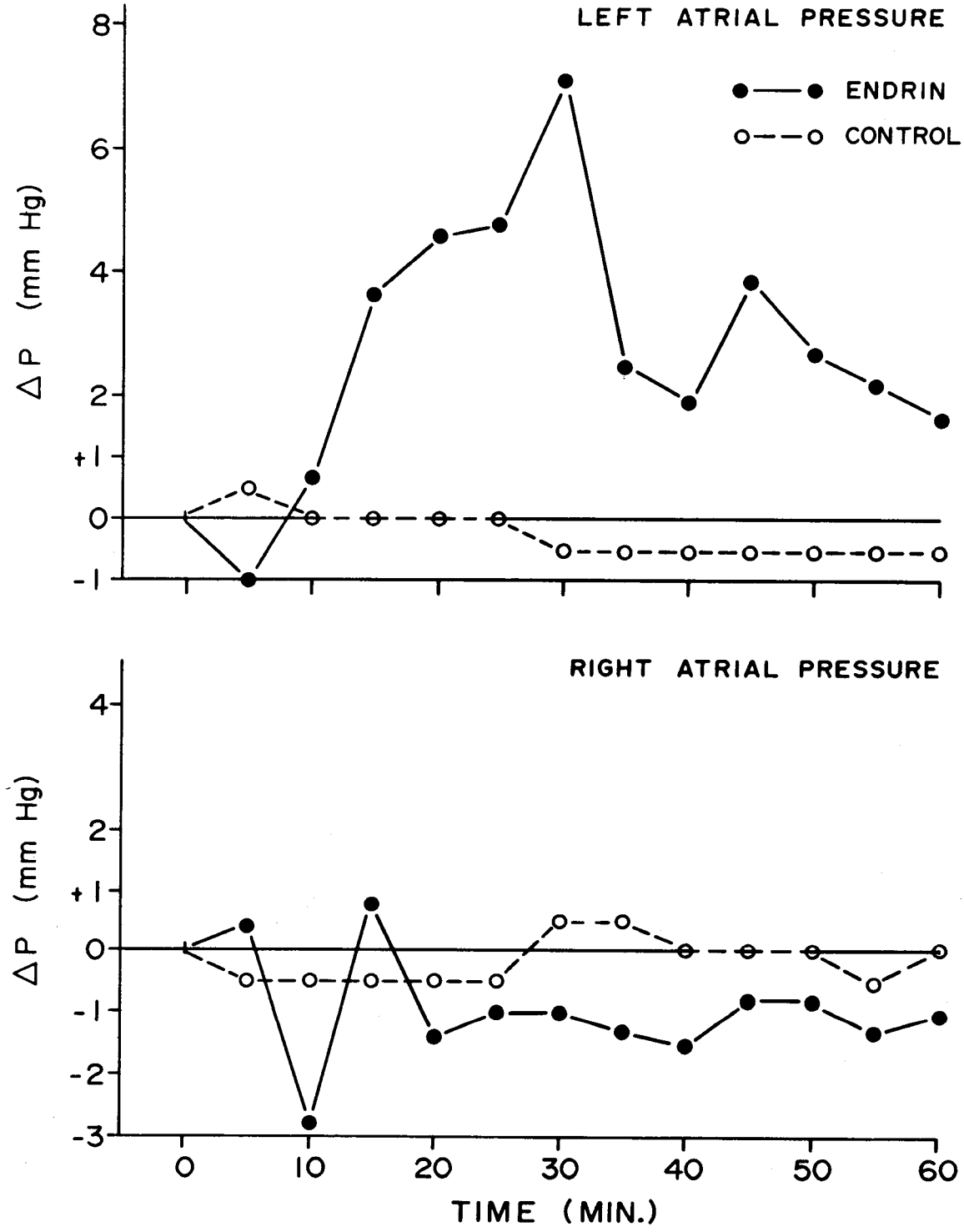

FIGURE 3. Effect of endrin on atrial pressures in the dog (mean values are shown for seven experimental studies and six control experiments).

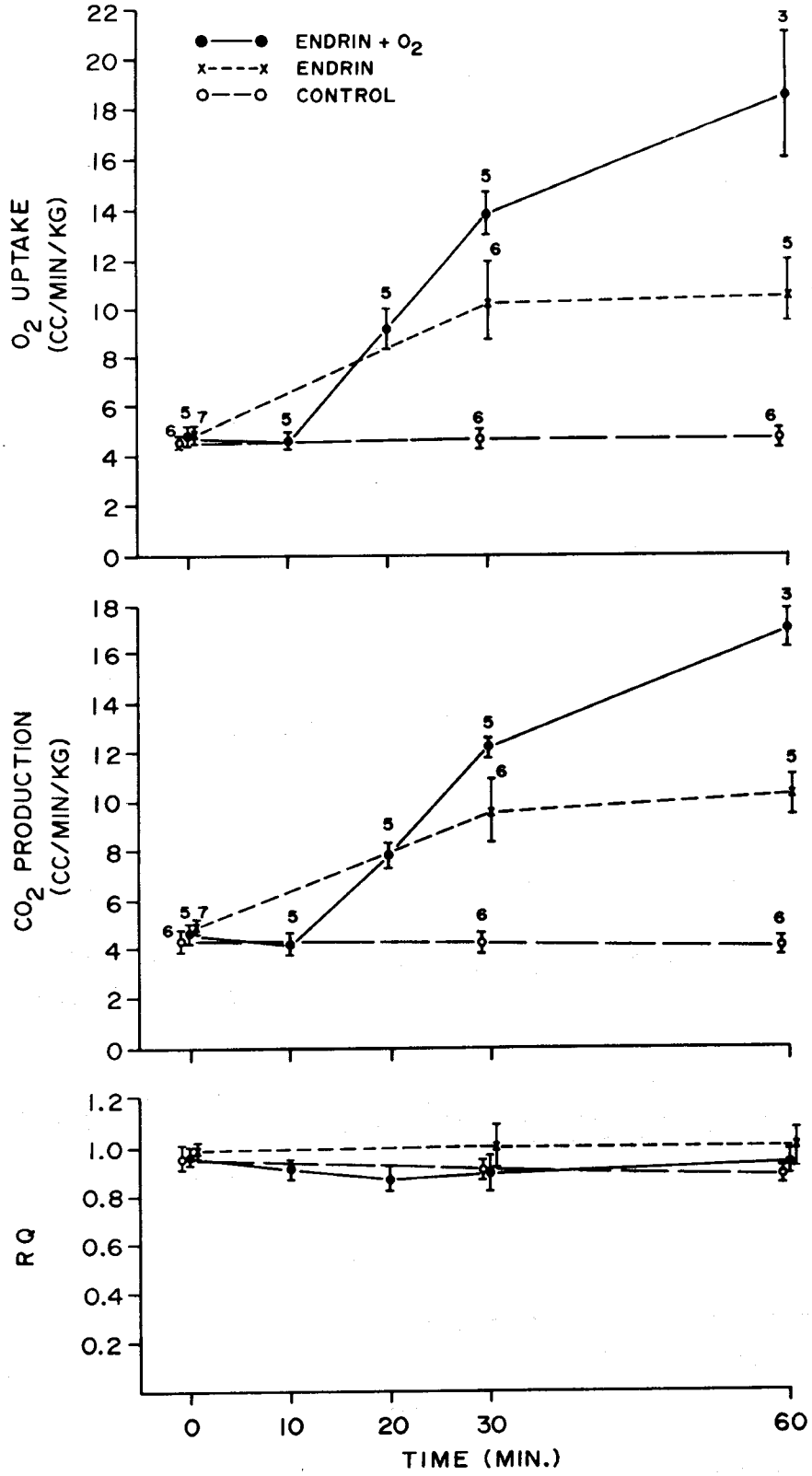

FIGURE 4. Effect of endrin and endrin plus oxygen on oxygen uptake and carbon dioxide production (M ±SE) (numbers of experiments performed are indicated in upper frames).

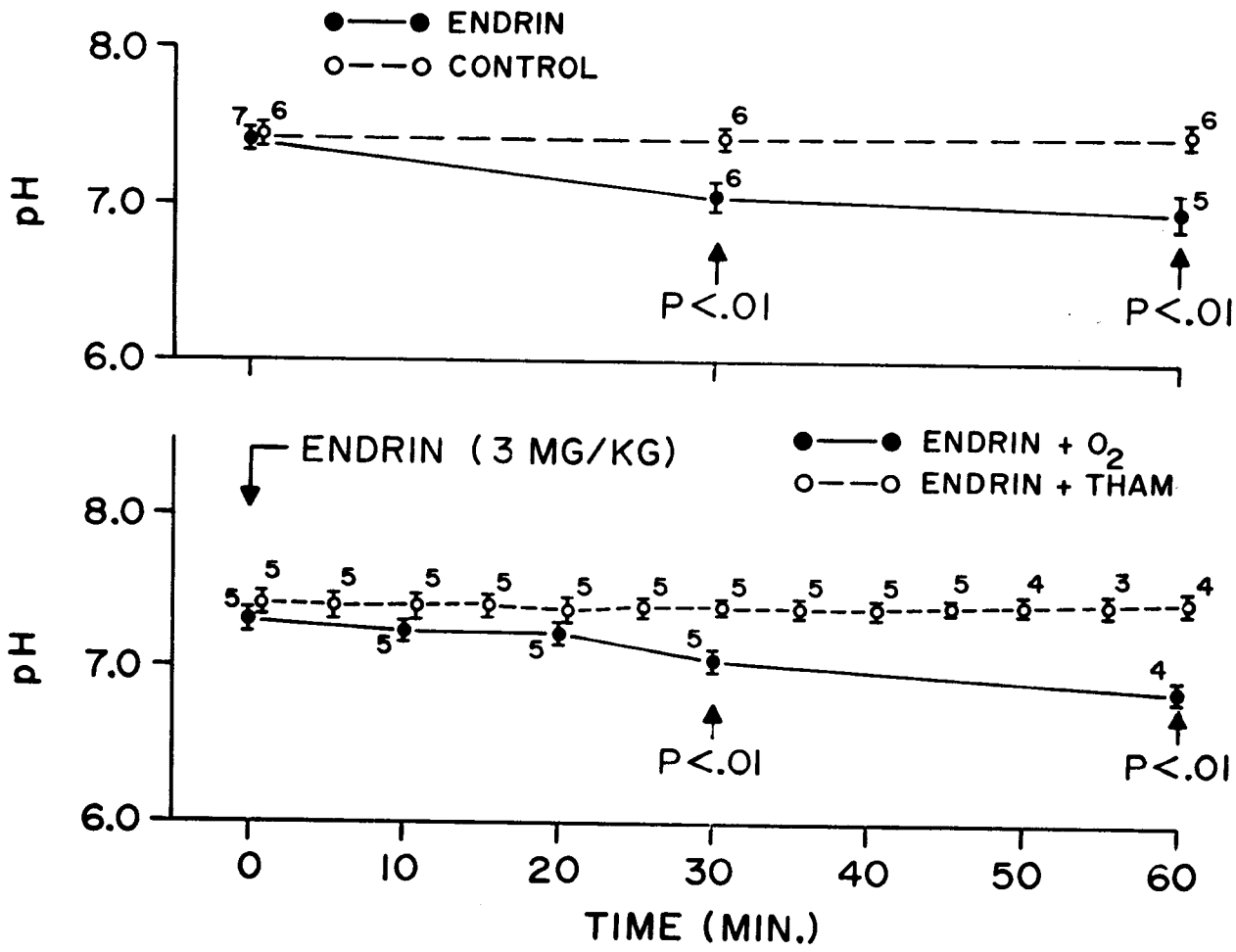

FIGURE 5. Changes in pH in dogs treated with endrin plus oxygen and endrin plus THAM (M ±SE) (numbers of experiments at each point are indicated by numbers).

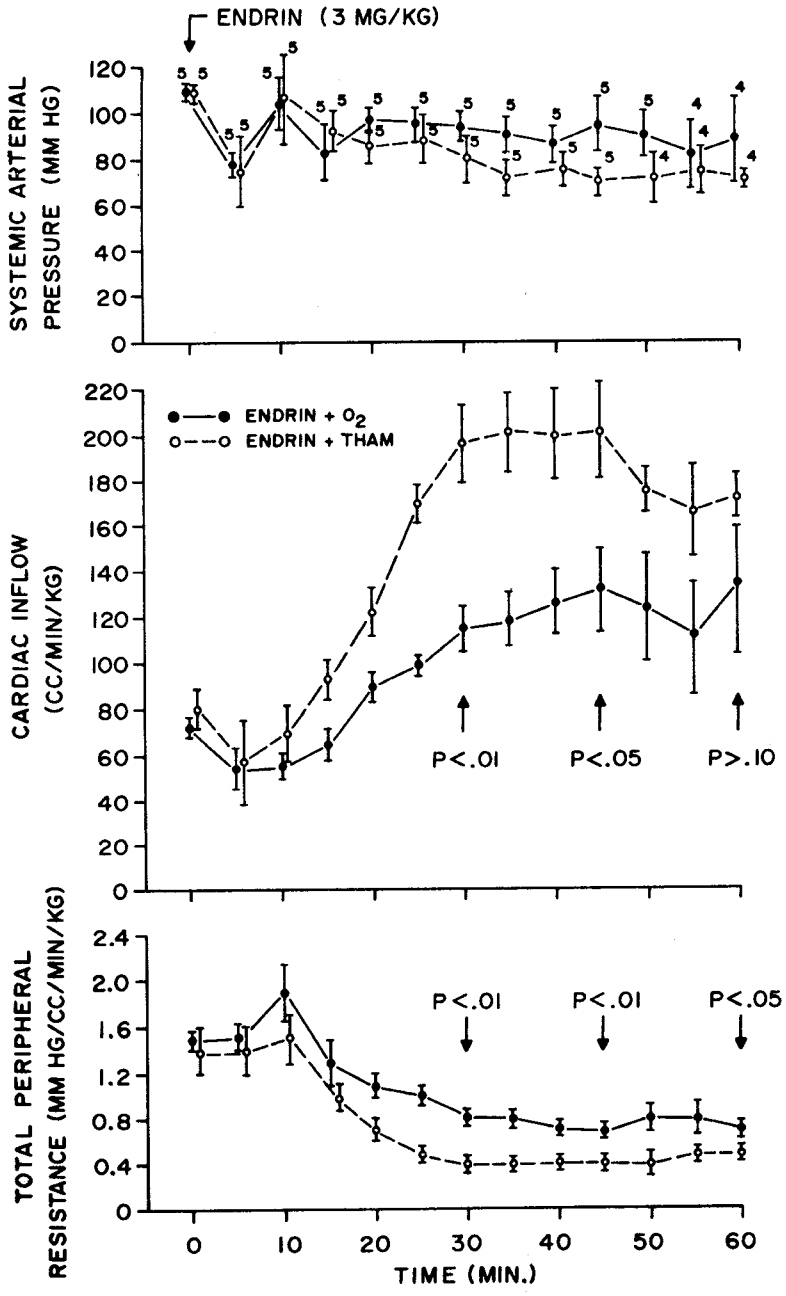

FIGURE 6. Changes in systemic hemodynamics in dogs treated with endrin plus oxygen and endrin plus THAM (M ±SE) (numbers of experiments are shown in upper frame).

Time, post-endrin (minutes)	*Oxygen administered (cc/min/kg) (M ±SE)*
0	530 ±79
10	563 ±49
20	650 ±119
30	744 ±31
40	781 ±20
50	810 ±43
60	747 ±33

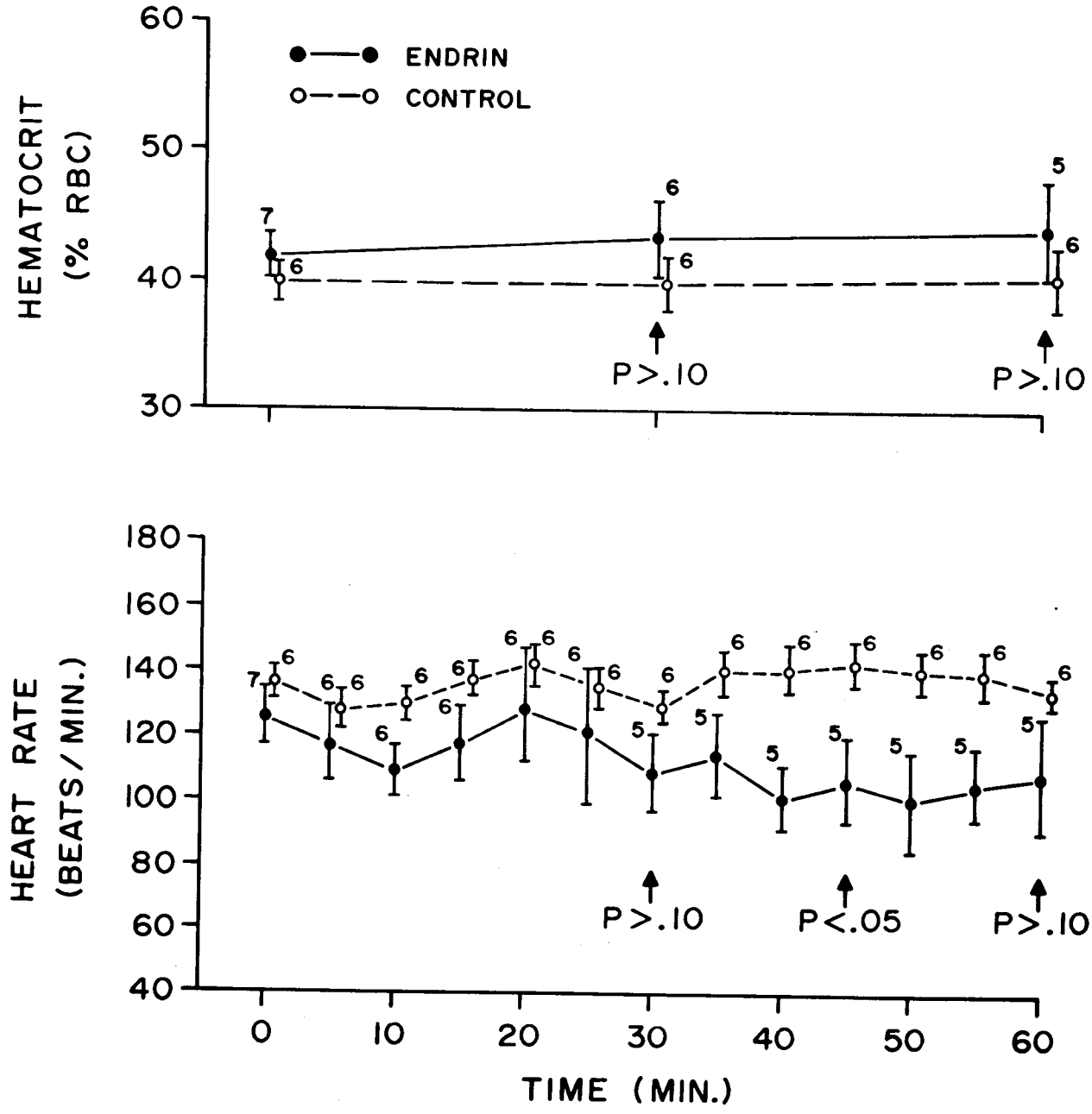

FIGURE 7. Effect of endrin on hematocrit and heart rate in dogs (M ±SE) (numbers of experiments indicated at each time of measurement).

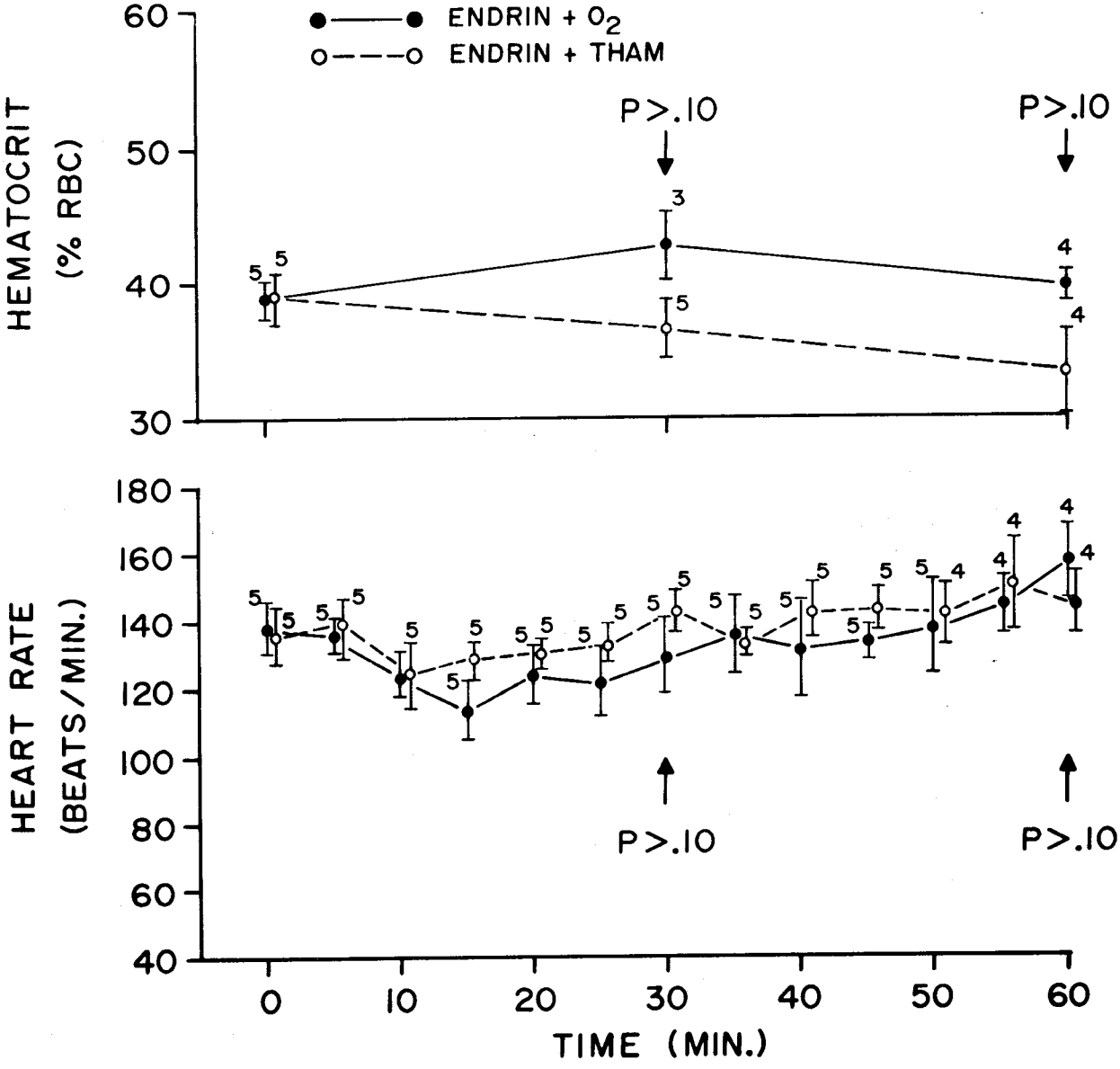

FIGURE 8. Changes in hematocrit and heart rate in dogs treated with endrin plus oxygen and endrin plus THAM (M ±SE) (numbers of experiments indicated on face of graph).

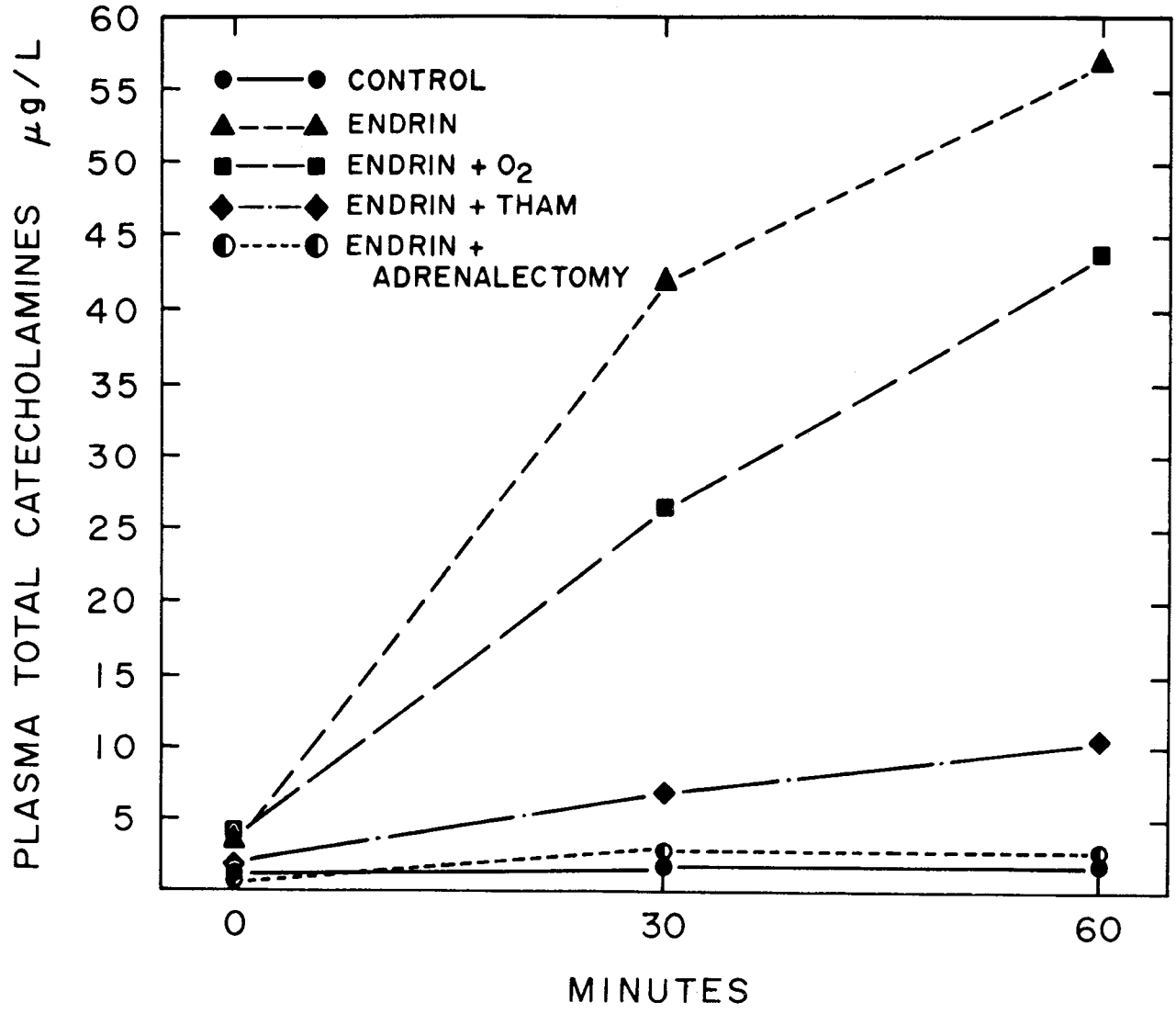

FIGURE 9. Effects of endrin and various treatment procedures on plasma total catecholamine concentrations (mean values).

PLASMA TOTAL CATECHOLAMINES μg/L (M ±SE)

Time (min)	Control	Endrin	Endrin plus oxygen	Endrin plus THAM	Adrenalectomized plus endrin
0	1.1 ±0.6 (N = 6)	3.4 ±3.3 (N = 6)	3.7 ±3.4 (N = 5)	1.5 ±1.0 (N = 5)	0.7 ±0.2 (N = 6)
+30	1.8 ±2.1 (N = 6)	41.5 ±38.9 (N = 6)	26.0 ±20.0 (N = 5)	6.6 ±5.2 (N = 5)	2.5 ±0.6 (N = 6)
+60	1.8 ±1.9 (N = 6)	56.8 ±36.4 (N = 4)	43.4 ±23.2 (N = 4)	10.3 ±9.6 (N = 4)	2.6 ±0.8 (N = 6)

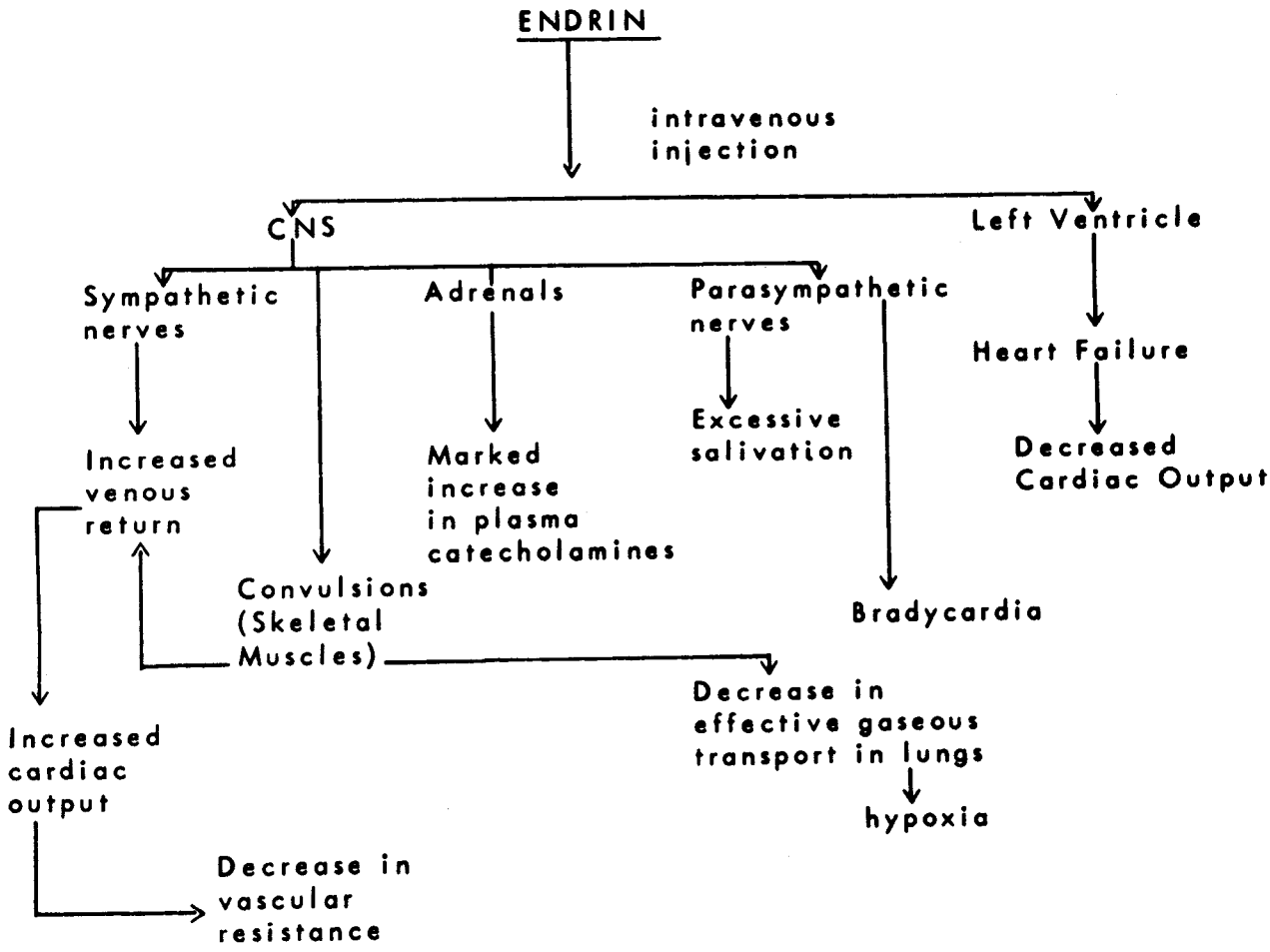

FIGURE 10. Suggested schema for action of endrin in dog.

TABLE 1. Endrin toxicity studies in dogs.

Dose (mg/kg)	1	2	3	5	10
No. animals	5	5	8	6	5
Mean survival times (hr)	Permanent survivors	24	20	3	2
Percent mortality	0	40	75	100	100

TABLE 2. Effect of endrln on isolated heart (M ±SE).

A. Experimentals (five preparations)

Time (min)	ABP* (mm Hg)	RAP (mm Hg)	LAP (mm Hg)	Cardiac output (cc/min/kg)
0	91 ±5.5	6.9 ±3.0	8.4 ±4.1	74 ±5.1
20	94 ±6	5.2 ±2.1	10.3 ±4.3	76 ±6.4
40	94 ±6.1	5.3 ±2.2	14.3 ±4.6	72 ±5.2
60	92 ±6.8	5.6 ±2.3	30.0 ±4.0	67 ±5.8

B. Controls (five preparations)

Time (min)	ABP* (mm Hg)	RAP (mm Hg)	LAP (mm Hg)	Cardiac output (cc/min/kg)
0	90 ±4.8	2.1 ±2.0	3.6 ±1.2	80 ±2.5
20	90 ±4.2	3.0 ±2.3	3.2 ±0.8	79 ±3.1
40	86 ±7.4	3.1 ±2.8	2.8 ±0.8	78 ±3.1
60	85 ±7.5	3.2 ±3.1	3.8 ±0.9	76 ±4.05

*ABP=arterial blood pressure.
RAP=right atrial pressure.
LAP=left atrial pressure.

TABLE 3. Effect of endrin on blood concentrations of oxygen and carbon dioxide (M ±SE).

	Time (min)	Oxygen (vol %)		Carbon dioxide (vol %)	
		Artery	Vein	Artery	Vein
Control	0	17.13 ±0.92	10.78 ±1.16	26.49 ±1.00	33.25 ±1.17
	+30	16.81 ±0.90	10.29 ±1.25	26.63 ±1.01	32.48 ±1.14
	+60	17.10 ±0.92	10.65 ±1.20	24.77 ±1.28	30.44 ±1.32
Endrin	0	17.31 ±0.73	10.03 ±0.78	26.41 ±1.83	32.43 ±2.46
	+30	10.84 ±1.67	2.12 ±1.39	28.96 ±2.39	37.41 ±2.05
	+60	11.21 ±1.75	1.83 ±1.12	19.97 ±3.23	30.45 ±1.45
Endrin + oxygen	0	14.30 ±1.26	7.53 ±1.35	32.35 ±2.48	38.86 ±2.34
	+30	16.64 ±1.21	4.32 ±1.48	30.87 ±2.15	37.23 ±5.56
	+60	14.73 ±2.03	2.34 ±0.68	28.46 ±4.61	43.56 ±1.11

TABLE 4. Effect of endrin following acute bilateral adrenalectomy on hemodynamic parameters in dogs (mean values, five animals).

Time postendrin (min)	Mean systemic arterial pressure (mm Hg)	Cardiac inflow (cc/min/kg)	Total peripheral resistance (mm Hg/cc/min/kg)
0	94	62	1.5
5	77	55	1.4
10	78	66	1.2
15	89	92	1.0
20	78	102	0.9
25	74	113	0.7
30	68	118	0.6
35	65	109	0.6
40	67	102	0.7
45	68	98	0.7
50	67	91	0.8
55	65	93	0.8
60	61	83	0.8