AN ANALYSIS OF THE MECHANISMS OF PENTOBARBITAL INDUCED MYOCARDIAL DEPRESSION BY A STUDY OF ELECTROLYTE DISTRIBUTION

by

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Abstract

Continuous infusions of barbiturates in artificially respired animals result in profound cardiovascular depression with terminal cardiac arrest. In an attempt to elucidate the nature of this cardiac depression at a cellular level, tissue electrolyte analyses were performed on mammalian hearts in failure following infusion of the intact animal with sodium pentobarbital. The patterns of electrolyte distribution encountered were compared to those in animals receiving control saline infusions, and to those in cats subjected to partial myocardial ischemia through ligation of the left coronary artery. Significant species differences in resistance to the cardiolethal effect of pentobarbital were observed. On the basis of differences in effects on electrolyte distribution and pattern of failure, the conclusion was reached that myocardial ischemia was probably not responsible for pentobarbital failure in the cat and the dog. In cat auricles, pentobarbital exerted a selective action on electrolyte metabolism which may have been related to depression of atrial electrical activity. In addition, evidence was found of a direct action of pentobarbital on cardiac contractility independent of Na and K distribution. Control electrolyte values provided support for the hypothesis that electrolyte distribution is related to cardiac automaticity.

Approved:

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I. INTRODUCTION

The ability of large doses of barbiturates to produce profound depression and eventual cardiac arrest in dogs has been attributed to a direct depressant action on the contractile force of the myocardium (Daniel et al, 1956). Electrocardiographic (ECG) recordings during sodium pentobarbital (PB) infusion showed little change in pattern other than a terminal bradycardia. However, when the negative inotropic action of PB was antagonized by infusion of sympathomimetic amines, permitting large amounts of the barbiturate to be administered, atrial standstill and A-V block occurred. Holland (1954) has reported that PB alters the rate of K transport in isolated rabbit and guinea pig auricles in concentrations which produce a 50-80% reduction in the amplitude of contraction. In addition, the changes encountered in the intracellular amounts of Na and K following previous stimulation (Hajdu, 1953), and the administration of digitalis (Szent-Gyorgyi, 1953, and Conn, 1956) and epinephrine (Szent-Gyorgyi, 1953) have been suggested to underlie changes in cardiac contractility.

The present investigation was undertaken in order to discover whether or not the cardiac failure induced by PB was associated with alterations in tissue electrolytes in the intact animal. In the dog, no significant changes were encountered, in keeping with the earlier finding of little change in electrical activity. In the cat, however, the response to continuous

infusions of PB followed a different course, and the cardiac lethal dose was significantly greater than in the dog. Bradycardia was pronounced, even without infusions of sympathomimetic amines, and terminal electrolyte changes were marked. The evidence suggests a selective action of PB on cat auricles.

II. METHODS

Cats

PB Infusion. Cats weighing 1-3.5 Kgm. were anesthetized, intraperitoneally and intravenously, with 30-45 mgm./Kgm. PB (Pentobarbital Sodium, U.S.P., British Drug Houses, 60 mgm./ml. in 10% ethyl alcohol). The trachea and the left carotid artery were cannulated, and a mercury manometer was arranged to measure arterial blood pressure. A control blood sample was withdrawn from the carotid artery. Infusions were made into the femoral vein by means of an adjustable rate pump. PB was given at the rate of 3 mgm./Kgm./min. in 0.95% NaCl (0.5 ml./min.). Control animals received 0.5 ml./min. of 0.95% NaCl and supplemental anesthetic doses of PB in alcohol. Heart rate, blood pressure, and respiratory rate were recorded every 5-10 minutes. After respiratory failure, artificial respiration was provided at the rate of 16 resp./min. in animals infused with PB. When the blood pressure fell below 15 mm. of Hg (or immediately before termination of the experiment in controls), 1000 I. U. Heparin (Connaught Laboratories) were given intracardially. A terminal blood sample was taken by heart puncture and the chest was opened. In control animals, the heart was removed at once. In PB infused animals, it was memoved when there was no longer a visible heartbeat.

Coronary Ligation. In one group of cats, prepared for infusion as above, partial myocardial ischemia was produced by interference with the coronary blood supply. The chest and

pericardium were opened after respiratory arrest, and the left coronary artery was dissected out above the level of its first major brances. When fifteen minutes had elapsed since respiratory failure and institution of artificial respiration, the left coronary was ligated, the PB infusion was discontinued, and the pericardium and chest were closed. In one animal (Cat # 13) the experiment was allowed to proceed to complete cardiac failure. In the remaining animals, the heart was removed when the left ventricle failed.

Dogs

PB Infusion. Dogs weighing 7-16 Kgm. were anesthetized with 25-40 mgm./Kgm. PB in alcohol and prepared and infused in the same manner as the cats. In both control and experimental animals, artificial respiration was started at the same time as the intravenous infusions which were given at a rate of 1.5 ml./min. At termination of the experiment, only the right auricular appendage and the apex of the left ventricle were removed.

Rabbits

PB Infusion. Rabbits whose hearts were to be used for tissue blood content estimations were prepared in the same manner as PB infused cats. The anesthetic dose required was 35-55 mgm./Kgm. PB, and the rate of the infusions varied from 0.5-1.56 ml./min.

Isolated Hearts. An attempt was made to study the effects of PB perfusion isolated rabbit hearts by a modified

Langendorff procedure. Owing to large losses of K and heart failure in control preparations, the reliability of the technique employed was questioned. Therefore, this phase of the project was abandoned in favour of more detailed studies in intact animals.

Analytical Methods

Tissue Blood Content. Tissue blood content was estimated by a modification of the method of Lowry and Hastings (1942). Readings of hemoglobin concentration were made on the Beckman DU Spectrophotometer. Since no attempt was made to distinguish between blood hemoglobin and tissue myoglobin, the concentrations reported are probably higher than those actually present. The average blood content of the auricles and left ventricles of various animal preparations are listed in Table I (see following page).

The maximum change in concentration of any tissue electrolyte which could be produced by the observed variations in blood content would be less than 3%. The contribution of blood electrolytes to total tissue concentrations was considered negligible, and corrections were not applied.

Tissue Electrolytes. Tissues were dissected free from gross deposits of fat and connective tissue, blotted lightly, and minced with scissors into small weighed vessels. Tissue water and electrolytes were determined by the techniques described by Daniel and Boyes (1956). Fat content was determined by the method of Lowry and Hastings (1942).

TABLE I

TISSUE BLOOD CONTENT OF VARIOUS ANIMAL PREPARATIONS

Animal	Preparation	# of tests	Ml. blood per Kgm. tissue (wet wt.) Auricles	Left Ventricle
Rabbit	Control	3	19.7 ± 2.4	32.5 ± 1.6
Rabbit	PB failure	5	14.8 ± 1.5	29.7 ± 4.9
Cat	Control	5	22.3 ± 2.1	23.0 ± 2.4
Cat	PB failure	1	45.3	20.4
Combined Average		14	20.7 ± 2.3	27.3 ± 2.2

Blood and Plasma Electrolytes. Whole blood samples were measured, weighed and dried for water and electrolyte analysis by the same methods as tissues. Plasma Cl was determined by the method of Asper, Schales, and Schales (1947). Na and K were determined by internal standard flame analysis on diluted plasma with the Janke flame spectrophotometer (Hald, 1951).

Calculations

All tissue electrolyte concentrations are reported in terms of fat free weights. Formulae for the derivation of intra- and extracellular water and electrolytes were taken from Manery (1954). It has been reported that the absolute values of extracellular volume derived from plasma and tissue electrolyte concentrations are unreliable for cardiac muscle (Robertson and Peyser, 1956). Therefore, conclusions arising from the data to be presented are based on comparisons of the relative sizes of the chloride and sodium spaces, and are subject to quantitative revision pending further evidence.

Formulae for statistical analysis were obtained from Snedecor (1946). Variability is expressed as the standard error of the mean. t was calculated, and the probability of a higher value of t of less than 0.05 ($P = < 0.05$) is considered to be significant in the comparison of any two groups.

III. RESULTS

Cardiovascular Depressant Effects of PB Infusions.

The comparative doses of PB required to produce anesthesia, respiratory arrest, and cardiac failure in cats, dogs, and rabbits are listed in Table II. (see following page)

The cardiac lethal dose of PB in the dog, which compares to that reported by Daniel et al (1956) when calculated on the basis of the free base, is significantly lower than in the cat or the rabbit. The ratio of respiratory arrest dose to anesthetic dose was less in the rabbit, but was similar in all species. The ratios of cardiac lethal doses to anesthetic doses were markedly different, being highest in the rabbit and lowest in the dog. The pattern of depression of heart rate and blood pressure also differed according to the species. Figures I and II on the following page show the heart rate and blood pressure, expressed as percentage of the control values, plotted against the mgm./Kgm. of PB infused. In the cat, heart rate decreased steadily throughout the course of the infusion and reached a minimum of 32% of normal just before cardiac arrest. In the dog and the rabbit, the decrease occurred at a slower rate, and the heart was beating at 65% of the control rate in the dog, and 48% in the rabbit when arrest occurred. In all species, the blood pressure fell rapidly to 20-30% of normal with the first 100 mgm./Kgm. of PB. In the cat and the rabbit, the rate of fall then decreased markedly and the animal survived until a considerably larger dose of PB had been infused.

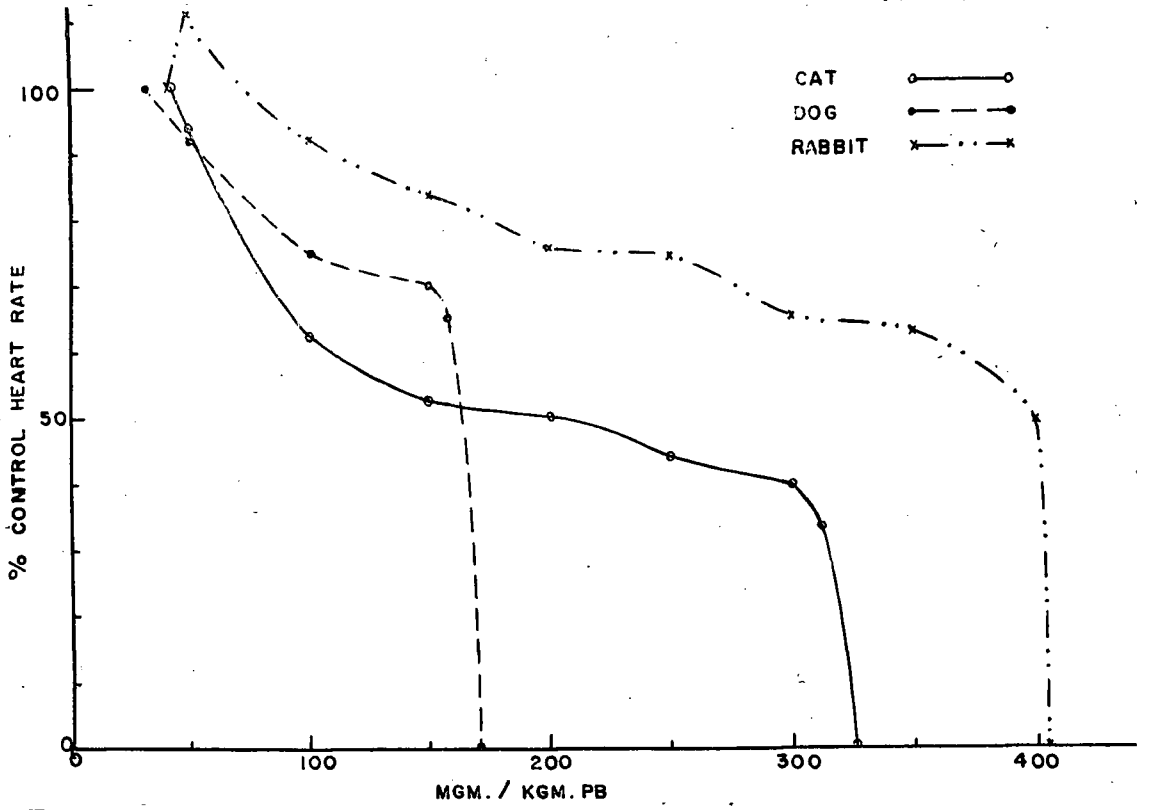

Figure I. Comparative effects of PB infusions on the heart rate of cats, dogs, and rabbits. (Rate of infusion, 3 mgm.PB/Kgm./min.)

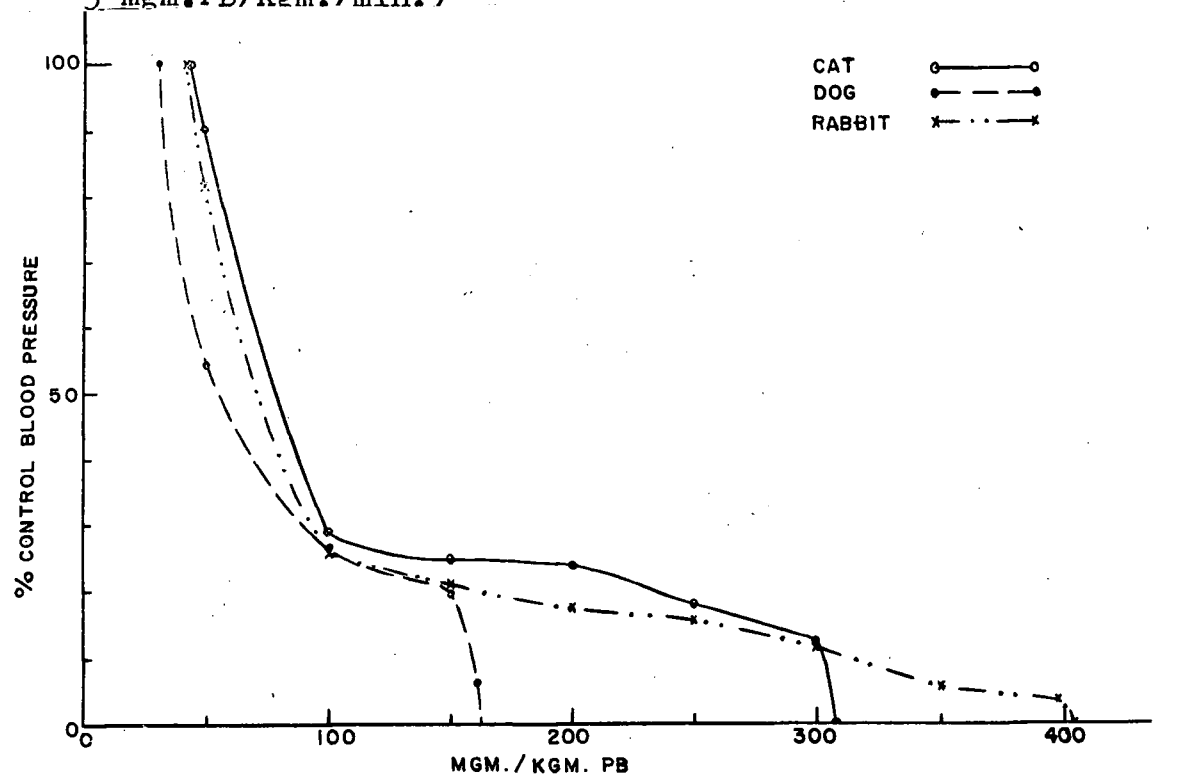

Figure II. Comparative effects of PB infusions on the blood pressure of cats, dogs, and rabbits. (Rate of infusion, 3 mgm.PB/Kgm./min.)

TABLE II

COMPARISON OF THE ANESTHETIC, RESPIRATORY DEPRESSANT, AND CARDIAC LETHAL DOSES OF PB IN CATS, DOGS, AND RABBITS

Animal	# of tests	Duration min.	Anesth. Dose mgm./Kgm.	Resp. Depressant Dose mgm./Kgm.	Ration to Anesth. Dose	Cardiac Lethal Dose mgm./Kgm.	Ratio to Anesth. Dose
Cat	6	94.9 ±9.9	43.4 ±1.2	74.4 ±5.9	1.72	326.1 ±29.5	7.52
Dog	5	43.9 ±4.1	30.0 ±0.0	(55.0)[a]	(1.84)	170.6 ±13.7	5.69
Rabbit	3	118.3 ±29.9	42.9 ±5.6	53.3 ±8.5	1.24	403.7 ±82.6	9.42

a From Daniel et al (1956)
Rate of infusion, 3 mgm. PB/Kgm./min.

In the dog, the blood pressure continued its rapid descent and the animal succumbed within a short time.

Effect of Reduced Coronary Flow in Cats.

The observed difference between cats and dogs in their response to PB infusions indicated the possibility that the greater resistance of the former might be due to the ability of the cat heart to withstand prolonged periods of reduced coronary flow attendant upon the extreme hypotension. With circulation obviously impaired, the concentration of PB reaching the heart from the femoral infusion site might be considerably reduced or non-existent, and eventual cardiac failure might be due to myocardial anoxia alone. In order to discover whether or not reduced coronary flow could produce the effects observed in PB infused cats, a series of animals were given sufficient PB to depress the blood pressure to the plateau level and were then subjected to ligation of the left coronary. The results are summarized in Table III. (see page 13).

In all cases, the animals subjected to coronary ligation survived longer than those infused with PB despite the fact that they maintained a similar degree of hypotension. Although the left ventricle had failed, the heart was still beating strongly enough to produce fluctuations in blood pressure. Figures III and IV on page 12 present graphically the difference in the pattern of cardiovascular depression.

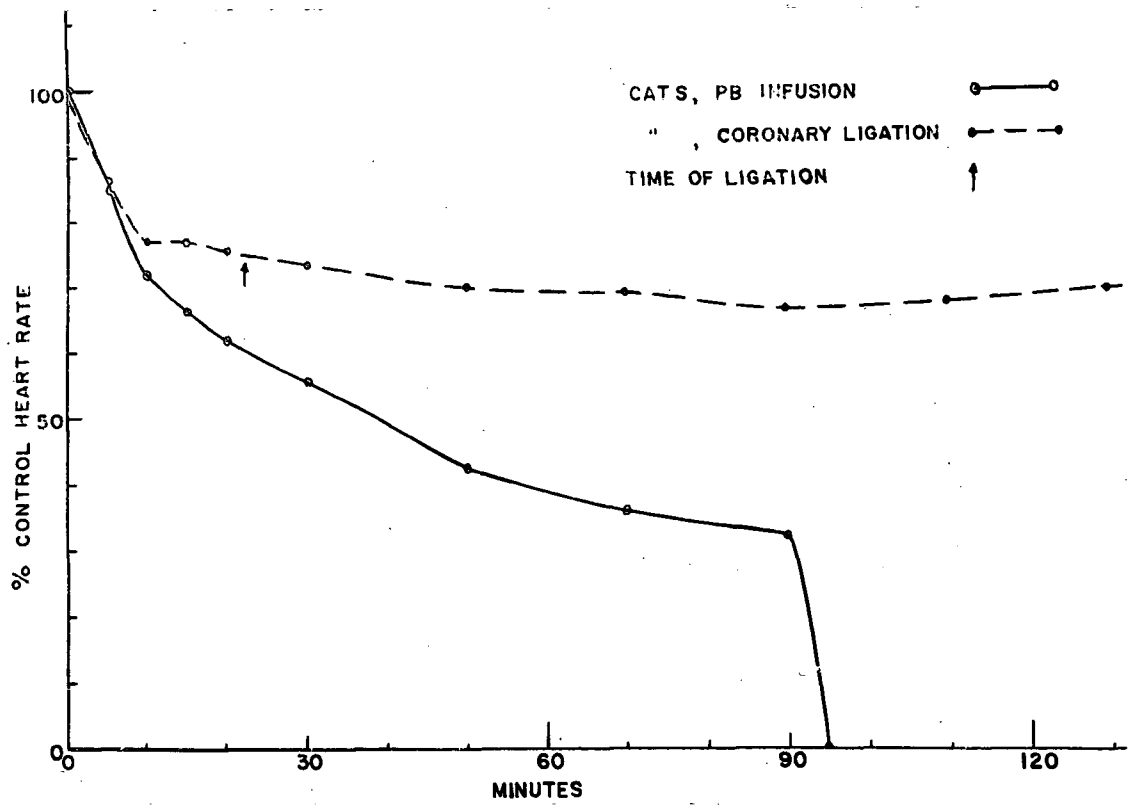

Figure III. Comparative effects of PB infusion and coronary ligation on the heart rate of cats.

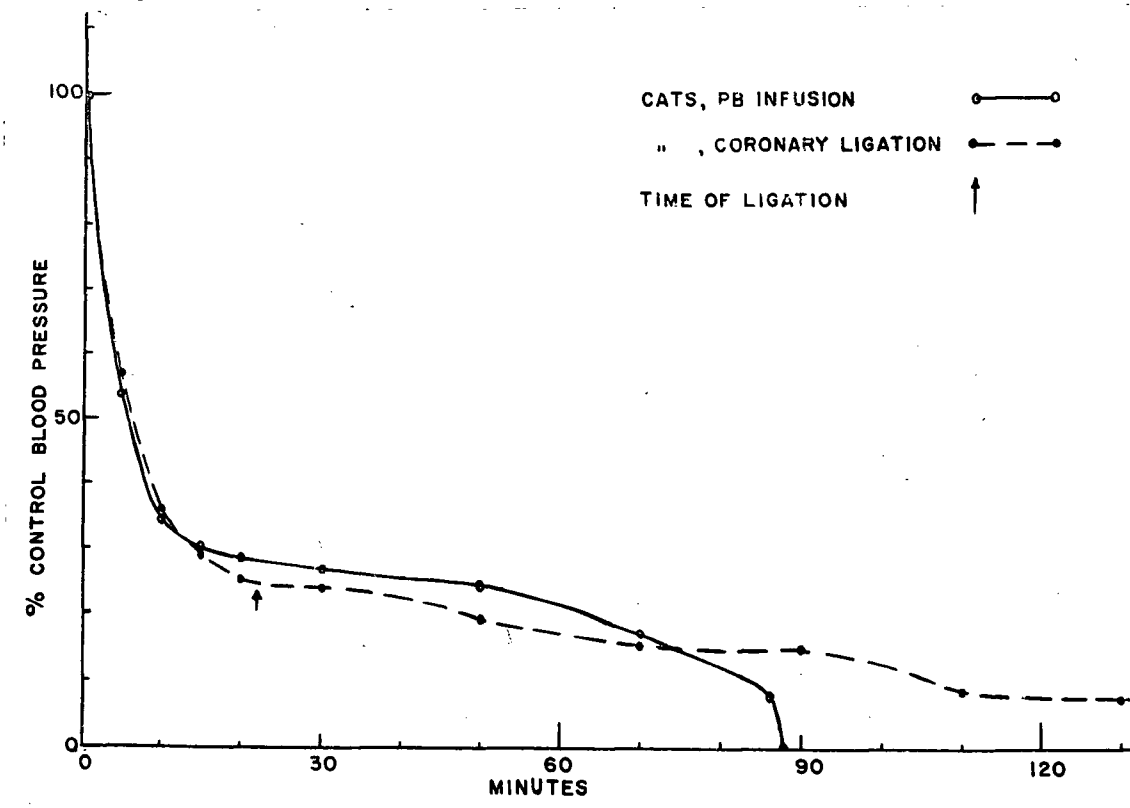

Figure IV. Comparative effects of PB infusion and coronary ligation on the blood pressure of cats.

TABLE III

EFFECTS OF CORONARY LIGATION ON THE CARDIOVASCULAR STATUS OF CATS

Animal	# of tests	Control Heart Rate beats/min.	Control Blood Press. mm. Hg.	Terminal Heart Rate beats/min.	Terminal Blood Press. mm. Hg.	Survival ⁂ Time min.
PB infused cats	6	165 ± 4	110 ± 4	0	0	69.5 ± 8.8
Coronary ligated cats	6	165 ± 6	110 ± 12	89 ± 20	9 ± 5	111.9 ± 21.5
Cat #13	1	180	107	0	0	248.0

⁂ Survival time after ligation of the coronary, or from 15 min. after respiratory arrest.

The blood pressure after coronary ligation followed the same course as in PB failure, but the plateau portion of the curve was considerably extended. The heart did not exhibit the gradual slowing that was characteristic of PB failure in cats, but was maintained above 60% of the control value. The results, as well as the electrolyte analyses reported below, indicated that myocardial ischemia did not duplicate the effects of PB infusion.

Effects of PB Infusion and Coronary Ligation on Tissue Electrolytes. Results of electrolyte analyses are listed in Tables IV (page15) and VI (page18) Data deriving from the assumption that Cl is confined to the extracellular space are reported in Tables V (page 16) and VII (page 19). The most important observations are summarized below.

Cat. In cat auricles, PB failure was accompanied by significant increases in the Na and Cl concentrations, together with a slight decrease in total tissue water, but no change in the K content. The sum of the cations was markedly increased. Na space was increased, as was Cl space to a lesser extent. The content of intracellular water was lower and the intracellular cations appeared to have increased in concentration. The increase in total tissue Na occurred in excess of the increase in Cl.

In cat ventricles, PB failure was associated with similar, but statistically insignificant, changes of a lesser magnitude..However, total tissue water in the right ventricle

TABLE IV

EFFECTS OF PB INFUSION AND CORONARY LIGATION (C.L.) ON THE ELECTROLYTE DISTRIBUTION IN CAT HEARTS

	Tissue	# of tests	Total H_2O Gm./Kgm. wet wt.	Cl mEq./Kgm. wet wt.	Na mEq./Kgm. dry wt.	Na mEq./Kgm. wet wt.	K mEq./Kgm. dry wt.	K mEq./Kgm. wet wt.
Control	Auricles	5	819 ± 4	63.6 ± 3.3	370 ± 14	66.8 ± 3.2	351 ± 12	63.4 ± 3.2
PB		6	811 ± 6	76.4 ± 4.0*	473 ± 33*	89.1 ± 6.4*	345 ± 33	65.2 ± 6.4
C.L.		6	783 ± 21	52.1 ± 1.2**↑↑	384 ± 12↑	70.3 ± 1.9↑	335 ± 12	61.2 ± 2.4
Control	Right Ventricle	5	799 ± 2	42.8 ± 1.9	239 ± 10	48.2 ± 2.4	338 ± 9	78.1 ± 2.3
PB		6	804 ± 4	50.7 ± 1.9*	275 ± 13	53.7 ± 2.3	390 ± 5	76.5 ± 2.3
C.L.		6	797 ± 3	41.5 ± 0.7↑↑	234 ± 6↑	47.5 ± 1.1↑	373 ± 9	75.6 ± 1.9
Control	Left Ventricle	5	794 ± 1	33.4 ± 1.6	203 ± 9	41.8 ± 1.6	383 ± 7	78.9 ± 1.4
PB		6	778 ± 23	40.5 ± 1.3**	217 ± 10	45.8 ± 3.2	397 ± 4	79.6 ± 1.8
C.L.		6	796 ± 3	31.4 ± 1.2↑↑	199 ± 7	40.6 ± 1.2	371 ± 11↑	75.5 ± 2.4

* Significant change (P=0.01-0.05) with respect to control value.
** Highly significant change (P=<0.01) with respect to control value

↑, ↑↑ Significance of changes with respect to PB infusion values.

TABLE V

DATA DERIVED FROM TABLE IV, ASSUMING ALL Cl TO BE EXTRACELLULAR

	Tissue	Cl space Gm. EC H_2O	Na space Gm. EC H_2O	IC H_2O Gm./Kgm. wet wt.	IC Na Gm./Kgm. IC H_2O	IC K Gm./Kgm. IC H_2O
Control	Auricles	476 ± 25	416 ± 21	403 ± 24	- a	156 ± 15
PB		544 ± 27	555 ± 34 ***	282 ± 23 ***	29.6 ± 21.0	228 ± 24 **
C.L.		389 ± 7 ** ↑↑↑	443 ± 11 ↑↑	407 ± 18 ↑↑↑	13.0 ± 7.0	150 ± 10 ↑↑
	Right					
Control	Ventricle	320 ± 12	301 ± 16	501 ± 15	-	154 ± 9
PB		362 ± 15 *	334 ± 12	470 ± 13	-	161 ± 7
C.L.		314 ± 8 ↑↑	295 ± 6 ↑↑	502 ± 6 ↑	-	148 ± 5
	Left					
Control	Ventricle	249 ± 11	261 ± 10	546 ± 10	5.08 ± 1.55	143 ± 4
PB		289 ± 11 **	285 ± 19	499 ± 29	5.95 ± 4.10	161 ± 14
C.L.		234 ± 9 ↑↑↑	249 ± 7	562 ± 6 ↑	4.95 ± 1.13	133 ± 6

a Absence of a value for intracellular Na indicates that a negative value was calculated.

* Change of borderline significance (P=0.05-0.1) with respect to control value.
** Significant change (P=0.01-0.05) with respect to control value.
*** Highly significant change (P=<0.01) with respect to control value.

↑, ↑↑, ↑↑↑ Significance of changes with respect to PB infusion values.

was slightly increased, and Cl space increased more than Na space in both ventricles, so that there was no apparent increase in intracellular Na concentration. The only significant changes from control values were in the increased tissue Cl in both ventricles, and increased Cl space in the left ventricle. The increased Cl space in the right ventricle was of borderline significance.

With coronary ligation, cat hearts showed very few electrolyte alterations from the controls, but again these were most pronounced in the auricles. Tissue Cl was significantly decreased, and minor decreases occurred in total water and K. Na concentration was slightly increased. The Cl space was significantly smaller while Na space expanded slightly.

In the ventricles, the changes occurring following coronary ligation were minor.

When the electrolytes in anoxic hearts, were compared to those in PB failed hearts, significantly lower concentrations of Cl and Na were found in anoxic auricles and right ventricles, and lower concentrations of Cl and K in left ventricles. Total water content tended to be less in all but the left ventricle, and K concentration was also decreased. Na space was smaller in all tissues, significantly so in the auricle and right ventricle. An even greater decrease in Cl space was observed, which was significantly

TABLE VI

EFFECTS OF PB INFUSIONS ON THE ELECTROLYTE DISTRIBUTION IN DOG HEARTS

	Tissue	# of tests	Total H_2O Gm./Kgm. wet wt.	Cl mEq./Kgm. wet wt.	Na mEq./Kgm. dry wt.	Na mEq./Kgm. wet wt.	K mEq./Kgm. dry wt.	K mEq./Kgm. wet wt.
Control	Auricles	5	819 ± 6	54.9 ± 0.9	369 ± 15	66.3 ± 1.4	374 ± 7	67.6 ± 2.7
PB		5	818 ± 6	53.9 ± 2.0	400 ± 27	72.0 ± 2.1*	362 ± 12	65.7 ± 2.7
Control	Left Ventricle	5	786 ± 4	30.9 ± 2.5	196 ± 15	41.9 ± 2.9	420 ± 27	89.7 ± 5.5
PB		5	786 ± 2	33.2 ± 2.6	202 ± 10	43.1 ± 1.9	419 ± 13	89.6 ± 2.7

* Change of borderline significance (P= 0.05-0.1) with respect to the control value.

TABLE VII

DATA DERIVED FROM TABLE VI, ASSUMING ALL Cl TO BE EXTRACELLULAR

	Tissue	Cl space Gm.EC H_2O per Kgm. wet wt.	Na space Gm. EC H_2O per Kgm. wet wt.	IC H_2O Gm./Kgm. wet wt.	IC Na mEq./Kgm. IC H_2O	IC K mEq./Kgm. IC H_2O
Control	Auricles	415 ± 10	427 ± 14	413 ± 11	10.6 ± 6.1	159 ± 8
PB		420 ± 16	458 ± 13	398 ± 11	16.4 ± 2.0	161 ± 5
Control	Left Ventricle	234 ± 20	269 ± 19	552 ± 18	10.2 ± 4.6	163 ± 15
PB		259 ± 21	274 ± 11	534 ± 16	11.1 ± 2.0	167 ± 8

less in all tissues. A significant increase in intracellular water and decrease in intracellular K occurred in the auricles of anoxic hearts, and this pattern was repeated to a lesser degree in the other tissues.

Dog. In dog auricles, the only change in PB failure was a borderline increase in tissue Na unaccompanied by increases in water or Cl.

Dog ventricles showed only minor changes following PB failure.

Effects of Saline and PB Infusions, and Coronary Ligation on Plasma and Blood Electrolytes. Analytical results are reported in Tables VIII (page 21) and IX (page 22). In the cat, terminal blood and plasma samples showed an increase in water and Cl and, after PB infusion, an increase in Na concentration over the initial samples. There was little change in K concentration. PB infusions were accompanied by significantly increased blood water and Cl and plasma Cl as compared to the control saline infusions. Following coronary ligation, the terminal changes were small in magnitude and tended to be opposite in direction to those occurring in PB infusion. In the dog, the only important change was an increase in water content of the terminal plasma samples following PB infusion.

TABLE VIII

EFFECTS OF SALINE AND PB INFUSIONS, AND CORONARY LIGATION (CL) ON WHOLE BLOOD ELECTROLYTES IN THE CAT

Infusion or Procedure	Sample	H_2O Gm./Kgm. wet wt.	Cl mEq./Kgm. wet wt.	Na mEq./Kgm. dry wt.	Na mEq./Kgm. wet wt.	K mEq./Kgm. dry wt.	K mEq./Kgm. wet wt.
None	Initial	828 ± 4	93.8 ± 1.8	760 ± 26	129 ± 2	25.5 ± 0.8	4.35 ± 0.10
Saline	Terminal	849 ± 9 **	97.5 ± 2.5	905 ± 84 **	133 ± 4	32.2 ± 1.8	4.82 ± 0.37 *
PB	Terminal	877 ± 6 *** ↑↑	111 ± 2 *** ↑↑↑	1180 ± 90 *** ↑	142 ± 4 ***	34.2 ± 1.8 ***	4.08 ± 0.12 ↑
CL	Terminal	833 ± 8 §§§	94.9 ± 3.4 §§§	777 ± 38 §§§	128 ± 3 §§	27.0 ± 2.8 §	1.44 ± 0.38

* Change of borderline significance (P = 0.05-0.1) with respect to initial control sample (no infusion).

** Significant change (P = 0.01-0.05) with respect to initial sample (no infusion).

*** Highly significant change (P < 0.01) with respect to initial sample (no infusion).

↑, ↑↑, ↑↑↑ Significance of changes with respect to control saline infusion.

§, §§, §§§ Significance of changes with respect to PB infusion.

TABLE IX

EFFECTS OF SALINE AND PB INFUSIONS AND CORONARY LIGATION (CL)

PON PLASMA ELECTROLYTES IN CATS AND DOGS

Animal	Infusion or Procedure	Sample	H_2O Gm./Kgm. wet wt.	Cl mEq./L.	Na mEq./L.	K mEq./L.
Cat	None	Initial	921 ± 1	121 ± 1	159 ± 1	3.67 ± 0.12
	Saline	Terminal	935 ± 3 **	122 ± 1	160 ± 1	3.83 ± 0.40
	PB	Terminal	942 ± 3 **	130 ± 2 ** ↑	162 ± 1 *	3.70 ± 0.12
	CL	Terminal	930 ± 2 ** §	122 ± 2 §	161 ± 3	3.98 ± 0.86
Dog	None	Initial	925 ± 2	116 ± 2	157 ± 1	4.35 ± 0.17
	Saline	Terminal	930 ± 3	120 ± 2	153 ± 2	4.34 ± 0.23
	PB	Terminal	934 ± 2 *	118 ± 1	156 ± 1	4.08 ± 0.31

* Significant change ($P = 0.01-0.05$) with respect to initial control sample (no infusion)

** Highly significant change ($P < 0.01$) with respect to initial control sample (no infusion)

↑ Significant change ($P = 0.01-0.05$) with respect to control saline infusion.

§ Significant change ($P = 0.01-0.05$) with respect to PB infusion.

IV. DISCUSSION

Species Variation in the Cardiovascular Effects of PB.

The resistance of cat and rabbit hearts to concentrations of PB greater than those required to produce arrest in dog hearts might be due in part to the ability of the former to survive in the presence of reduced blood pressure. This ability could result from better coronary flow at low arterial blood pressures, or from a smaller demand for the metabolites or oxygen supplied by the circulation. In dogs, ventricular tachycardia has been reported to follow severe myocardial ischemia, with ventricular fibrillation ensuing in a high percentage of cases (Harris, et al,1954). Ventricular tachycardia and fibrillation were not encountered during PB infusion. This fact, together with the electrolyte data discussed below, are taken as an indication that decreased coronary flow did not occur to any significant degree in dogs. Direct evidence in favour of the possibility that cat hearts require less coronary blood supply is presented by a comparison of the terminal tissue electrolytes in the right and left ventricles of cats subjected to left coronary ligation to controls and to one another. The values for both ventricles were similar to those in controls, and the left ventricle, which was presumably severely ischemic, showed values similar to those in the right ventricle, with the exception of an insignificant decrease in dry weight.K. Therefore, in contrast to the dog

heart, the metabolism of these tissues, insofar as it is reflected by electrolyte concentration, appears to be little influenced by the degree of tissue anoxia. Even the severe anoxia which was present in the left ventricle did not result in significant alterations from control electrolyte values.

These electrolyte data, combined with the fact that bradycardia was much more pronounced during PB infusion, are evidence that PB infusions acted differently from ischemia in producing cardiac depression in the cat heart. In rabbits, where the heart rate was well maintained, as it was in cats with coronary ligation, the possibility that anoxia was a major factor in producing cardiac arrest by altering the mechanisms concerned in electrolyte distribution cannot be excluded since electrolytes were not analyzed. In dog hearts, myocardial ischemia is probably unimportant in PB depression since ventricular tachycardia did not occur, and since the terminal decrease in intracellular K which has been reported in the presence of reduced ventricular pO_2 (Conn, 1956) was not encountered. Therefore it seems probable that the difference in species resistance to PB induced cardiovascular depression is due to some direct action or actions of the drug on heart tissue. The species differences observed here are cause for speculation as to the susceptibility of the human heart.

PB and Cardiac Electrolytes.

Cat Heart. The changes accompanying PB failure were most marked in the cat auricle. The net effect was an uptake of Na and Cl by the tissue and a loss of water from the cells into the interstitial fluid and plasma. Since tissue K was unaltered, and water was slightly decreased, the results must be due to an uptake of ions without water. The question arises as to whether or not these changes could be due to an expansion of the extracellular space with an osmotic shift of water caused by the hypertonic infusion fluid, The most extreme assumption which could be made is that the infusion fluid (containing 418 milliosmols per liter) accumulated behind the right auricle in the failing heart and replaced the interstitial fluid, thus forming a hypertonic extracellular fluid of theoretically infinite volume surrounding the cells of the right auricle. Even this extreme condition would account for only one-third of the actual loss of intracellular water which occurred. The extreme nature of the assumption is illustrated by (1) the fact that terminal plasma samples, taken by heart puncture, did not exhibit any great increase in Na concentration, (2) the fact that tissue analyses were performed on the combined right and left auricles, and (3) the fact that K did not move out of cells as is usually the case in osmotic dehydration. Therefore it can be concluded that, although the extracellular space probably expanded at the

expense of intracellular water in response to the hypertonicity of the infusion, PB exerted some other action directly on the mechanisms controlling the maintenance of intracellular water content.

An increase in tissue Na unrelated to that discussed above also occurred. It must be borne in mind that the absolute values for Na and Cl space discussed here may be subject to revision in view of the findings of Robertson and Peyser (1956), however, there is little reason to believe that such a revision would lead to marked changes in the relative volumes of distribution of these two ions. The calculated Na space expanded more than the Cl space, indicating that Na was penetrating some compartment from which Cl was excluded, probably the cells. Normally, when Na enters cells, due to some defect in the transport system which exchanges Na for K, the latter ion is lost. However, since tissue K remained unchanged, and extracellular K was not increased, one of two possibilities must have occurred. Either some anion other than Cl entered the cell with Na, or a cation other than K was lost. Either possibility would explain the increase in the extracellular concentration of Cl which was encountered, but there is insufficient evidence at present to determine the nature of the ions involved.

The facts which can be concluded from this study are that PB exerts a selective and direct action on the systems which regulate the intra- to extracellular ratios

of Na and water in the cat auricle. This action probably causes ultimate cardiac arrest, although there is no evidence that the negative inotropic effect of the drug in the ventricles has a similar basis.

The absence of an accumulation of K in the presence of PB induced heart failure is in apparent contradiction to the findings of Holland (1954). It would be of interest to know whether or not the decreased rate of loss of K from auricles beating in K-free media, which he reports in response to PB, could be due to a dilution of the external medium through loss of intracellular water.

Dog Heart. The lack of electrolyte alterations found in dog heart raises the possibility that such changes may be dependent upon the absolute concentration of PB present in the tissue. The dog heart appears to be susceptible to some other form of supression by PB, probably an impairment of contractility. This fact, combined with the lack of effect of PB on cat ventricular electrolytes, emphasizes the probability expounded by Green et al (1952) and Hoffman et al (1956) that changes in the contractility of mammalian heart may occur independently of changes in Na and K distribution.

In intact dogs and isolated heart-lung preparations, when ventricular contraction was supported by infusions of norepinephrine, large amounts of PB could be infused before cardiac failure occurred (Daniel et al, 1956). At the point of failure of antagonism, a series of ECG events characterized

by auricular standstill, A-V block and terminal idioventricular bradycardia were observed. In intact dogs, although not in heart-lung experiments, hyperkalemia also occurred. This ECG pattern is similar to that reported by McKusick (1954) to occur following intravenous infusion of Li salts. The latter author states that, while these changes could be due to the increased Li concentration, they could also be secondary to a decrease in the ratio of intra- to extracellular K. It would be of considerable interest to know whether or not changes in the intra- to extracellular ratio of K occur in dog auricles at the point of failure of norepinephrine antagonism to PB. It would be equally informative to investigate the ECG changes at PB failure, and the effects of norepinephrine infusion in cats.

The effect of PB on the electrical excitability of nervous tissue has been a point of considerable interest (Gruber et al, 1938, Eccles, 1946 and Brooks and Eccles, 1947.) In view of the changes encountered in Na and water metabolism in cat auricles, microelectrode studies might provide evidence regarding an effect of PB on membrane and action potentials in this tissue.

The Relation of Tissue Electrolytes in Different Chambers of the Heart to Function.

Control values for cardiac electrolytes, which are in general agreement with those reported by Robertson and Peyser, (1951) and Manery (1954) are of considerable interest.

In the auricles, Clspace tends to be larger than Na space, whereas, in the left ventricle, the Na space is always the larger. This is an indication of a greater proportion of Cl-containing cells, or a higher intracellular Cl concentration, in the auricles, Daniel and Boyes (1956) found similar evidence of the intracellular position of Cl in the fundus of the uterus, which is the pacemaker area of that organ. The apparent proportion of intracellular Cl varied inversely with the distance from the pacemaker area. It appears that intracellular Cl may be associated with automaticity in excitable tissues.

V. SUMMARY

1. A species difference in resistance to the cardiovascular depressant effects of continuous PB infusions has been found. The cardiac lethal dose for dogs was 171, for cats 326, and for rabbits 404 mgm./Kgm. of NaPB.

2. Myocardial ischemia resulting from the hypotensive effects of PB is probably not the cause of cardiac arrest in PB intoxicated cats and dogs.

3. Electrolyte studies indicate a selective action of PB on the control of intracellular Na and water concentrations in cat auricle.

4. The possible relation of PB induced electrolyte changes to some other direct action of PB on the myocardium is discussed. Evidence is produced in support of the hypothesis that changes in cardiac contractility may occur independently of changes in Na and K distribution.

5. The distribution of electrolytes in different cardiac tissues indicates that tissues with greater automaticity, such as the auricles, may contain more intracellular Cl.

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