# EFFECT OF ACUTE CARDIAC TAMPONADE
# ON T-1824 SPACE, THIOCYANATE SPACE, CIRCULATING RED CELL VOLUME
# AND PLASMA PROTEINS

by

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# INTRODUCTION

Acute heart failure is a common clinical problem, but the fundamental circulatory changes are difficult to study in the human because of the difficulty of obtaining adequate control data and the emergency nature of the situation. The use of experimental animals has provided a great deal of information on the hemodynamic affects of acute failure of the heart, but there are still many unanswered questions about this and other aspects of the problem.

One of these aspects which has been considered by several investigators is the possibility of a change in blood volume and distribution resulting from acute heart failure. The consideration of the possibility of a change in blood volume has resulted from the observations of the altered hemodynamics following acute failure. Thus far, the results obtained have been somewhat inconclusive and contradictory.

The purpose of this investigation was to determine whether or not there is a change in blood volume following acute heart failure produced by cardiac tamponade in the dog.

When the heart suddenly fails, the initial result is a reduction in cardiac output. Compensatory vasoconstriction may maintain arterial pressure for a time, but if output continues to fall, arterial pressure will also fall in spite of the increased peripheral resistance. Coincident with these changes is a rise in systemic and pulmonary venous pressure. The reason for this rise has been debated, but the most generally accepted explanation is that the elasticity of the arterial tree plus vasoconstriction has resulted in a shift of blood to the venous sides of the pulmonary and systemic circuits.[1] With these facts in

mind, the possibility of a change in blood volume may now be considered.

The present day concept of the exchange of fluid between blood and and tissues originated with Starling [2] and may be summarized as follows: The exchange of fluid and solutes between the intravascular and interstitial compartments occurs in the capillary beds. The net movement of fluid into or out the capillary is essentially a filtration process and, therefore, the rate and direction of net movement depends mainly on the difference in pressure across the capillary wall. This difference in pressure is the resultant of the colloid osmotic pressure of the plasma and the hydrostatic pressure within the capillary. The plasma colloid osmotic pressure tends to cause a movement of fluid into the capillary, and the hydrostatic pressure tends to cause an outward movement of fluid.

The pressure exerted by the tissues surrounding the capillary modify the effect of the hydrostatic pressure in the capillary on the direction of fluid movement, and the small amount of protein in the interstitial fluid causes an osmotic pressure which modifies the effect of the plasma colloid osmotic pressure on fluid movement.

Since tissue pressure and colloid osmotic pressure along the length of the capillary will remain fairly constant, the amount of hydrostatic pressure at a given point should determine the direction of net fluid movement. Thus, at the arterial end of the capillary, where hydrostatic pressure is greater than plasma colloid osmotic pressure, fluid will move out of the capillary; and at the venous end of the capillary, where hydrostatic pressure is less than plasma osmotic pressure, fluid will move into the capillary.

One of the most definitive experiments offered to prove this foregoing concept is that of Pappenheimer [3] who used the change in weight of a perfused limb as a measure of the net rate and direction of movement of transcapillary fluid exchange. He was able to vary plasma colloid osmotic pressure, arterial pressure and venous pressure independently of one another, and found that the weight of the limb changed in the direction and amount that would be predicted on the basis of Starling's hypothesis.

Since in acute heart failure there is a fall in arterial and a rise in venous pressure, it is possible that there are alterations in pressure along the capillary which might affect a change in blood volume (or more specifically, plasma volume) due to an imbalanse in fluid exchange.

There are three possibilities concerning plasma volume in this situation. First, if the pressure in the arterial side of the capillary is lowered more than the pressure in the venous side is elevated, net fluid movement would be inward resulting in an increase in plasma volume. Second, if the pressure rise on the venous side overbalances the fall in pressure on the arterial side, net fluid movement would be outward, causing a decrease in plasma volume. Third, if the opposite changes in arterial and venous pressures were such as to result in no change in net fluid movement, plasma volume would not change.

It is very difficult to predict which of these three situations will obtain in an animal subjected to acute heart failure because of the many uncontrolable variables. For example, the venous stasis might

cause anoxia of the capillary endothelium and result in a great increase in capillary permeability, allowing the escape of not only fluid but large protein molecules as well. Furthermore, arteriolar constriction will affect the magnitude of the pressure transmitted to the capillaries from the arterial tree.

To further complicate the situation, the various capillary beds in the body differ from one another in permeability and perhaps in susceptibility to anoxia. For instance, the capillaries in the liver are highly permeable to protein while those in skeletal muscle are only slightly permeable to these large molecules.[4]

It must be emphasized at this point that the factors governing a possible change in blood volume in acute heart failure differ considerably from those involved in a more chronic state of failure. Blood volume in chronic failure is affected not only by changes in the capillary beds, but also by sodium and water retention. Any rapid change in blood volume resulting from acute heart failure can be only the result of a shift of fluid between the vascular and extravascular compartments, with no change in the total body sodium or water.

As stated earlier, the initial effect of heart failure is a reduction in cardiac output. There are four basic methods for producing this reduction in the experimental animal. First, the myocardium may be damaged so that the heart is weakened and can no longer eject a normal amount of blood. This damage may be produced by ligation of a coronary artery, by thermocoagulation of part of one or both ventricles, or by injection of toxic substances either into the blood stream or

directly into the myocardium. Second, valvular lesions may be produced which impair the efficiency of the heart in pumping blood or which impede the inflow or outflow of the ventricles. This is most commonly done by surgically producing a combination of pulmonic stenosis and tricuspid insufficiency, but may also be done by other combinations of valvular damage. Third, the venous return or the arterial outflow may be impeded. Partial ligation or obstruction of one or both vena cavae will produce this result. The pulmonary artery or the aorta may also be partially ligated or obstructed for this purpose. Finally, by external compression of the heart, cardiac filling may be impaired and output reduced. This compression may be achieved by placing an irritating substance such as cellophane or talc within the pericardial sac, resulting in chronic constrictive pericarditis; or by injecting fluid or air into the pericardial sac, producing acute cardiac tamponade.

Each of these methods has its advantages and disadvantages, and the various methods may produce somewhat different end results. Damaging the myocardium is relatively easy to do, but has the disadvantage of resulting all too often in rapid death of the animal if the damage is too extensive, or no effect whatsoever when not enough damage is produced. Valvular lesions are difficult to produce, but will usually result in heart failure which is of the chronic type. Obstruction of the great veins or arteries is accomplished with ease but has the disadvantage of producing a situation where the animal is just on the borderline between life and death before a complete syndrome of heart failure is produced. For example, occlusion of the pulmonary artery up to 60% of

its cross sectional area has no effect on systemic arterial or venous pressure, but when a slightly greater occlusion is produced, venous pressure rises, while arterial pressure falls precipitously and the animal may die very suddenly.(5) Constrictive pericarditis may be produced quite easily but takes several weeks to develop, producing chronic heart failure. Cardiac tamponade may also be produced easily and has an advantage that none of the other methods have; namely, that the arterial pressure may be controlled quite precisely by adjusting the volume of fluid or air within the pericardial sac.

For the purposes of this investigation, a method was desired which would produce acute failure. Furthermore, it was desirable that the degree of reduction in cardiac output could be controlled to some extent. If any changes in blood volume did result, it would be further desirable to be able to remove the cause of heart failure and observe the changes as the circulation regained its normal status. The method of cardiac tamponade seemed to meet these requirements better than any of the others and was, therefore, employed in this investigation.

## Review of the Literature

This section is divided into two parts. The first part will be mainly concerned with work which has been done on hemodynamic changes in cardiac tamponade. The second part will be a review of investigations dealing with blood volume changes in acute heart failure.

Hemodynamic Changes in Cardiac Tamponade. The fact that compression of the heart in human beings can have serious consequences has been recognised by clinicians for at least two centuries. The first written observations on acute cardiac compression were those of the Italian anatomist, Morgagni,[6] in 1751. He noted that the pericardial sac was often distended with blood in persons dying after receiving penetrating wounds of the heart, and concluded that death in these cases was not caused by the heart wound itself but was due to compression of the heart by blood which had escaped from the wound. In 1884, the German physician, Rose,[6] presented a series of cases in which death was due to acute hemopericardium. Utilising the French word "tamponade", meaning compression, Rose coined the term "herz tamponade" to describe cases such as these.

Cardiac tamponade in the human is manifested by several signs which are related to changes in hemodynamics. The three principle signs of acute tamponade have been summarized by Beck[7] and are generally known as "Beck's triad." The triad consists of: 1) falling arterial pressure, 2) rising venous pressure and, 3) a small quiet heart. Patients with tamponade will also usually have a weakening of the arterial pulse during inspiration and a strengthening during expiration. This

variation of pulse strength with respiration is an exaggeration of a normal physiological event known as "pulsus paridoxicus" and is considered pathognomic of cardiac compression.

Clinical cases of tamponade are almost always due to a penetrating wound of the heart, but may also be due to a rapid accumulation of fluid in the pericardial sac. Between 100 and 200 cc. of blood or fluid in the sac will produce signs of tamponade in the adult human if the bleeding or effusion is rapid.[7] The pericardium is quite distensible and may accommodate as much as 1 or 2 liters if the process of accumulation is slow. Chronic cardiac compression usually results from a slowly accumulating pericardial effusion and is complicated by fluid and electrolyte retention. Beck points out that the signs of acute and chronic compression differ in that patients with chronic compression will have ascites and a large liver as well as an elevated venous pressure.

Experimental work on cardiac tamponade has been done for a variety of reasons. Earlier investigators were interested mainly in the causes and results of the hemodynamic changes. More recent work has been concerned with the effects of tamponade on respiration and metabolism, and with attempts to improve the therapy of tamponade in clinical cases.

The earliest experimental work done on tamponade was by the Italian physiologists, Luciani and Stefani, in 1871.[8] These investigators were primarily interested in the action of the vagus in modifying the diastolic size of the heart. In order to study this problem they needed some means of bringing the circulation to a temporary standstill, after which they could stimulate one or both vagi and determine the effect on

the circulation. They made a pericardial fistula in dogs and connected the pericardial cavity to a pressure bottle filled with saline. Carotid arterial and superior vena caval pressures were recorded. When fluid was allowed to flow into the pericardium, the venous pressure increased and the arterial pressure decreased. When enough fluid had been introduced to stop the circulation, the arterial pressure fell to 15-20 mm. Hg. and the venous pressure rose to 12-18 cm. $H_2O$. At the moment that circulation ceased, the vagi were stimulated, and it was noted that arterial pressure rose. The conclusion of these investigators was that the vagus excites active dilatation of the heart, allowing an increased diastolic volume to be accommodated which, when expelled, will cause a rise in arterial pressure. This conclusion is not tenable in the light of more recent information concerning the action of the vagus on the heart, but a discussion of alternative explanations is beyond the scope of this paper.

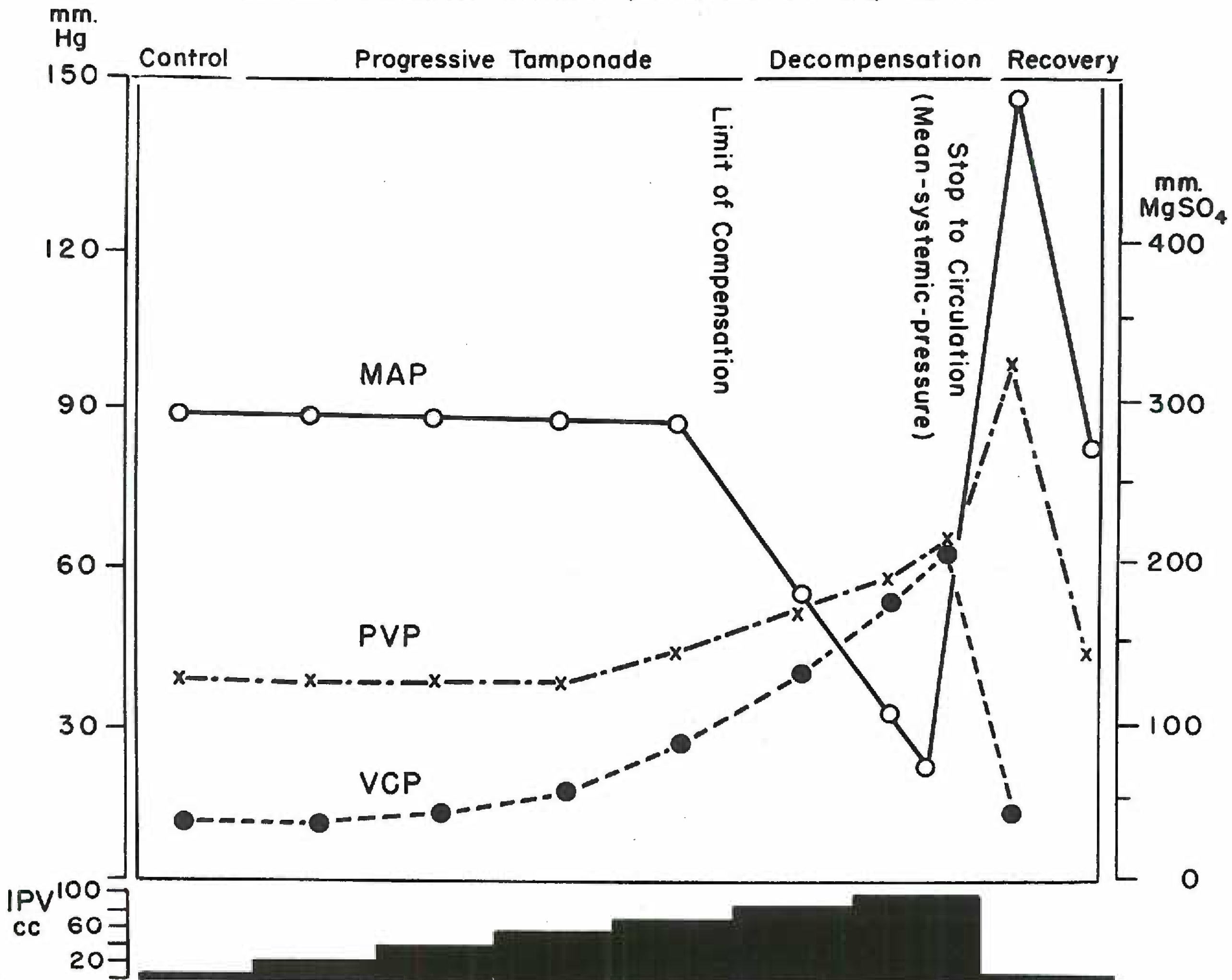
The first detailed work on hemodynamic changes in cardiac tamponade was done by Starling (9) in 1897. Starling was interested in pressure changes occurring in various parts of the circulation as a result of acute heart failure. He employed dogs in his investigations, injecting measured amounts of oil into the pericardial sac and observing the changes in pressure in the portal vein, vena cava and carotid artery. In a typical experiment, the injection of 20 cubic centimeters of oil had no effect on any of the pressures. An additional 20 cc. caused a slight rise in caval pressures with no change in arterial or portal pressure. Thirty additional cubic centimeters caused a further small increase in caval pressure, a slight increase in portal vein pressure, but still no

Figure 1

A graphic summary of Starling's data on acute cardiac tamponade in the dog.

MAP = mean arterial pressure
PVP = portal vein pressure
VCP = inferior vena caval pressure
IPV = intrapericardial volume

Mean arterial pressure is read on the left ordinate in mm. of mercury. Portal vein and inferior vena caval pressures are read on the right ordinate in mm. of $MgSO_4$.




This chart was prepared by Dr. Clare Gray Peterson from Starling's original data.

change in arterial pressure. The addition of 20 cubic centimeters more, making a total of 90 cubic centimeters, caused a marked change in all pressures. Arterial pressure rapidly dropped to half its original value, and portal vein and caval pressure rose markedly. Circulation stopped completely when 10 additional cubic centimeters of oil were injected. At the moment that circulation stopped, all pressures were approximately the same.

A few seconds after the circulation had ceased, the oil was completely removed from the pericardial sac. As soon as circulation recommenced, arterial pressure rose far above the original control level, fell rapidly to slightly below control and then gradually returned to normal. Vena caval pressure quickly fell to the control level, but portal vein pressure rose to almost twice the level it had attained when circulation ceased. After this marked rise, portal pressure returned slowly to normal.

The preceding data are summarized in Figure 1.

Starling's explanation for these pressure changes is as follows: Arterial pressure is maintained by peripheral vasoconstriction until cardiac output is so low that arterial pressure falls. When the circulation ceases, the pressure throughout the vascular system is the same — this pressure is known as the mean systemic pressure. After the tamponade is removed, arterial pressure overshoots its original level because of the increased peripheral resistance due to vasoconstriction. Portal vein pressure rises because of vasoconstriction within the liver combined with the increase in portal flow that occurs when the tamponade is removed. Both arterial and portal vein pressures return to normal

as gradual vasodilation occurs.

Kuno,[10] using a heart lung preparation, confirmed Starling's observations and in addition studied the changes in intrapericardial pressure during tamponade. He noted that when intrapericardial pressure was increased enough to equal the pressure in the great veins, circulation was brought to a standstill; and that if venous pressure was then raised, circulation was restored. Kuno also measured cardiac output in his preparations and found that it began to drop as soon as the first small increments of fluid were added. Further addition of fluid caused a further drop in output.

Pressures in the pulmonary artery, pulmonary veins and right atrium were measured by Metcalfe [11] in dogs using the catheterization technique. As the intrapericardial pressure was increased, pulmonary artery pressure began to fall and pulmonary venous and right atrial pressure began to rise. When the degree of tamponade was sufficient to halt circulation, Metcalfe noted that pulmonary artery and venous pressures, as well as systemic arterial and venous pressure, approached the same value of about 20 mm. Hg. (mean systemic pressure)

Nerlich [12] has divided the hemodynamic changes during tamponade into four phases. The first phase starts with the introduction of fluid into the pericardial sac and continues until two-thirds of the maximum volume which can be accommodated by the sac has been introduced. During this phase, there is cardioacceleration, progressive decrease in stroke volume, cardiac output and pulse pressure and an increase in total peripheral resistance. The second phase begins with an addition of a further small volume of fluid and is characterised by a precipitous drop in

arterial pressure and the abrupt onset of bradycardia. The reason for the bradycardia is not clear since it persists after vagotomy, but diminished coronary blood flow may be a contributing cause. Phase three occurs when enough fluid is added to cause cardiac output to fall to practically nothing. Heart rate and arterial pressure are lowest during this period. Phase four is the recovery period after the removal of the pericardial fluid. In this period, heart rate and arterial pressure return to normal, but pulse pressure and cardiac output are not quite restored.

Experimental work on hemodynamic changes in acute tamponade has resulted in two schools of thought concerning the fundamental circulatory defect. Cohnheim [13] in 1889 stated that the increase in intrapericardial pressure causes an obstruction to the inflow of blood to the heart at the point where the great veins enter the pericardium, and that the fall in cardiac output was due to this diminished venous inflow.

Beck [7] currently supports this view on the basis of his observations that the intrapericardial portions of the systemic and pulmonary veins appear collapsed when intrapericardial pressure is increased, while the extrapericardial portions of these veins appear distented.

Another viewpoint was introduced by Starling [9] who concluded that there is an interference with diastolic expansion of the ventricles by the increased intrapericardial pressure, resulting in decreased ventricular filling and consequent decreased output.

Isaacs [14] had recently presented evidence that this hypothesis is probably correct. He found that there was no measurable pressure gradient between the vena cavae and right atrium or between the pulmonary veins and left atrium. Furthermore, when effective ventricular filling pressure was calculated by subtracting intrapericardial pressure during ventricular diastole from mean atrial pressure, it was found that effective filling pressure was greatly reduced. Isaacs feels that the decreased effective filling pressure of the ventricles results in a shorter diastolic fiber length and a consequent weakening of contraction in accordance with Starling's law of the heart.

Nerlich's [12] observation that pressure in both the atria and ventricles is elevated at the end of ventricular diastole would support the view that the decrease in cardiac output is due to the reduced diastolic capacity of the heart.

Studies of pressure changes within the heart during tamponade also show that during ventricular systole the intrapericardial pressure is reduced. This reduction in intrapericardial pressure facilitates atrial filling by increasing the pressure gradient between the veins and atria. As pericardial pressure is increased, ventricular filling is reduced and, consequently, ventricular systole results in a smaller decrease in pericardial pressure and an attenuation of the facilitating effect on atrial filling. Nerlich points out that this situation leads to a vicious cycle which eventuates in a reduction of cardiac output and arterial pressure to the critically low levels. This cycle is diagramed as follows:

Diminishing veno-atrial gradient
↓ ↑
attenuated facilitating effect
↑
decreased systolic discharge

The cause of the "pulsus paradoxicus" observed during tamponade has been described by Katz.[15] He found that when the pericardium is distended by fluid, the intrapericardial and intra-auricular pressures vary less during respiration than does the pressure in the great veins outside the pericardium. Thus, during inspiration the pressure in the great veins drops markedly while the intrapericardial pressure changes only slightly. This results in a lowered pressure gradient between the great veins and the right atrium, which leads to a decreased inflow of blood to the heart and a consequent decreased output. During expiration, the pressure in the great veins increases, causing an increased pressure gradient and an increased venous inflow with a temporary increase in output.

Some mention should be made of changes observed in respiration and renal hemodynamics during tamponade. Respiratory minute volume is increased markedly during severe tamponade.[16] The arterial oxygen saturation remains normal, but the venous saturation drops so that the arteriovenous oxygen difference is increased.[17] The alkali reserve is decreased and the blood lactic acid is increased.[15] These changes indicate a stagnant anoxia on the basis of heart failure.

Post[18] investigated the changes in renal hemodynamics during tamponade and found that as cardiac output fell, renal resistance increased. However, the total peripheral resistance increased more, so

that the renal fraction of cardiac output was increased. Glomerular filtration rate and renal plasma flow decreased. Sodium reabsorption increased, and Post feels that this is due to the decreased sodium load resulting from the decreased filtration rate. However, the increased pressure in the renal veins may also contribute to the increase in sodium reabsorption.[19]

The preceding information on changes during tamponade is summarized in Figure 2.

Blood Volume Changes in Acute Heart Failure

Clinical studies. Many investigators have studied blood volume changes in patientswith heart failure. However, most of these patients have been in chronic congestive failure in which the blood volume is elevated by sodium and water retention.

There is relatively little information available on blood volume changes in acute heart failure in clinical cases. The main reason for this is that these cases are all of an emergency nature and little time is available for doing volume studies. Furthermore, adequate control data are almost impossible to obtain. Many of the patients with acute heart failure have pre-existing chronic failure after the acute episode. Almost all of the cases studied have had acute heart failure due to coronary occlusion.

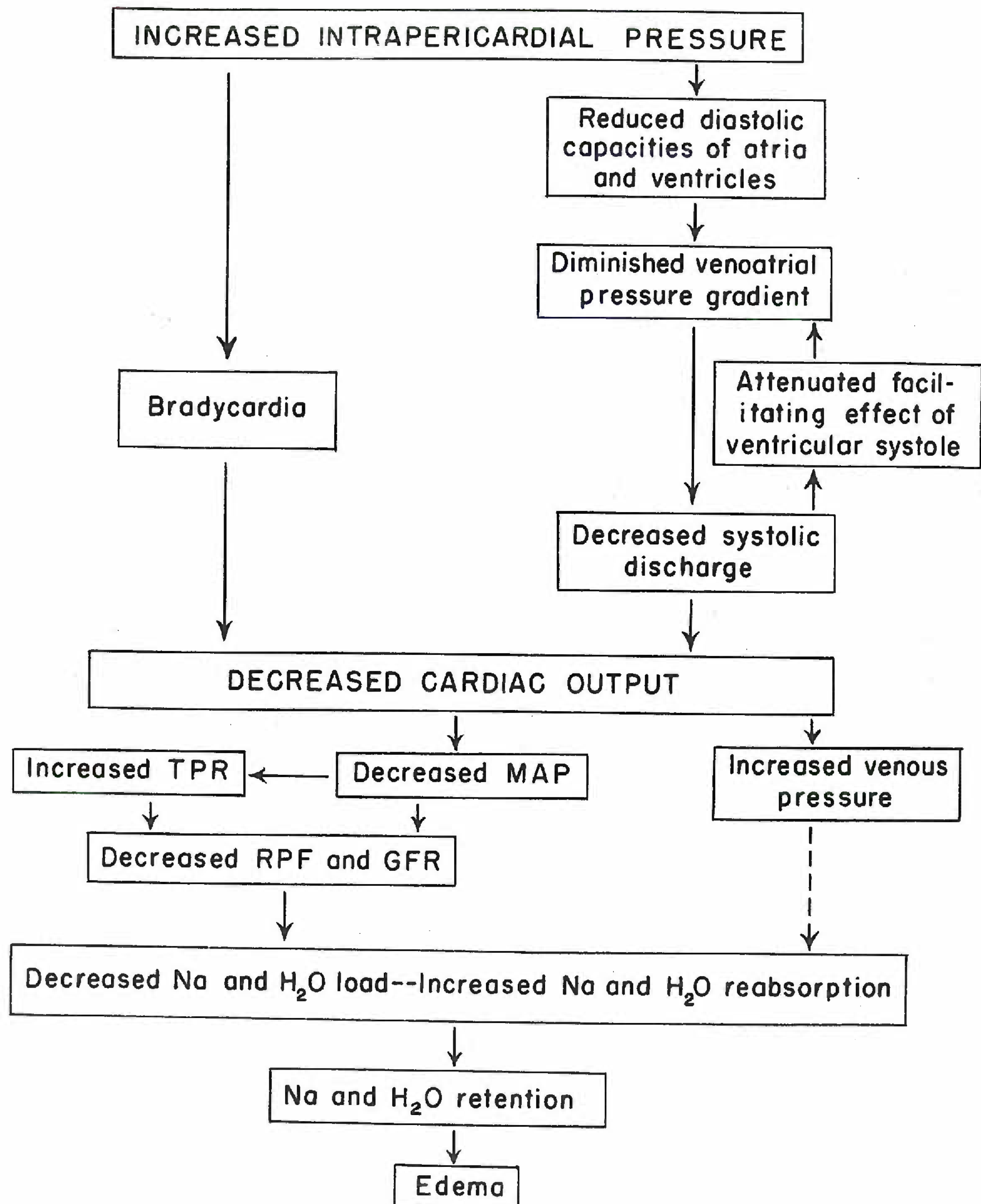
Agress [20] has reported blood volume measurements in a group of patients with coronary occlusion. He classified this group as being in severe, moderate or non-shock. Venous pressure was either normal or somewhat elevated in all three groups. Blood volume studies were done

Figure 2

A summary of the hemodynamic and renal consequences of cardiac tamponade.

MAP = mean arterial pressure
TPR = total peripheral resistance
RPF = renal plasma flow
GFR = glomerular filtration rate

This chart was prepared by Dr. Clare Gray Peterson.


HEMODYNAMICS OF CARDIAC TAMPONADE
INCREASED INTRAPERICARDIAL PRESSURE
Reduced diastolic capacities of atria and ventricles
Diminished venoatrial pressure gradient
Attenuated facilitating effect of ventricular systole
Bradycardia
Decreased systolic discharge
DECREASED CARDIAC OUTPUT
Increased TPR
Decreased MAP
Increased venous pressure
Decreased RPF and GFR
Decreased Na and $H_2O$ load--Increased Na and $H_2O$ reabsorption
Na and $H_2O$ retention
Edema

with T-1824. The patients with severe shock had lower T-1824 spaces than the other groups. After recovery, their blood volumes increased by 16%.

Stead and Ebert[21] found that patients in acute failure had a slightly lower T-1824 space than normal patients. There was some evidence of hemoconcentration, since the hematocrit and plasma protein values were slightly higher than normal. Most of their patients had pulmonary edema.

Cameron[22] reports little change in T-1824 space over a period of several weeks in patients who had acute failure at the time of the initial measurement.

One group of patients with cardiac tamponade from stab wounds of the heart has been studied by Warren.[23] There was no significant change in hematocrit values after treatment. No other studies dealing with blood volume were done.

Experimental studies. There is a large amount of information in the literature on blood volume studies in animals with experimentally produced heart failure. Here again, as in the clinical studies, most of the work deals with heart failure of the chronic type. However, some work has been done on volume studies in acutely produced failure.

Hemodynamic and blood volume changes in dogs following acute ligation of the left anterior descending coronary artery have been studied by Gross and Mendlowitz.[24] Cardiac output was found to drop considerably, but there was only a slight drop in arterial pressure and a slight rise in venous pressure. These changes occurred a few minutes after the ligation and persisted for about 24 hours. After this interval, the

dogs either died or recovered completely. The hematocrit and plasma protein values increased slightly one to two hours after the ligation. Plasma volume measurements with congo red indicated a slight decrease in the same time period.

The effects of an injection of zinc hydroxide directly into the myocardium of dogs have been investigated by Meyers.[25] The zinc hydroxide produces an area of myocardial necrosis and consequent heart failure if the area is large enough. Most of the injections were made into the wall of the left ventricle. Cardiac output, measured by dye dilution, and arterial pressure fell markedly soon after the injection. Plasma volume was measured with T-1824 before and about three hours after the injection, using an 8 minute mixing period. The results indicated a fall in T-1824 space of about 14 ml/Kg. Hematocrit values rose from 5-10%, and the calculated red cell volume did not change. Meyers concludes that acutely developing hypotension is similar to traumatic shock in that cardiac output and plasma volume fall _pari passu_.

No work has been done specifically on blood volume changes in cardiac tamponade, but some of the data in the literature suggest that there may be changes. Cannon[17] produced tamponade in cats for the purpose of investigating the problem of shock. He noted that there was a 5-20% drop in the hematocrit values in one to three hours, and suggests that this may indicate hemodilution.

Starling[9] measured limb volume with a plethysmograph during his experiments, and found that the limb volume decreased during tamponade.

His interpretation of this finding is that the pressure in the capillaries and small veins is directly proportional to the arterial pressure, so that when arterial pressure falls, limb volume also falls due to the lowered capillary pressure.

# METHODS

Experimental Preparation. Mongrel dogs weighing between 9 and 15 Kg. were used as experimental animals. Cardiac tamponade was produced in these animals by injection of normal saline into the pericardial sac through a polyethylene catheter.

The surgical preparation was as follows: anesthesia was produced by a dose of 30 mg/Kg. of intravenous nembutal. The chest was opened through an incision between the 4th and 5th ribs and respiration maintained by a mechanical respirator connected to an intratracheal tube or a tracheal cannula. A small cut was made in the pericardium and a silk purse-string suture placed around the edges of the cut. The polyethylene catheter (size PE 190) was then inserted through the cut and the purse-string suture drawn tightly around the catheter and tied.

In the first few dogs, the catheter was installed one or two weeks prior to the actual experiment. The distal end of the catheter was brought out through a stab wound in the anterior chest wall, sutured to the skin and the chest wall closed. It was felt that this procedure would allow the dog to recover somewhat from the trauma of the operation and would also save time on the day of the experiment. The dogs all recovered well from the operation, but the procedure was abandoned because many times the catheter was found to be plugged with fibrin or was kinked and broken off inside the chest.

The most satisfactory method was found to be installation of the catheter at the time of the experiment. Usually, the chest was left open throughout the experiment and the animal maintained on artificial

respiration. In a few instances, the chest was closed and the animal allowed to breath spontaneously.

It was most desirable to produce a comparable degree of tamponade in each dog. This could be done by producing the same rise in venous or intrapericardial pressure or the same fall in arterial pressure in each experiment. Since arterial pressure was the easiest to record graphically, it was used as an index of the amount of circulatory embarrassment. Femoral arterial pressure was recorded with a citrate-mercury manometer, writing on a slowly moving kymograph drum. Saline was injected into the intrapericardial catheter from a large syringe until mean arterial pressure was approximately fifty per cent of the control level. This level was maintained by the addition or subtraction of small increments of fluid as required. The tamponade was maintained for thirty minutes to two hours. At the end of this time the tamponade was discontinued by aspirating the saline from the intrapericardial catheter with a syringe.

All blood samples were arterial and were withdrawn either from an indwelling Cournand needle or a cannula tied into the femoral artery. Heparin was used as an anticoagulant.

Experiments were performed on a total of 23 dogs. Five dogs were splenectomized ten days to two weeks prior to the experiment, and two dogs were splenectomized at the time of the experiment.

<u>Volume Measurements</u>

<u>General discussion</u> An unknown volume of fluid may be measured by the introduction of a known amount of a substance into the fluid and

then measuring the concentration of the substance after it has distributed itself throughout the unknown volume. This procedure utilizes the dilution principle of volume measurement and may be expressed in an equation:

$$C_1 V_1 = C_2 V_2$$

in which $C_1$ = concentration of substance in its vehicle solution
$V_1$ = volume of vehicle containing substance
$C_2$ = concentration of substance in unknown volume
$V_2$ = unknown volume

The unknown volume $V_2$ is then equal to the quotient of $C_1 V_1 / C_2$. When measuring fluid compartments in biological systems, the product of $C_1 V_1$ is known as the dose, and the volume of the unknown compartment is then equal to the quotient of dose/$C_2$.

The substances used in measuring body fluid compartments are usually foreign chemical compounds which remain in the compartment, or isotopes of elements which normally are found only in the compartment to be measured.(26) Thus, total body water may be measured with deuterium oxide or antipyrine, and extracellular fluid volume with sodium thiocyanate and radioactive chloride or bromine compounds. Plasma volume is measured with substances which will attach to the plasma proteins and thus theoretically remain intravascularly. T-1824 (Evans blue,) $I^{131}$ albumin and $Cr^{51}Cl_2$ are used for this purpose.(26, 27) T-1824 and $Cr^{51}Cl_2$ both are bound to plasma albumin when injected into the blood stream. Red cell volume may also be measured by utilizing compounds which are taken up only by the red cells. Carbon monoxide is the only non-radioactive substance used.(28) Radioactive isotopes used to tag red

cells include $P^{32}$, $Fe^{59}$ and $Cr^{51}$.[28] For red cell tagging, the $Cr^{51}$ is in the form of sodium chromate.[29]

Fluid compartment measurement is complicated by two main factors: 1) determination of the time required for mixing of the injected substance and, 2) escape of the substance from the compartment which it is supposed to measure. Ideally, mixing time may be determined by observing the time required for the concentration of the measuring substance to reach a constant level. This is possible only with red cell volume measurements since the cells do not escape from the vascular compartment.

The substance used to measure plasma and extracellular fluid volume do escape from these compartments, thus making the determination of mixing time more difficult.[28,30] This is especially true of plasma volume determination, since albumin leaks out of certain parts of the capillary bed, such as the liver, rather rapidly. If the disappearance curve of labeled albumin is plotted graphically, it is evident that at least two rates of disappearance are involved. The initial rate of disappearance is rapid and is considered to represent mixing of the tagged protein within the vascular compartment. This mixing time has been estimated by various investigators to be from less than 60 seconds to 10 minutes.[26] The second rate of disappearance is much slower and is felt to represent the escape of albumin from the blood stream. Thus, the entire disappearance curve of labeled albumin is exponential in type and if plotted on semilogarithmic paper will be a straight line.

The most widely used method of determining the concentration of

labeled albumin when it is completely mixed in the plasma is that of Gregersen.[31] The disappearance curve is plotted on semilogarithmic paper and the straight line is extrapolated backward to zero time. The concentration at zero time is considered to be that concentration which would result if the albumin were mixed instantaneously throughout the plasma volume.

Another method used simply assumes that mixing is complete by ten minutes and that escape of albumin is negligible in this time.[26] This method is, of course, invalid if mixing is prolonged due to slowing of the circulation or any other cause.

The principle of backward extrapolation of the disappearance curve may be employed with substances used to determine extracellular fluid volume. Since mixing in this compartment seems to be complete by sixty minutes, concentrations at this time are usually used in determining the volume instead of constructing a disappearance curve.[32]

Changes in plasma or extracellular fluid volume may be measured by one of two methods. The first method assumes that the total amount of the measuring substance present in the compartment does not change and that only the volume of the compartment changes.[31] In this method, a control disappearance curve is constructed after a single injection of the measuring substance. This curve is then extrapolated backward for the zero time concentration and forward for the predicted concentrations during the time when the compartment volume is expected to change. If the observed concentrations fall above the forward extrapolated portion of the curve, this is interpreted as meaning that the compartment volume

must have decreased. The reverse is true if the observed concentrations fall below the curve. Changes in volume are then calculated by the equation:

$$\text{Volume at time } t = \frac{\text{Predicted concentration at time } t}{\text{Observed concentration at time } t} \times \text{Volume at zero time}$$

The disadvantage of this method is that the measuring substance may escape from the compartment at the same time that the volume changes. This would cause a decrease in the concentration of the substance and a falsely high value for the compartment volume.

The second method of determining volume changes will not be affected as much by changes which may occur in the total amount of the measuring substance present. This method depends on the re-injection of the measuring substance at the time when the volume is expected to change.[33] Disappearance curves may be constructed for both the initial and re-injection measurements. The concentration of the measuring substance is determined just before the re-injection and subtracted from the concentration after the injection to give the concentration of the substance resulting from the new injection.

Plasma Volume Measurements with T-1824. Plasma volume was measured in 13 dogs with T-1824, using either the single or the re-injection method for estimations of changes in volume. The T-1824 used was from a single lot with a peak absorption at 610 mu. The concentration of the injected dye was 0.5% and the dose was usually 0.04 cc/Kg. A volumetric pipette was used to measure the dose into a syringe barrel connected to a three-way stopcock. This stopcock was attached to a needle placed in a vein and to a second syringe. The dye in the syringe barrel was drawn into this

second syringe and then injected through the stopcock and needle into the vein. The syringe barrel was rinsed several times with saline which was also injected in order to wash any dye remaining in the stopcock or needle into the vein.

Disappearance curves were constructed by determining dye concentrations of samples drawn at ten to fifteen minute intervals for thirty to sixty minutes. The optical density of the dyed plasma samples was read at 610 mu. in a Beckman model B spectrophotometer and the dye concentrations determined from a standard curve.

Since the volume of distribution of T-1824 albumin appears to be larger than the true plasma volume, the term "T-1824 space" will be used in this paper. The term "plasma volume" will be used to refer to plasma volume calculated from the $Cr^{51}$ red cell volume.

$Cr^{51}$ red cell volume measurements. Red cells tagged with $Cr^{51}$ were used to measure red cell volume in 13 dogs. Five cc. of blood were drawn from the dog the day before the experiment and incubated with 25 uc/Kg. of body weight of $Na_2Cr^{51}O_4$ (Rachromate, Abbott) for one hour at 37 C. The cells were then washed, resuspended in normal saline and stored overnight in the refrigerator. The next morning the cells were washed again with saline, resuspended and drawn into the syringe to be used for injection.

The dose of tagged cells given was determined, using a method worked out by Dr. Tyra Hutchens.[34] A standard solution of $Cr^{51}$ was prepared by placing approximately the same amount of $Cr^{51}$ as had been incubated with the cells in 5 cc. of water and drawing this solution into the same

size syringe used for the tagged cells. Both syringes were then counted in exactly the same position above a well-type scintillation counter. The syringe containing the standard solution was then emptied into a 500 cc. volumetric flask and the cells in the other syringe were given intravenously to the dog. Both empty syringes were then counted in the same position as before. One cc. of the diluted standard solution was placed in a counting vial and counted in the well chamber. The counts per second obtained from this vial were then multiplied by 500 to give the total counts/sec. present in the standard. This quantity was multiplied by the ratio of the counts/sec. of the full minus the empty dose syringe to the counts/sec. of the full minus the empty standard syringe to give the dose in counts/sec. injected into the dog. The equation for the calculation of the dose is:

$$\text{Dose in cps} = \frac{\text{cps full dose syringe} - \text{cps empty dose syringe}}{\text{cps full std syringe} - \text{cps empty std syringe}} \times \text{total cps std.}$$

Blood samples were drawn after a fifteen or thirty minute mixing period. Samples were also drawn at various times during tamponade. In 6 dogs a second injection of cells was given during tamponade. Both the first and second doses were approximately the same. Blood samples were thoroughly mixed and one cc. carefully pipetted into a counting vial containing two cc. of water. The vials were counted in the same manner as the standard vial.

Total blood volumes and red cell volumes were calculated by dividing the dose by the counts/sec./cc. of whole blood and red cells respectively. The cps/cc. of red cells was calculated by dividing the cps/cc. of whole blood by the hematocrit. When a second injection was

used, the cps/cc. of red cells resulting from this injection was determined by subtracting the cps/cc. of red cells before the injection from the total cps/cc. of cells after the injection. Mixing after the second injection was assumed to be complete when the cps/cc. of red cells reached a constant level.

Extracellular fluid volume measurements with thiocyanate (SCN) Disappearance curves for SCN were established during the control period in 4 dogs. The dose given was 0.4 cc/Kg. of a 5% solution of Na SCN and was measured with a volumetric pipette. Samples were drawn at fifteen minute intervals for sixty minutes before tamponade was started, and at twenty minute intervals during tamponade. Changes in the SCN space were estimated by comparing the observed to the predicted concentration from the extrapolated control curve.

Twenty per cent trichloroacetic acid was added to the plasma samples to precipitate the proteins. Five per cent ferric nitrate was added to the supernatant solution for color development, and the samples read at 470 mu. in the spectrophotometer. SCN concentrations were obtained from a standard curve.

Plasma protein determinations. Total plasma protein was determined on samples drawn at various intervals before, during and after tamponade in 17 dogs. Weichselbaum's[35] modification of the biuret method was used. This method was standardized by the use of solutions whose protein concentration had been determined by the micro Kjeldahl technique.

The difference required for significance at the 0.05 level was determined to be ±0.16 Gm.% for the Weichselbaum method.

Hematocrits. The hematocrit of all samples obtained from 21 dogs was determined by placing the thoroughly mixed blood in Wintrobe tubes and centrifuging at 3000 RPM for thirty minutes. No correction was made for trapped plasma.

## EXPERIMENTAL RESULTS

This section will be divided into two parts. The first part will deal with the results obtained in the unsplenectomized group of dogs, and the second part will deal with results obtained in the splenectomized group.

The various detetarminations described in the preceding section were not done on all of the dogs. In order to avoid confusion as to the size of the experimental group involved in each determination, the following tabulation is presented:

| | Tot. No. Dogs | HCRT. | Single inj. T-1824 | Reinj. T-1824 | SCN | Tot. Prot. | Single inj. $Cr^{51}$ | Reinj. $Cr^{51}$ |
|---|---|---|---|---|---|---|---|---|
| UNSPLENECT. | 16 | 15 | 9 | 3 | 4 | 11 | 3 | 3 |
| SPLENECT. | 7 | 7 | | 1 | | 6 | 4 | 3 |

A complete tabulation of data is given in the appendix.

A. Unsplenectomized Dogs

T-1824 space measurements with a single injection. The initial experiments with T-1824 were done using the single injection technique. A control disappearance rate was established with at least three samples drawn at ten to fifteen minute intervals. Tamponade was then produced and samples drawn every ten to fifteen minutes for an hour or more. After discontinuation of the tamponade, one or two more samples were obtained over an hour period.

The disappearance curves obtained in 9 dogs were of three types. In the first type, the observed concentrations of the dye during tamponade fell consistently above the extrapolated control disappearanse curve. The calculated T-1824 space from the observed concentrations showed a substantial decrease from control levels. Five dogs showed disappearance curves of this type. Control T-1824 spaces in these dogs ranged from 34 to 59 cc/Kg. and the calculated decrease ranged from 7 to 10 cc/Kg. after forty-five to sixty minutes. The T-1824 space appeared to remain lowered after removal of the tamponade.

The opposite direction of change was shown by the second type of curve observed in 2 dogs. In this type, the observed dye concentration fell consistently below the extrapolated control curve. Control T-1824 spaces in these 2 dogs were 44 and 48 cc/Kg. and the calculated increase was approximately 10 cc/Kg. in each dog. In one dog, the dye concentration remained below the extrapolated curve after removal of the tamponade, and in the other, the concentration returned to extrapolated values.

The third type of curve was also observed in 2 dogs whose T-1824 spaces were 44 and 57 cc/Kg. In this type, the observed dye concentration coincided with the extrapolated control curve, indicating no change in T-1824 space.

There was no apparent difference in the experimental preparation of these three groups of animals or in the control levels of arterial pressure. A satisfactory degree of tamponade was obtained in all of the dogs, and the mean arterial pressures during tamponade were approximately the same. None of the dye samples were visibly hemolized and all control plasmas were clear. The dosage of dye on a weight basis was equivalent in all groups. It should be further noted that the hematocrits in all three groups showed the same direction of change.

The three types of curves are shown in representative examples in Figure 3.

In view of the inconsistency of the results with a single injection of dye, this method was abandoned and the re-injection technique used instead. The results obtained with this method will be discussed in a later section.

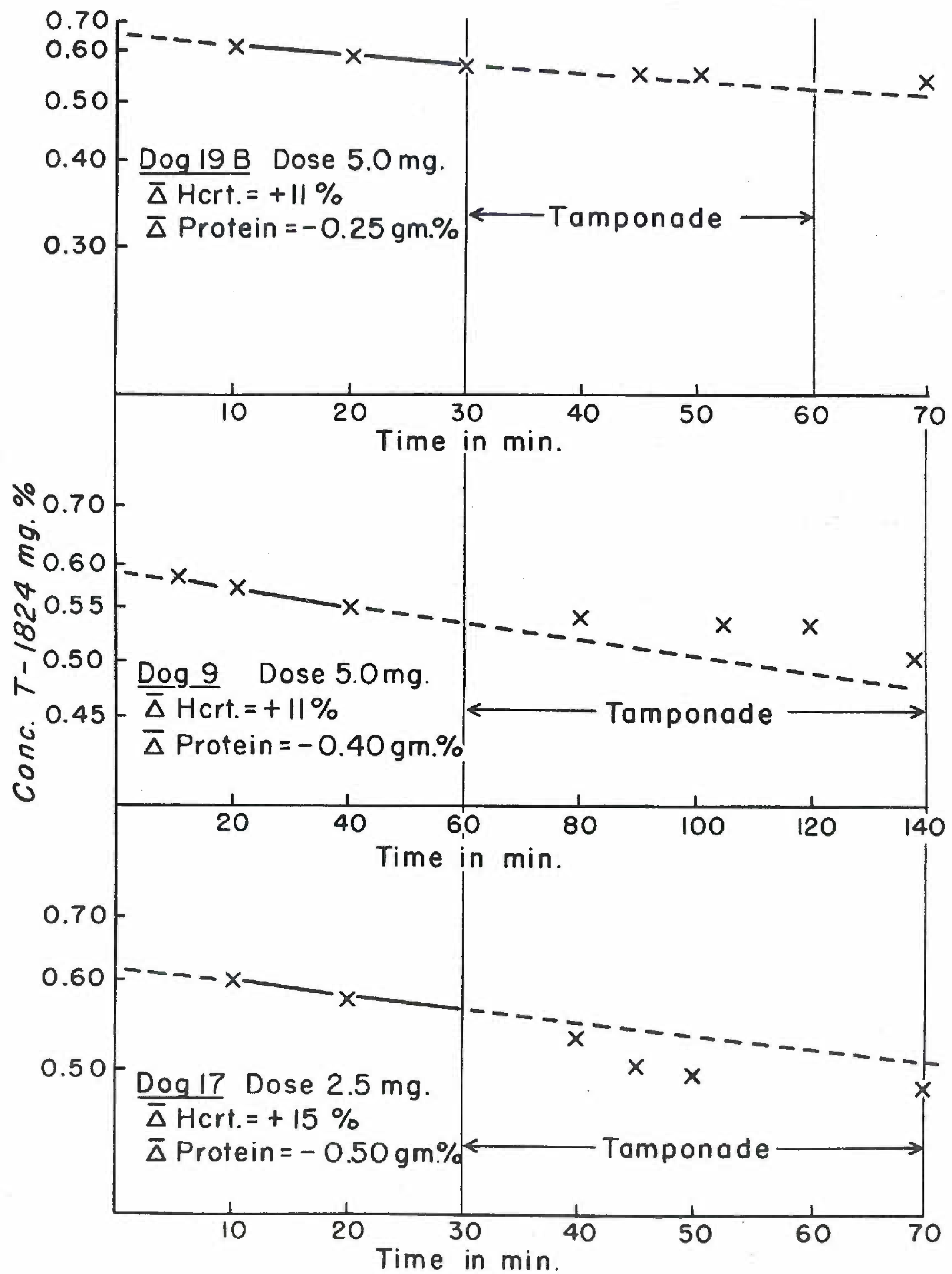
<u>Hematocrit changes.</u> Hematocrits were determined on each blood sample obtained in 15 experiments. In all of these experiments the hematocrit showed a marked increase after tamponade. Usually, the increase reached a maximum point by ten minutes after the tamponade had been produced and maintained this level for the duration of the tamponade.

The range of the per cent increase of the hematocrit above control levels was quite large. In the first ten minutes after tamponade, the range of the per cent increase was from + 5% to + 50%. This wide range

Figure 3

Three types of T-1824 disappearance curves obtained furing cardiac tamponade. Note that in the first graph, the dye concentrations during tamponade coincide with the forward extrapolated portion of the control curve. In the second graph, the dye concentrations fall above the extrapolated control curve; while in the third graph, the concentrations fall below the extrapolated control curve. The direction of change in the hematocrits and total plasma protein was the same in all three dogs.

This figure demonstrates the unreliability of the extrapolation method in extimating changes in plasma volume in this type of experiment.


THREE TYPES OF T-1824 DISAPPEARANCE CURVES
Conc. T-1824 mg. %
0.70
0.60
0.50
0.40
0.30
Dog 19 B Dose 5.0 mg.
Δ̄ Hcrt. = +11 %
Δ̄ Protein = -0.25 gm.%
Tamponade
10
20
30
40
50
60
70
Time in min.
0.70
0.60
0.55
0.50
0.45
Dog 9 Dose 5.0 mg.
Δ̄ Hcrt. = +11 %
Δ̄ Protein = -0.40 gm.%
Tamponade
20
40
60
80
100
120
140
Time in min.
0.70
0.60
0.50
Dog 17 Dose 2.5 mg.
Δ̄ Hcrt. = + 15 %
Δ̄ Protein = - 0.50 gm.%
Tamponade
10
20
30
40
50
60
70
Time in min.

was typical for samples drawn later during tamponade. The mean percentage increase over control levels at ten minutes after tamponade was 19.6%, and at twenty, forty and sixty minutes, varied only slightly from this level.

After the tamponade was removed, the hematocrit appeared to drop somewhat but seldom returned to control levels by one hour. The mean per cent increase over the original control level was about 14% at both thirty and sixty minutes after the removal of the tamponade.

There was apparently no correlation between the original control level of the hematocrits and the percentage increase during tamponade. The control hematocrits vs. the percentage increase are plotted on a scatter graph in Figure 4A.

The mean change of the hematocrits in the unsplenectomized dogs is shown graphically in Figure 5.

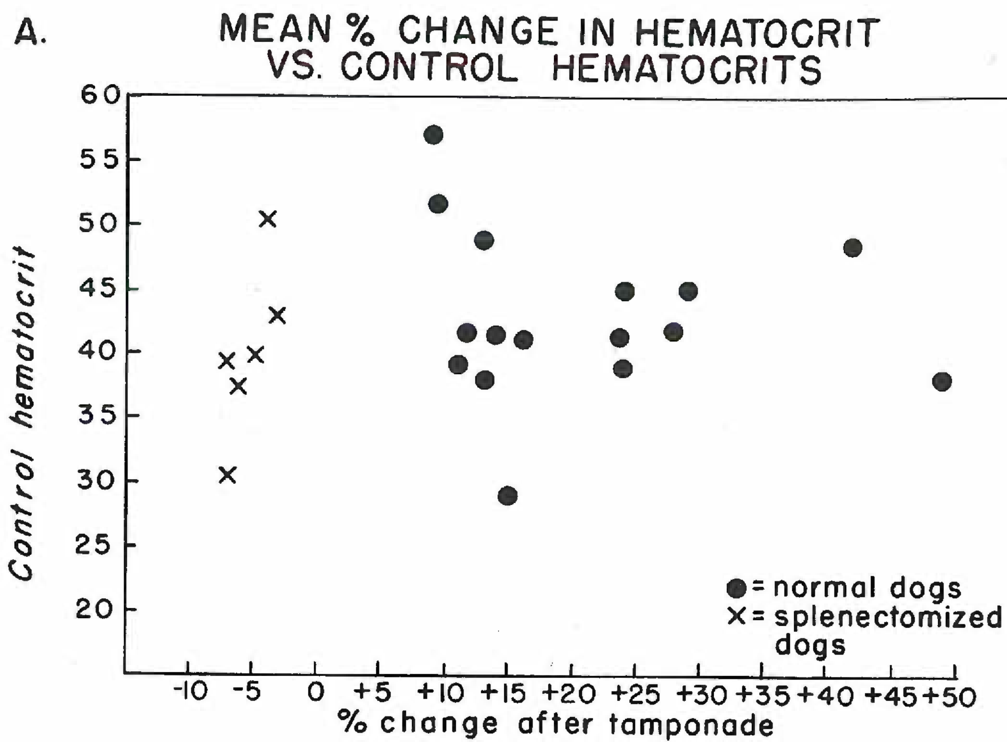
T-1824 space measurements by re-injection. Plasma volume during tamponade was measured by re-injection of T-1824 in 3 unsplenectomised dogs. The control T-1824 space was determined by injecting a dose of dye and establishing a disappearance curve over a period of thirty minutes, using blood samples drawn at ten minute intervals. Tamponade was then produced and maintained for one hour or more. Dye was re-injected at times ranging from twenty to eighty minutes after the start of the tamponade. A blood sample for dye determination was drawn immediately before the re-injection. A new disappearance rate was established by drawing samples at ten to fifteen minute intervals for forty minutes or more. The new volume was determined by extrapolation of the disappearance

Figure 4

A. A comparison of the control hematocrits and the mean percentage change in hematocrit of each dog after tamponade in the unsplenectomized and splenectomized dogs.

B. A comparison of the control total plasma protein and the mean percentage change in protein of each dog after tamponade in the unsplenectomized and splenectomized dogs.

These graphs demonstrate that there is no correlation between the original level of the hematocrit or total plasma protein and the amount of change in these values after the initiation of tamponade.


A.
MEAN % CHANGE IN HEMATOCRIT
VS. CONTROL HEMATOCRITS
Control hematocrit
60
55
50
45
40
35
30
25
20
● = normal dogs
X = splenectomized
dogs
-10 -5 0 +5 +10 +15 +20 +25 +30 +35 +40 +45 +50
% change after tamponade

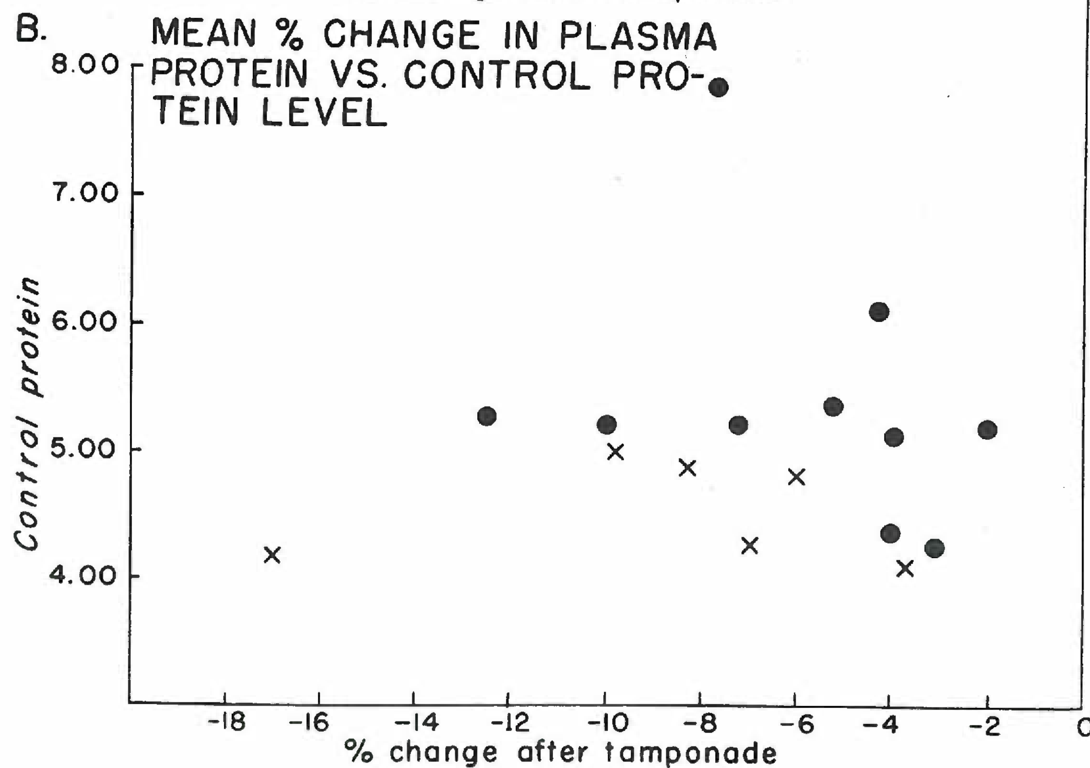
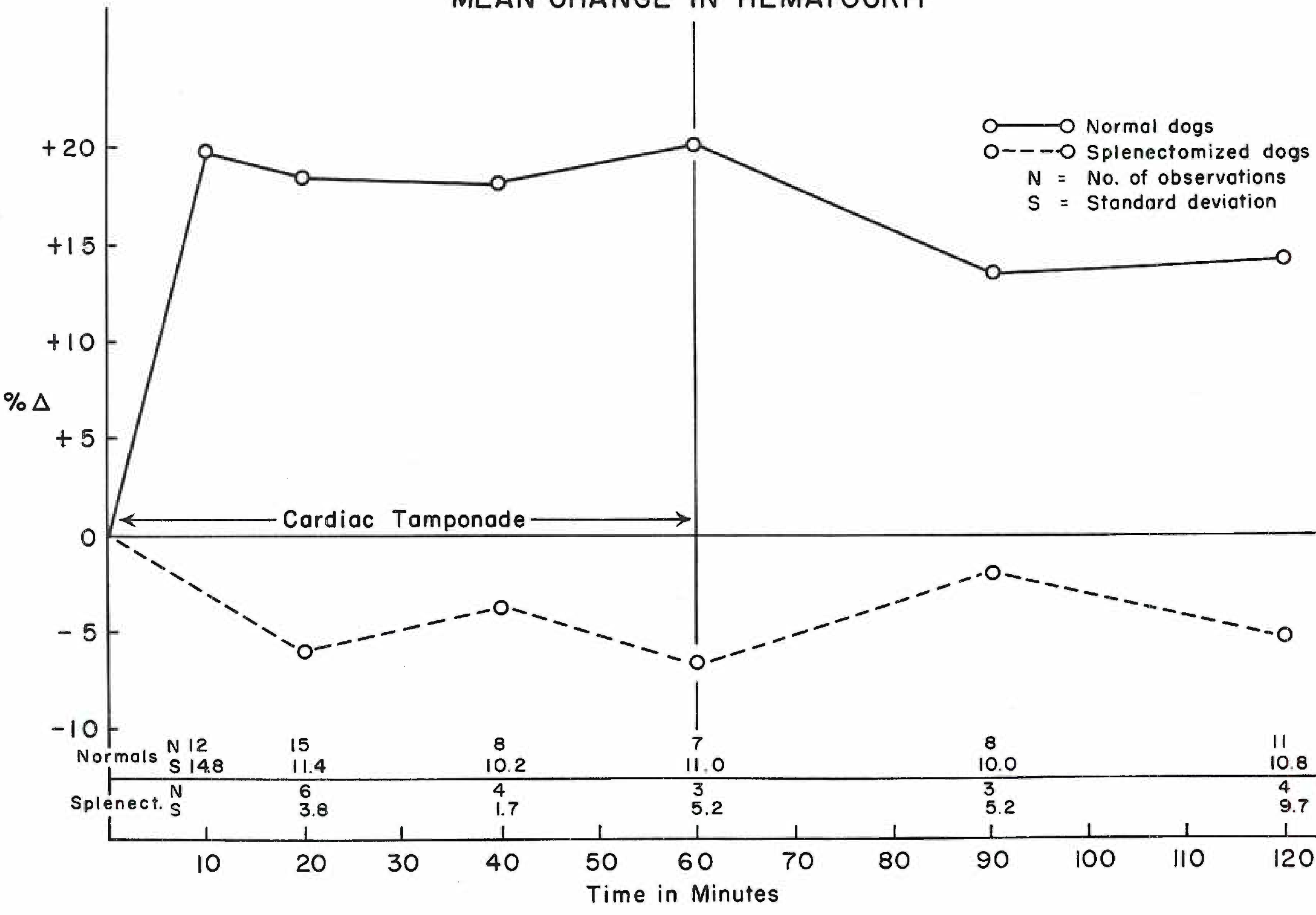

B.
MEAN % CHANGE IN PLASMA
PROTEIN VS. CONTROL PRO-
TEIN LEVEL
Control protein
8.00
7.00
6.00
5.00
4.00
-18 -16 -14 -12 -10 -8 -6 -4 -2 0
% change after tamponade

Figure 5

Mean change in hematocrit values in splenectomized and unsplenectomized dogs during and after tamponade. The ordinate is in percentage change from control levels. The difference between the mean changes of the two groups of dogs is significant at all of the time intervals.


MEAN CHANGE IN HEMATOCRIT
Normal dogs
Splenectomized dogs
N = No. of observations
S = Standard deviation
+20
+15
+10
% Δ
+5
0
-5
-10
Cardiac Tamponade
Normals N 12 S 14.8
15 11.4
8 10.2
7 11.0
8 10.0
11 10.8
Splenect. N S
6 3.8
4 1.7
3 5.2
3 5.2
4 9.7
10
20
30
40
50
60
70
80
90
100
110
120
Time in Minutes

curve back to the time of re-injection and subtraction of the concentration of dye just before the re-injection from the extrapolated value.

There was no apparent difference between the slopes of the control and re-injection disappearance curves. The T-1824 space during tamponade showed a definite decrease from the control level, and the hematocrit increased as described before. Total red cell volume calculated from the hematocrits and T-1824 spaces showed no apparent change before or during tamponade. All measurements were corrected for blood removal. These results are summarized in Table 1.

Extracellular fluid volume measurements with thiocyanate (SCN). Thiocyanate disappearance curves were established in 4 unsplenectomized dogs by injection of SCN and withdrawal of blood samples at ten to fifteen minute intervals for at least one hour before the tamponade was started. During and after tamponade, samples were drawn every fifteen to twenty minutes.

Most of the observed SCN concentrations during tamponade coincided with the extrapolated control disappearance curves. A few concentrations showed small and very inconsistent deviations from the extrapolated values.

A typical SCN disappearance curve is shown in Figure 6.

Plasma protein changes. Total plasma proteins were determined in 11 unsplenectomized dogs. At least one control determination and determinations at twenty minute intervals during and after tamponade were made.

All dogs showed a decrease in total plasma protein during tamponade.

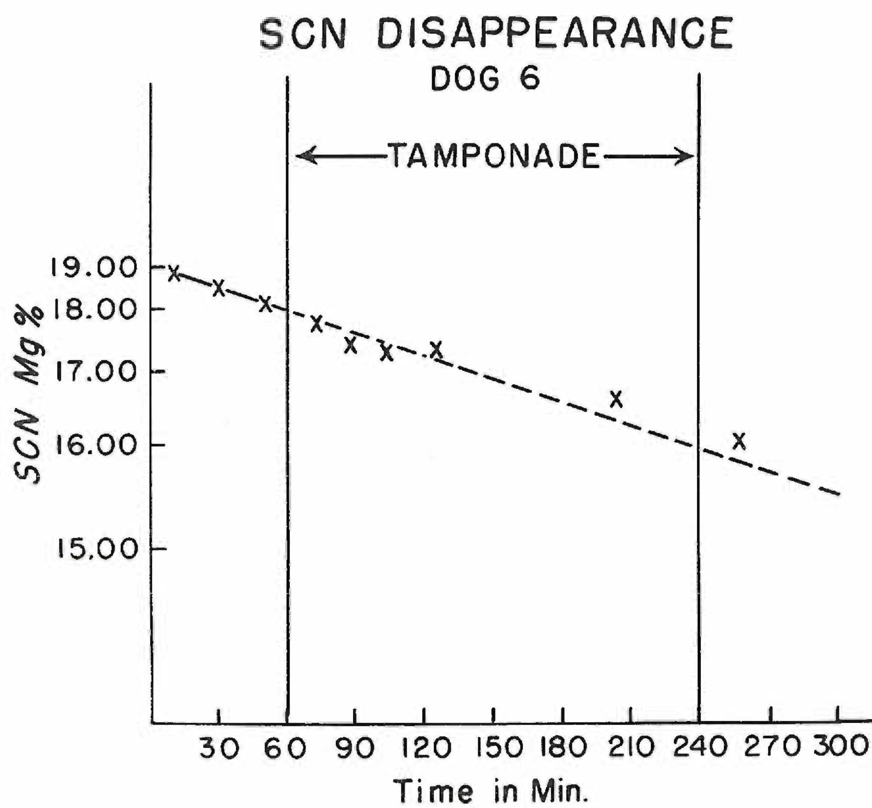
Table 1

CHANGE IN T-1824 SPACE BY RE-INJECTION IN
3 UNSPLENECTOMIZED DOGS

| | | T-1824 SPACE cc/Kg. | HCRT. | RBC cc/Kg. (calc.) |
|---|---|---|---|---|
| DOG 9 | CONTROL | 45.8 | 41.5 | 31.1 |
| | 80' TAMP. | 36.1 | 45.0 | 30.1 |
| DOG 12A | CONTROL | 60.5 | 41.5 | 41.2 |
| | 50' TAMP. | 39.4 | 52.0 | 42.7 |
| DOG 19B | CONTROL | 57.4 | 39.0 | 36.7 |
| | 20' TAMP. | 46.7 | 44.5 | 36.0 |
| | $\overline{\Delta}$ | - 14.2 | + 7.0 | - 0.1 |

Figure 6

A typical thiocyanate disappearance curve. The deviations of SCN concentrations from the forward extrapolated portion of the control curve are not significant.



SCN DISAPPEARANCE
DOG 6
TAMPONADE
19.00
18.00
17.00
16.00
15.00
SCN Mg %
30 60 90 120 150 180 210 240 270 300
Time in Min.

Twenty minutes after beginning tamponade, the mean decrease was 0.34 gm. with a range of 0.17 to 0.83 gm. Forty minutes after beginning tamponade the mean decrease was 0.38 gm.; range 0.15 to 0.96 gm.

Thus, the fall in protein did not seem to be progressive as the duration of tamponade increased. Total protein values appeared to return toward, but never reached control levels by one hour after the tamponade was removed.

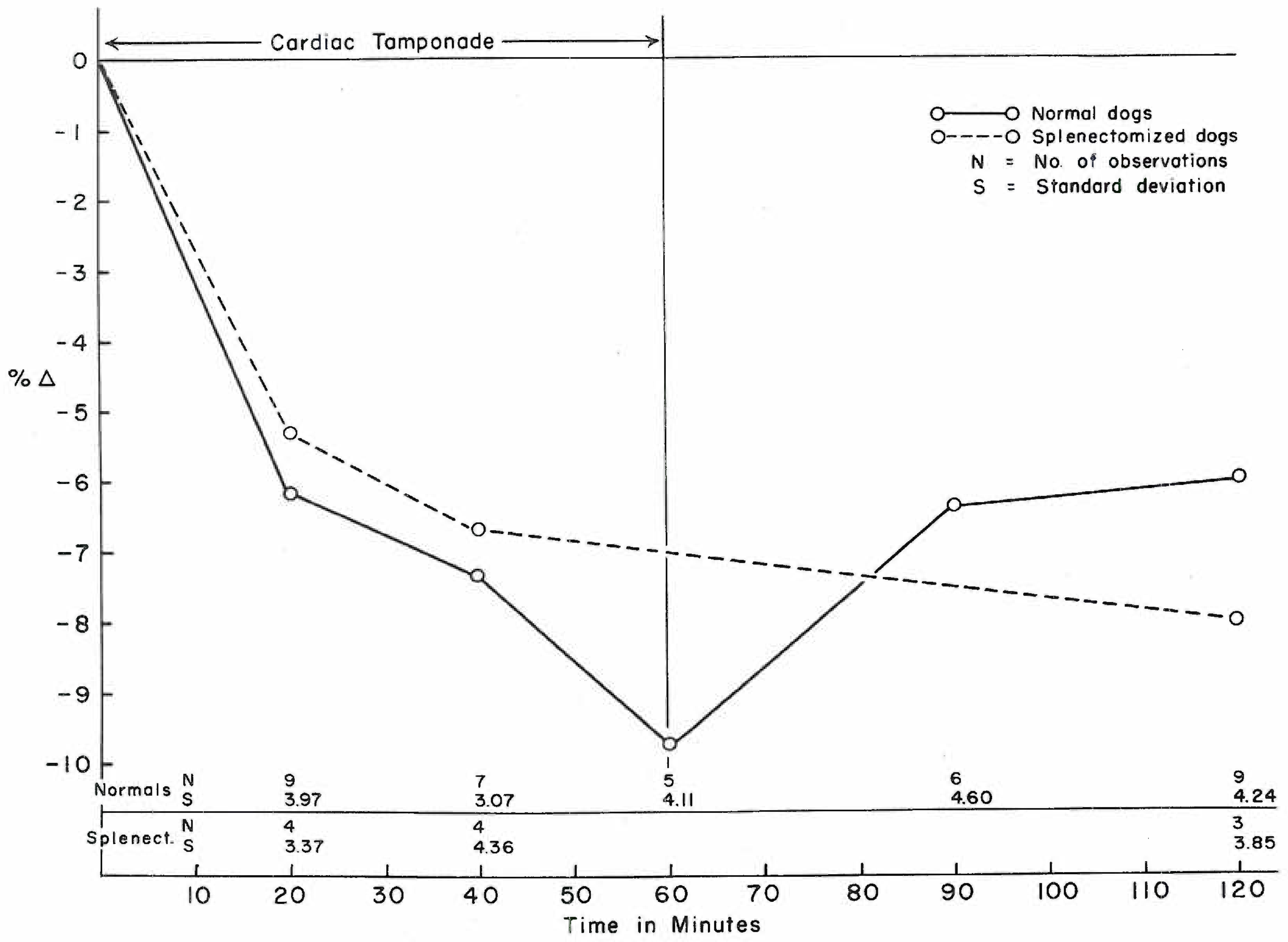
The change in total protein is presented graphically in Figure 7. Since control protein levels differed considerably, the changes are calculated as the percentage change from control levels. There was no apparent correlation between the original control levels and the per cent change as shown by the scatter graph in Figure 4B.

Red cell volume measurement with $Cr^{51}$ tagged red cells. $Cr^{51}$ tagged cells were used to measure red cell volume in six unsplenectomized dogs. In three of these dogs, tagged cells were re-injected during tamponade.

In the three dogs in which a single injection of tagged cells was given, a fifteen minute initial mixing period was allowed before a sample was drawn and the tamponade started. After tamponade in these dogs, the counts per second per cc. of red cells decreased, indicating a dilution of the circulating blood volume by unlabeled cells. In all three dogs, the cps/cc. of red cells had reached a constant level by twenty minutes after the beginning of tamponade. The calculation of red cell volume in these animals indicated an increase over control levels of from 4 to 7 cc. of red cells per Kg.

Figure 7

Mean change in total plasma protein in splenectomized and unsplenectomized dogs during and after tamponade. The ordinate is in percentage change from control values. The difference between the mean changes of the two groups is not significant at any of the time intervals.


MEAN CHANGE IN PLASMA PROTEINS
Cardiac Tamponade
Normal dogs
Splenectomized dogs
N = No. of observations
S = Standard deviation
% Δ
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
Normals N 9 7 5 6 9
S 3.97 3.07 4.11 4.60 4.24
Splenect. N 4 4 3
S 3.37 4.36 3.85
10 20 30 40 50 60 70 80 90 100 110 120
Time in Minutes

In the three dogs in which cells were re-injected during tamponade, a thirty minute mixing period was allowed during the control period before drawing a sample and starting tamponade. Twenty minutes after starting the tamponade, another blood sample was drawn and the second injection of tagged cells given. Further samples were drawn at ten minute intervals for forty-five minutes to one hour. The mixing of the second dose of injected cells was assumed to be complete when at least two successive samples had the same cps/cc. of red cells.

There was no change in the cps/cc. of cells between the samples drawn immediately before and twenty minutes after the initiation of tamponade in these dogs. Thus, when a thirty minute mixing period was used, there was no dilution of the circulating red cell volume by unlabeled cells.

The results obtained with one injection of tagged cells are shown in Table 2.

The red cell volumes during tamponade calculated on the basis of the re-injection of tagged cells did not differ significantly from control levels. The mean control red cell volume in the three dogs in which cells were re-injected was 33.7 cc/Kg., and the mean red cell volume after re-injection was 31.4 cc/Kg. The difference of 2.3 cc/Kg. had a 95% confidence interval of $\pm$ 4.7 cc/Kg. and was therefore not significantly different from zero.

Changes observed in viscera during tamponade. There was no observable change in the lungs of the animals with open chests during tamponade. No signs of pulmonary edema were present.

Table 2

$Cr^{51}$ RED CELL VOLUME AND CALCULATED PLASMA VOLUME WITH ONE INJECTION OF $Cr^{51}$ TAGGED RED CELLS

| | | COUNTS/Sec per cc RBC | $Cr^{51}$ RBC SPACE cc/Kg. | HCRT. | CALCULATED PV cc/Kg |
|---|---|---|---|---|---|
| DOG 20 (15' initial mixing) | Control | 120.1 | 28.4 | 38.0 | 46.4 |
| | Tamp. 1' | 104.0 | 32.7 | 50.0 | 32.7 |
| | 10' | 98.6 | 34.6 | 57.0 | 26.1 |
| | 30' | 97.9 | 34.9 | 56.0 | 26.5 |
| | After Tamp. 10' | 97.9 | 34.9 | 50.0 | 35.0 |
| | 65' | 97.9 | 34.9 | 44.5 | 43.5 |
| DOG 19B (15' initial mixing) | Control | 112.3 | 32.7 | 39.0 | 49.8 |
| | Tamp. 10' | 102.2 | 34.9 | 44.5 | 43.6 |
| | After Tamp. 20' | 100.5 | 35.5 | 44.5 | 44.2 |
| | 60' | 99.4 | 36.1 | 45.0 | 44.1 |
| DOG 21 (15' initial mixing) | Control | 204.5 | 26.1 | 41.0 | 37.3 |
| | Tamp. 10' | 192.5 | 27.8 | 42.5 | 37.3 |
| | 20' | 171.0 | 31.1 | 48.0 | 33.8 |
| | 40' | 169.0 | 31.5 | 52.0 | 28.5 |
| | After Tamp. 10' | 172.9 | 30.7 | 48.0 | 33.3 |
| DOG 27 (30' mixing) | Control | 122.5 | 30.5 | 49.0 | 31.8 |
| | Tamp. 20' | 120.3 | 31.1 | 54.0 | 26.4 |
| DOG 28 (30' mixing) | Control | 96.0 | 48.2 | 57.5 | 35.7 |
| | Tamp. 20' | 97.2 | 47.7 | 60.0 | 31.8 |
| DOG 23 (30' mixing) | Control | 119.5 | 20.8 | 29.0 | 50.9 |
| | Tamp. 20' | 118.0 | 21.2 | 33.0 | 43.0 |

In 2 dogs, the abdomen was opened and the viscera observed or palpated during tamponade. The bowel quickly became a dark purplish-red color and the liver appeared somewhat darker. There was no noticeable enlargement of the liver. The spleen was very large before tamponade had been produced, and when fluid was injected into the pericardial sac, the spleen was observed to contract vigorously. After a minute or so, the spleen relaxed again, but each time an increment of fluid was injected intrapericardially, splenic contraction would occur. When the arterial pressure had fallen to 50% of control levels, the spleen became maximally contracted for the duration of the tamponade. After removing the tamponade, the spleen again relaxed, but never achieved its original size.

B. Splenectomized Dogs

Hematocrit changes. Tamponade was produced in 7 splenectomized dogs. In all animals, the hematocrit decreased slightly during tamponade. This decrease ranged from -2 to -12 per cent of the control values in the first twenty minutes after the initiation of tamponade, with a mean decrease of -6%. The decrease did not appear to be progressive, since at 60 minutes after the start of tamponade the mean decrease was still -6% with a range of -2 per cent to -11 per cent.

After removal of the tamponade, the hematocrit values showed more variability in the direction of change. In 4 dogs, samples were taken sixty minutes after removal of the tamponade. Two of the hematocrits were slightly lower than they had been during the tamponade; one had returned to the control level and one was slightly greater than the

control level.

As in the unsplenectomized dogs, there was no apparent correlation between the control level of hematocrits and the percentage change during tamponade (see Figure 4A)

The mean change of hematocrits in the splenectomized dogs is shown graphically in Figure 5.

Red cell volume measurement with $Cr^{51}$ tagged red cells. Red cell volume was measured with a single injection of tagged cells in 4 splenectomized dogs. The initial mixing period was thirty minutes. Samples were drawn at twenty minutes after starting the tamponade. There was no difference in counts per cc. of red cells between the control and tamponade samples, indicating that there was no dilution by unlabeled cells.

Red cell volume during tamponade was measured in 3 splenectomized dogs by re-injection of tagged cells, using the same procedure and time intervals described for the unsplenectomized dogs. The mean control red cell volume was 31.3 cc/Kg. and the mean red cell volume during tamponade was 29.9 cc/Kg. The difference between means of 1.4 had 95% confidence limits of $\pm$ 2.5 and thus was not significantly different from zero.

It should be noted that the time required for mixing of the tagged cells during tamponade in both the normal and splenectomized dogs ranged from fifteen to thirty-five minutes with a mean of about twenty-five minutes in both groups.

Plasma volume. Plasma volume was calculated from the hematocrit and $Cr^{51}$ red cell volume in 7 splenectomized dogs. Changes in plasma volume were calculated on the assumption that red cell volume did not change, and thus the changes in the hematocrit were due to changes in plasma volume. The mean control plasma volume was 45.7 cc/Kg. and the mean maximum plasma volume during tamponade was 51.8 cc/Kg. The difference between means was 6.1 and the 95 confidence limits $\pm$ 3.4; therefore, the increase was significantly different from zero.

The data on red cell volumes obtained by re-injection of tagged cells in normal (unsplenectomized) and splenectomized dogs and plasma volume in splenectomized dogs is shown in Table 3.

The mean change in plasma volume of the splenectomized dogs for each twenty minute interval during tamponade was calculated and is shown graphically in Figure 8.

Effect of injecting blood during tamponade. An attempt was made to eliminate the possibility that red cells are trapped in some way during tamponade, thus causing the decrease in hematocrit in the splenectomized dog. This was done by injecting blood of high hematocrit after tamponade was well established. None of the cells in the injected blood were labeled with $Cr^{51}$.

This experiment was performed on 2 dogs. In the first dog, the spleen was removed and as much blood as possible squeezed out of it. In this way, 125 cc. of blood with a hematocrit of 50.5% was obtained. The control arterial hematocrit was 40.0%. After splenectomy, a dose of $Cr^{51}$ tagged cells was given and thirty minutes allowed for mixing. Tamponade was then produced, and twenty-five minutes later a blood sample

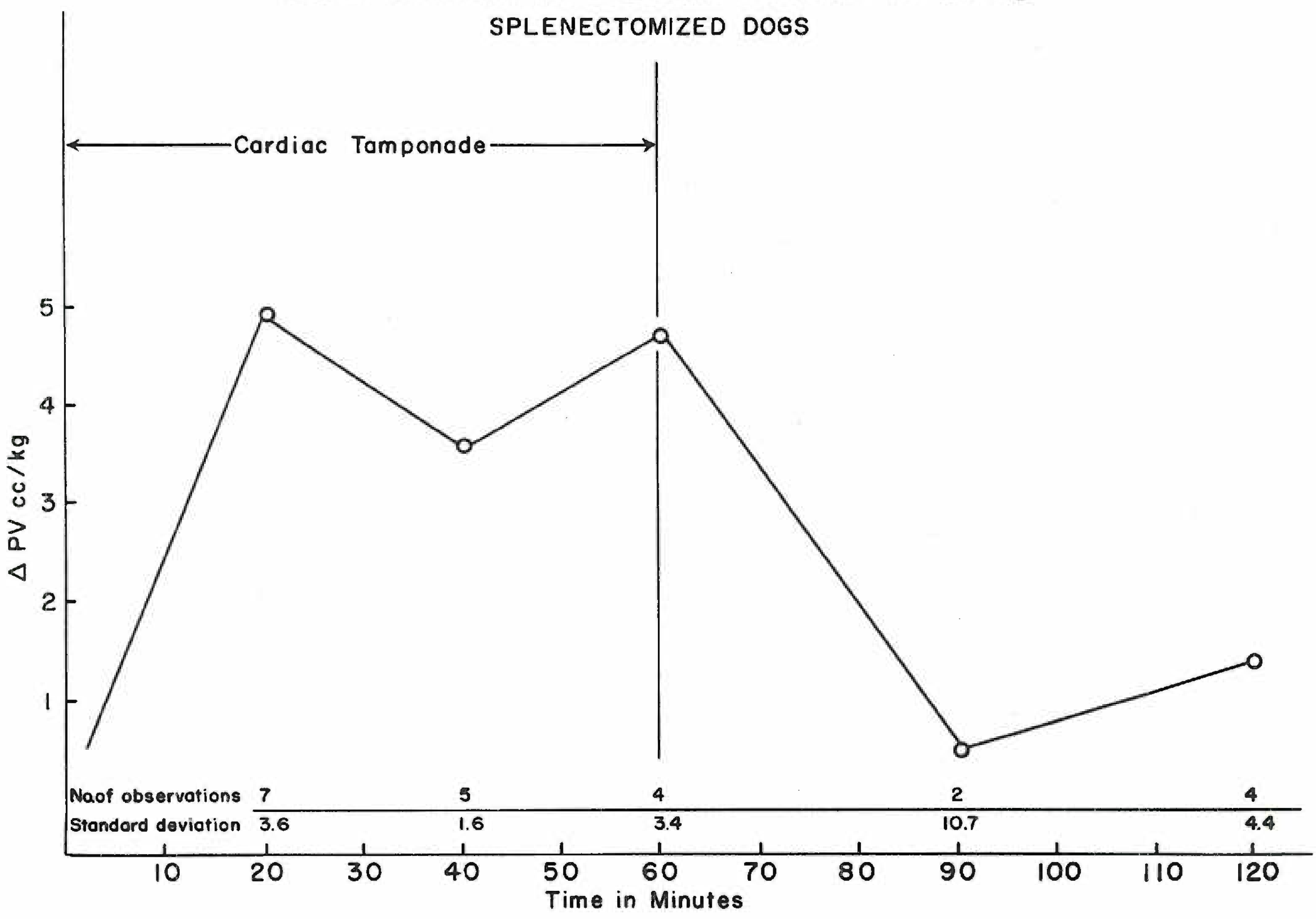
Table 3

| | CONTROL MEAN RBC cc/Kg. | MEAN RBC cc/Kg. AFTER TAMPONADE | DIFFERENCE |
|---|---|---|---|
| 3 NORMAL DOGS | 33.7 | 31.4 | 2.3 ± 4.7 |
| 3 SPLENECT. DOGS | 31.3 | 29.9 | 1.4 ± 2.5 |

| | CONTROL MEAN PV cc/Kg. | MEAN PV cc/Kg. AFTER TAMPONADE | DIFFERENCE |
|---|---|---|---|
| 7 SPLENECT. DOGS | 45.7 | 51.8 | 6.1 ± 3.4 |

Figure 8

Mean change in plasma volume in the splenectomized dogs. The plasma volumes were calculated from the hematocrit values and the $Cr^{51}$ red cell volumes.


MEAN CHANGE IN PLASMA VOLUME IN cc/kg
SPLENECTOMIZED DOGS
Cardiac Tamponade
Δ PV cc/kg
5
4
3
2
1
No.of observations 7 5 4 2 4
Standard deviation 3.6 1.6 3.4 10.7 4.4
10 20 30 40 50 60 70 80 90 100 110 120
Time in Minutes

was drawn and the splenic blood injected. The hematocrit before injection was 38.0%. Five minutes after injection, the hematocrit was 39.5; and fifteen minutes after, the hematocrit was 40.5%. $Cr^{51}$ red cell volume increased by 92 cc. and plasma volume by 96 cc. fifteen minutes after injection.

Since 62.5 cc. of cells and 62.5 cc. of plasma were injected, the hematocrit should have changed from 38.0 to 40.0. This figure agrees well with the observed hematocrit. Both the red cell volume and plasma volume increased more than the predicted value. This may have been due to errors in pipetting and counting the blood samples.

In the second dog, the spleen was removed and blood expressed, but a great deal of this blood clotted and could not be used. More blood was obtained from the dog by venesection, centrifuged and some of the plasma removed. One hundred and thirty-five cc. of blood with an hematocrit of 70.0% was obtained in this way. The control arterial hematocrit was 38.5%. The same procedure in blood volume measurement and blood injection was followed as in the first dog.

Before injection of the blood, the hematocrit was 37.0%. Fifteen minutes after the injection, the hematocrit was 43.0%. On the basis of the injection of 95 cc. of cells and 40 cc. of plasma into a volume of 231 cc. of cells and 395 of plasma, the hematocrit should have risen to 43.0%, which agrees with the observed value. Blood volume rose 92 cc. and plasma volume rose 32 cc., which agrees fairly well with the predicted values.

This data is shown in Table 4.

Plasma proteins. Total plasma proteins were determined in 6 of

Table 4

CHANGE IN BLOOD VOLUME OF SPLENECTOMIZED DOGS
AFTER TAMPONADE AND INJECTION OF BLOOD

| | | HCRT. | $Cr^{51}$ SPACE cc. | CALC. PLASMA VOL. cc. |
|---|---|---|---|---|
| | Control | 40.0 | 249 | 373 |
| | Tamp. 25' | 38.0 | 260 | 425 |
| DOG 29 10.0Kg. | 35' | 125 cc. blood, hcrt. 50.5% given I.V. | | |
| | 40' | 39.5 | 342 | 524 |
| | 60' | 40.5 | 353 | 521 |
| | Control | 38.5 | 231 | 368 |
| | Tamp. 20' | 37.0 | 231 | 395 |
| DOG 30 9.9 Kg. | 30' | 135 cc. blood, hcrt. 70.0% given I.V. | | |
| | 35' | 43.5 | 333 | 433 |
| | 45' | 43.0 | 329 | 437 |

the splenectomized dogs. Control determinations and one or two determinations during tamponade were made. In 3 of the dogs, a determination was made sixty minutes after the tamponade was discontinued.

The splenectomized dogs seemed to show the same direction and degree of change in respect to the total protein as the unsplenectomized dogs. Twenty minutes after the beginning of tamponade, the mean decrease from control levels in 4 dogs was 0.28 gm. with a range of 0.10 to 0.40 gm. Forty minutes after beginning tamponade, the mean decrease in 4 dogs was 0.43 gm. and the range 0.15 to 0.69 gm. Sixty minutes after tamponade was discontinued, the mean decrease was 0.52 gm. and the range 0.22 to 0.68 gm.

These changes in total protein are shown graphically as the percentage change from control levels in Figure 7. As in the unsplenectomized dogs, there was no apparent correlation between the control levels and the per cent change as shown by the scatter graph in Figure 4B.

Since plasma volume apparently increases in the splenectomized dogs, the possibility that the fall in total protein is due to plasma dilution should be considered. It is possible to predict the change in protein by calculating the amount of dilution that has occurred on the basis of the change in hematocrit. This may be demonstrated by a simple example: Assume a total blood volume of 100 cc., a plasma and red cell volume of 50 cc. and a protein concentration of 5.0 gm. Since the hematocrit is 50%, the total amount of protein present is (1-.50) 5.0 = 2.5 gm. If plasma volume now increases so that the hematocrit drops to 40%, the total blood volume will now be 50/.40 = 125 cc. and the new plasma

volume will be 125-50 = 75 cc. The new protein concentration will then be 2.5/75 = 3.33 gm. This calculation may be condensed in an equation:

$$\text{Predicted protein} = \frac{\text{Control protein (1-control Hcrt.)}}{\left(\frac{\text{Control Hcrt.}}{\text{New Hcrt.}}\right) 100 - \text{control Hcrt.}}$$

Table 5 presents the control hematocrit and protein, the lowest observed protein during tamponade and the hematocrit observed with this protein value for each splenectomized dog. The predicted protein has been calculated for each dog, and the difference between the observed and the predicted values is listed in the right hand column. The mean of this difference is -0.07 gm.%, and the standard error of the mean is 0.037. The 95% confidence interval is obtained by multiplying the standard error of the mean by the appropriate t value, and is found to be ± 0.095. Thus, the difference between the observed and predicted protein values is not significant, and one may conclude that the drop in protein could be due to plasma dilution.

T-1824 space. T-1824 space was determined before and during tamponade by re-injection in one splenectomized dog. The dye was re-injected forty-five minutes after tamponade had started. $Cr^{51}$ red cell space was also measured by re-injection at the same time that the T-1824 was re-injected.

The T-1824 space did not change significantly during tamponade, but the hematocrit dropped, so red cell volume calculated from the T-1824 space and the hematocrit showed a decrease. The $Cr^{51}$ red cell space did

Table 5

PLASMA PROTEIN CHANGES IN SPLENECTOMIZED DOGS

| | CONT. HEMAT. | CONT. PROT. GM% | NEW HEMAT. | OBSERV. PROT. GM% | PREDICT. PROT. GM% | OBSERV. MINUS PREDICT. |
|---|---|---|---|---|---|---|
| DOG 22 | 43.0% | 4.50 | 42.0% | 4.15 | 4.33 | -0.18 |
| DOG 24 | 37.5% | 3.85 | 36.0% | 3.62 | 3.62 | 0.00 |
| DOG 25 | 30.5% | 4.15 | 29.5% | 4.00 | 3.96 | +0.04 |
| DOG 26 | 39.5% | 4.33 | 35.0% | 3.50 | 3.60 | -0.10 |
| DOG 29 | 40.0% | 4.80 | 38.0% | 4.40 | 4.42 | -0.02 |
| DOG 31 | 50.5% | 5.00 | 48.0% | 4.38 | 4.53 | -0.15 |
| | | | | | | $\bar{X}$= -0.07±0.10 |

not change, and calculated plasma volume therefore increased during tamponade. The data from this experiment are shown in Table 6.

C. <u>Comparison of splenectomized and unsplenectomized Dogs</u>.

The difference between the per cent changes in the hematocrit of the two groups of dogs is highly significant at all time intervals. Table 7 shows the t values for each interval.

The difference between the per cent changes in total plasma proteins in the two groups at corresponding time intervals during and after tamponade is not significant at any of the intervals. The t test for each intervals is shown in Table 8.

The control mean arterial pressure ranged from 90 to 140 mm. Hg. in both groups. During tamponade, the range of pressures was 30 to 90 mm. Hg. After removal of the tamponade, the arterial pressure always rose, but seldom reached control levels by one hour. The range of pressures one hour after the removal of tamponade was approximately 80 to 130 mm. Hg. in both groups.

Table 6

COMPARISON OF CHANGES IN T-1824 AND $Cr^{51}$ TAGGED RED CELL SPACES BY RE-INJECTION
DOG 31 (SPLENECTOMIZED)

| | T-1824 SPACE cc/Kg. | HCRT. | RBC cc/Kg. (calc. from T-1824) | $Cr^{51}$ RBC cc/Kg. | PV cc/Kg. (calc. from $Cr^{51}$ RBC Sp.) |
|---|---|---|---|---|---|
| CONTROL | 40.3 | 50.5 | 41.1 | 29.5 | 29.0 |
| 45' TAMP. | 39.6 | 48.5 | 36.5 | 29.1 | 32.0 |

Table 7

T TEST FOR DIFFERENCE BETWEEN % CHANGES IN HEMATOCRITS OF 15 NORMAL AND 6 SPLENECTOMIZED DOGS

| | $\bar{X}$ Diff.of % Change | Std. Error of Diff. | T | p of This Diff. in same Direction |
|---|---|---|---|---|
| TAMPONADE 20' | 25.0 | 3.36 | 6.71 | .001* |
| 40' | 22.1 | 3.71 | 5.08 | .001* |
| 60' | 26.4 | 5.13 | 5.13 | .001* |
| AFTER TAMPONADE 30' | 15.6 | 6.87 | 2.27 | .05 * |
| 60' | 19.7 | 5.86 | 3.37 | .001* |

* SIGNIFICANT AT .05 LEVEL OR BETTER

Table 8

T TEST FOR DIFFERENCE BETWEEN % CHANGE IN PLASMA PROTEINS OF 15 NORMAL AND 6 SPLENECTOMIZED DOGS

| | $\bar{X}$ Diff.of % Change | Std. Error of Diff. | T |
|---|---|---|---|
| TAMPONADE 20' | 0.9 | 2.14 | 0.42 |
| 40' | 0.3 | 2.25 | 0.13 |
| AFTER TAMPONADE 60' | 2.7 | 5.86 | 0.47 |

## DISCUSSION AND CONCLUSIONS

Comparison of the results in the unsplenectomized and splenectomized dogs reveals one striking difference between the two groups. This difference is the opposite direction of change in the hematocrit values during tamponade. Since the presence or absence of the spleen was the only difference in the preparation of these groups, the difference in the hematocrit changes in the unsplenectomized dogs must have been due to splenic contraction. Splenic contraction was definitely observed to occur as mentioned previously.

The spleen is known to be a blood reservoir in the dog. According to Barcroft,[36] the dog spleen may contain up to one-fourth of the total blood volume. Furthermore, barbiturate anesthesia will cause marked engorgement of the spleen with consequent trapping of even more of the total blood volume.[37]

Splenic blood has a much higher proportion of red cells than arterial or mixed venous blood. Thus, splenic contraction will cause a marked increase in arterial hematocrit and cell count.[38]

Splenic contraction may be initiated by the injection of epinephrine intravenously, by ether anesthesia, by hemorrhage or by fright.[36] In short, any stressful situation is liable to cause the emptying of the splenic reservoir into the general circulation. The rise in hematocrit in the unsplenectomized dogs is most easily explained on this basis.

There was a decrease in the hematocrit of the splenectomized dogs during tamponade. This decrease could be due to three causes. First, plasma volume could be increasing, causing hemodilution. Second, some

of the red cells might be trapped somewhere in the circulation, causing a drop in the arterial hematocrit.

The third possible cause of this decrease in hematocrit requires a brief discussion. The hematocrit of minute vessel blood is considered by many investigators to be lower than the arterial hematocrit. This opinion is supported by direct observation of capillary blood in situ [4] and by tissue analysis for tagged red cells and $I^{131}$ albumin which had been injected into the blood stream.[39] Thus, there is supposed to be a "plasma envelope" surrounding an axial flow of blood in capillaries, arterioles and venules.

The third possible cause of the decrease in hematocrit might be the mixing of this "plasma envelope" with the rest of the circulating plasma due to the "wringing out" of these small vessels by vasoconstriction.

The first of these three causes seems the most acceptable for the following reasons: In the first place, the drop in total plasma protein was no different than the drop predicted on the basis of the fall in hematocrit; i.e., this drop in protein could have been due to simple plasma dilution.

In the second place, an injection of blood of high hematocrit during tamponade resulted in an increase in arterial hematocrit the same as that predicted on the basis of the addition of this blood to the measured blood volume. Thus, none of the injected cells were trapped.

In the third place, the mixing of the "plasma envelope" with the circulating plasma could not cause as great a decrease in the hematocrit as was usually observed; assuming that minute vessel blood volume is 10-15%

of the total blood volume, and has an hematocrit 10-15% lower than the arterial hematocrit.

The change in plasma volume in the unsplenectomized dogs was apparently opposite in direction to that in the splenectomized group. This was true of results obtained with both T-1824 and $Cr^{51}$ tagged red cells. There is no obvious reason for plasma volume actually changing in opposite directions in these two groups; therefore, the possibility that the decrease in plasma volume in the unsplenectomized dogs is an artifact must be considered.

The measurement of plasma volume changes by a single injection of T-1824 is obviously not reliable. Three types of disappearance curves were found in dogs whose hematocrits all increased and whose total plasma proteins all decreased. It is possible that slight degrees of hemolysis or actual changes in plasma optical density could have caused the dye concentrations to appear falsely high.

The re-injection of T-1824 showed a substantial decrease in T-1824 space. It should be recalled that when $Cr^{51}$ tagged red cells were injected during tamponade, the average time required for mixing was approximately twenty-five minutes. This is far greater than the normal mixing time. The same situation probably applies to the mixing of T-1824. If the disappearance curve is still in the mixing period, backward extrapolation of this curve will give falsely high values for the zero time concentration of the dye. The T-1824 space would then be falsely low.

The results obtained with re-injection of T-1824 in one splenectomized dog help to strengthen the suspicion that the dye is incompletely

mixed. The T-1824 space did not change, but plasma volume must have increased because the $Cr^{51}$ red cell space was constant while the hematocrit decreased.

The results obtained with $Cr^{51}$ tagged red cells demonstrate several points. One point is that more than 15 minutes are required to insure complete mixing of the total red cell volume with the tagged cells. It is apparent that in 15 minutes some of the blood in the spleen has not been mixed, since splenic contraction causes a dilution of the arterial blood with unlabeled cells.

Another point is that red cell volume does not change in either the unsplenectomized or the splenectomized dogs during tamponade. This is not surprising, because the only possible direction of change could be a decrease due to stagnation of blood at some point where it is unavailable for mixing with tagged cells. An increase is not possible because the total red cell mass is already mixed with the tagged cells, as has been shown by Gibson.(39)

The most important point relevant to this discussion is that calculations of changes in plasma volume from changes in hematocrit values are not valid in situations where splenic contraction is occurring. The reason for this is that the rise in hematocrit caused by splenic contraction causes an artifactual decrease in plasma volume. The hypothetical example shown in Figure 9 demonstrates this point. The calculated plasma volume in this example decreases, although no actual change in either total red cell or plasma volume has occurred. Thus, a slight increase in actual plasma volume would be completely masked and a marked increase would be underestimated.

Figure 9

## CONTRACTION OF AN ENGORGED SPLEEN AS A CAUSE OF APPARENT CHANGES IN PLASMA VOLUME

HYPOTHETICAL EXAMPLE:

1) MEASURED RBC VOLUME: 1000 cc.

2) 200 cc. OF CELLS ARE IN SPLEEN; 800 IN GENERAL CIRCULATION

3) SPLENIC HEMATOCRIT: 70%; ARTERIAL HEMATOCRIT: 50%

| 86 |
| --- |
| 200 |

200/286 = 70%

| 800 |
| --- |
| 800 |

800/1600 = 50%

4) ACTUAL TOTAL BLOOD VOLUME: 1886 cc; CALCULATED TBV; 1000/.50 = 2000 cc.

5) ACTUAL PLASMA VOLUME: 886 cc; CALCULATED PV: 2000-1000 = 1000 cc.

6) IF THE SPLEEN NOW CONTRACTS AND EMPTIES ITSELF, THE ARTERIAL HEMATOCRIT WILL NOW BE:

| 886 |
| --- |
| 1000 |

1000/1886 = 53%

7) CALCULATED TBV IS NOW 1000/.53 = 1886 cc.

8) CALCULATED PV IS NOW 1886-1000 = 886 cc.

9) APPARENT DECREASE IN PV = 1000-886 = 114 cc.

The changes in total plasma protein in the unsplenectomized dogs suggest that plasma volume is actually increasing by the same amount as in the splenectomized dogs. The direction and amount of change in total protein was the same in both groups.

Since cardiac tamponade produces a stagnant anoxia, the question of changes in capillary permeability due to anoxia should be considered. Anoxia of the capillary wall will cause a decrease in the permeability of the endothelium and escape of fluid and large protein molecules. Anoxic anoxia or stagnant anoxia have been reported to cause a fall in plasma volume and protein concentration.(40,41) The albumin fraction has been noted to fall more than globulin, probably because the smaller size of the albumin molecule allows it to escape more readily.

The results of this investigation do not suggest that capillary anoxia plays any great part in causing the changes in plasma volume or protein concentration. The increase in plasma volume would be very difficult to explain on the basis of altered permeability alone. The fall in protein concentration in most of the dogs is adequately explained on the basis of plasma dilution, with no escape of protein. The detection of disproportionate changes in albumin would require more accurate methods than were available.

Since tamponade is a type of acute heart failure, one might expect to see an increase in plasma volume in other types of acute failure. this does not seem to be the case, according to results obtained by other workers (see review of literature.) Since these other investigators also performed their experiments on dogs under barbiturate anesthesia, it is

probable that the apparent hemoconcentration which was reported was due in part to splenic contraction. The decrease in T-1824 spaces might have been due to inadequate mixing, as it probably was in this investigation.

The demonstration of an increase in plasma volume agrees with the results obtained by Cannon,(17) whose work on cats indicated hemodilution during tamponade.

The fact that the SCN space does not appear to change would indicate that the increase in plasma volume is due to a shift of fluid from the interstitial to the intravascular compartments, with no change in the total extracellular fluid volume.

As stated in the introduction, a change in plasma volume during acute heart failure could be explained on the basis of a change in the direction in net fluid movement across the capillary wall due to alterations in pressure along the length of the capillary. An increase in plasma volume during acute failure means that the fall in pressure at the arterial end of the capillary bed must overbalance the rise in pressure at the venous end. The fall in pressure at the arterial end is probably contributed to by arteriolar constriction, which in itself will cause a fall in intracapillary pressure. Whether or not all of the capillary beds in the body participate to the same degree in the above changes is a question that cannot yet be answered with any assurance.

## SUMMARY

This investigation was undertaken in an effort to determine whether or not there is a change in blood volume resulting from acute heart failure. The possibility of a change was considered in the light of the altered hemodynamics consequent to acute failure. Cardiac tamponade was chosen as a method of producing heart failure in dogs.

T-1824 spaces, red cell volumes, hematocrits and total plasma proteins were determined before, during and after tamponade in splenectomized and unsplenectomized dogs.

The results obtained led to the following conclusions:

1. Acute heart failure produced by cardiac tamponade is followed by splenic contraction which results in an increase in the arterial hematocrit of from 5 to 50 per cent. This increase is due to the discharge of cell-rich blood from the spleen which is initially engorged as a result of nembutal anesthesia.
2. The increase in hematocrit causes an artifactual decrease in plasma volume when plasma volume is calculated from the red cell volume.
3. T-1824 space measurement during tamponade is complicated by a greatly prolonged mixing time.
4. The hematocrit in the splenectomized dog decreases by a mean of 6 per cent following tamponade. This decrease is due to a mean increase in plasma volume of 6 cc/Kg.
5. Red cell volume does not change following tamponade in either the splenectomized or unsplenectomized dogs.

6. Total plasma protein decreases following tamponade in both the splenectomized and unsplenectomized dogs. The decrease in the splenectomized dogs is no greater than the amount that would be predicted on the basis of plasma dilution. There was no significant difference between the decrease in total protein in the two groups. The conclusion is therefore reached that plasma volume also increases in the unsplenectomized dog.

7. The changes in hematocrit, plasma protein and plasma volume are apparently at or near maximum by ten minutes after the tamponade is initiated. Removal of the tamponade after it has been maintained for one hour results in a tendency for the values to return to control levels. However, control values are seldom reached by one hour.

8. The increase in plasma volume is felt to be due to the fall in pressure at the arterial end of the capillary bed overbalancing the rise in pressure at the venous end. No definite evidence of a change in capillary permeability was seen.

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## TABULATION OF DATA

| Dog. No. | Time in Min.<br>C=control<br>T=tamponade<br>RT=remove T. | MAP<br>mm. Hg. | Hcrt. | T-1824<br>Space<br>cc/Kg. | SCN<br>Space<br>cc/Kg. | Total<br>Protein<br>Gm.% |
|---|---|---|---|---|---|---|
| 5 | C | 90 | | | 141 | |
| | T40 | 30 | | | 141 | |
| | 60 | 30 | | | 141 | |
| 6 | C | 108 | 51.5 | | 147 | |
| | T10 | 50 | 54.0 | | 147 | |
| | 20 | 50 | | | 147 | |
| | 40 | 50 | 55.0 | | 147 | |
| | 60 | 50 | 53.0 | | 147 | |
| | 120 | 50 | 56.0 | | 144 | |
| | 180 | 50 | | | 142 | |
| | RT190 | 94 | 62.0 | | 145 | |
| | 210 | 94 | 66.0 | | 139 | |
| | 240 | 94 | 56.0 | | 139 | |
| 9 | C | 140 | 41.5 | 45.8 | 158 | 5.50 |
| | T10 | 62 | 46.0 | | 158 | 5.16 |
| | 20 | 62 | 46.0 | | 158 | 5.16 |
| | 30 | 62 | 46.0 | 36.1 | 158 | |
| | 60 | 62 | 42.5 | | 158 | 5.00 |
| | 80 | 62 | 45.0 | | 158 | |
| | 120 | 40 | 42.5 | | 158 | 4.90 |
| | RT130 | 82 | 42.0 | | 158 | 4.70 |
| | 140 | 82 | 41.0 | | 166 | |
| | 160 | 82 | 42.0 | | 166 | 4.80 |
| 10 | C | 120 | 49.5 | | 158 | 5.28 |
| | T10 | 38 | 71.0 | | 158 | 4.80 |
| | 20 | 38 | 69.5 | | 158 | 4.45 |
| | 30 | 38 | 67.5 | | 158 | 4.70 |
| | 60 | 38 | 69.5 | | 165 | 4.50 |

| Dog. No. | Time in Min. C=control T=tamponade Rt=remove T | MAP mm. Hg. | Hct. | T-1824 Space cc/Kg. | SCN Space cc/Kg. | Total Protein Gm. % |
|---|---|---|---|---|---|---|
| 12a | C | 124 | 41.5 | 60.5 | | 5.48 |
| | T5 | 64 | 48.0 | | | 5.30 |
| | 15 | 60 | 47.0 | | | 5.20 |
| | 25 | | 53.0 | 39.4 | | 5.23 |
| | 35 | | 51.5 | | | 5.23 |
| | 50 | | 52.0 | | | 4.95 |
| | 65 | | 53.0 | | | 5.20 |
| | 75 | 60 | | | | 5.20 |
| | RT85 | 92 | 54.0 | | | 5.13 |
| | 100 | 84 | 50.0 | | | 5.13 |
| | 130 | | | | | 5.10 |
| 12b | C | 114 | 38.0 | | | 6.07 |
| | T5 | 60 | 47.0 | | | 5.85 |
| | 15 | 60 | 47.0 | | | |
| | 25 | 60 | 44.0 | | | 5.78 |
| | RT30 | | 44.0 | | | 5.65 |
| | 40 | 114 | 43.0 | | | |
| | 70 | 114 | 45.0 | | | 5.90 |
| | 80 | 110 | 45.0 | | | 5.90 |

| | Time in Min. | MAP mm. Hg | Hct. | T-1824 Space cc/Kg. | $Cr^{51}$ RBC vol. cc/Kg | Total Protein Gm. % |
|---|---|---|---|---|---|---|
| 13 | C | 150 | 44.0 | | | 7.80 |
| | T5 | 90 | 53.5 | | | |
| | 10 | | | | | 7.80 |
| | 20 | 90 | 54.5 | | | 7.50 |
| | 35 | 90 | 55.5 | | | |
| | 45 | | 55.5 | | | 7.25 |
| | 55 | 90 | 55.0 | | | 6.95 |
| | 70 | 90 | | | | |
| | RT85 | 130 | 51.0 | | | 7.15 |
| | 115 | | 52.5 | | | |
| | 175 | 130 | 51.5 | | | 7.15 |

| Dog No. | Time in Min. | MAP mm.Hg. | Hcrt. | T-1824 Space cc/Kg. | $Cr^{51}$ RBC vol. cc/Kg | Total Protein Gm. % |
|---|---|---|---|---|---|---|
| 16 | C | 152 | 41.0 | | | 5.15 |
| | T10 | 66 | 53.0 | | | 5.05 |
| 17 | C | 140 | 38.5 | | | 5.20 |
| | T5 | 54 | 45.5 | | | 4.88 |
| | 10 | | 45.0 | | | |
| | 20 | 50 | 44.5 | | | 4.73 |
| | 30 | | 43.5 | | | |
| | 35 | 50 | 43.5 | | | 4.63 |
| | RT70 | 94 | 42.5 | | | |
| | 140 | | 42.5 | | | |
| | 250 | | 43.0 | | | 4.72 |
| 19a | C | 140 | 38.5 | | | 5.20 |
| | T10 | 70 | 47.0 | | | 5.35 |
| | 25 | 70 | 46.0 | | | 4.95 |
| | 30 | | 52.0 | | | |
| | RT80 | 130 | 41.0 | | | 5.20 |
| 19b | C | 140 | 39.0 | 57.4 | 32.6 * | 5.25 |
| | T10 | 88 | 44.5 | | 36.0 | 5.05 |
| | 20 | | 42.0 | 46.7 | | |
| | RT30 | 138 | 41.0 | | 36.5 | |
| | 40 | | 40.5 | | | |
| | 80 | | 45.0 | | 37.1 | 5.15 |
| 20 | C | 156 | 38.0 | | 27.4 * | |
| | T10 | 84 | 50.0 | | 32.7 | |
| | 30 | | 57.0 | | 34.7 | |
| | RT40 | 150 | 50.0 | | 34.9 | |
| | 95 | | 44.5 | | 34.8 | |
| 21 | C | 128 | 41.0 | | 26.0 * | 4.57 |
| | T10 | 50 | 42.5 | | 27.7 | 4.43 |
| | 20 | | 48.0 | | 31.3 | 4.40 |
| | 40 | 50 | 52.5 | | 31.6 | 4.25 |
| | RT50 | 140 | 48.0 | | 30.9 | 4.43 |
| | 85 | 120 | 45.0 | | 33.6 | 4.43 |

* Single injection of tagged cells; 15 min. mixing period

| Dog No. | Time in Min. | MAP mm.Hg. | Hcrt. | T-1824 Space cc/Kg. | $Cr^{51}$ RBC vol. cc/Kg | Total Protein Gm. % |
|---|---|---|---|---|---|---|
| 23 | C | 120 | 29.0 | | 20.8 * | 4.55 |
| | T20 | 70 | 33.0 | | 21.2 | |
| | 40 | | 31.0 | | 19.5 # | 4.40 |
| | 50 | 70 | 33.0 | | 22.4 | |
| | 65 | | 36.0 | | 22.4 | 4.40 |
| | RT125 | 80 | 41.5 | | | |
| | 165 | 80 | 43.5 | | | 4.15 |
| 27 | C | 100 | 49.0 | | 30.5 * | |
| | T20 | 50 | 54.0 | | 31.1 | |
| | 45 | 50 | 54.5 | | 28.1 # | |
| | 65 | | 54.0 | | | |
| | RT115 | 100 | 48.5 | | | |
| 28 | C | 110 | 57.5 | | 48.2 * | |
| | T20 | 50 | 60.0 | | 47.7 | |
| | 45 | | 62.0 | | 40.5 # | |
| | 65 | 50 | 63.0 | | 43.7 | |
| | RT110 | 96 | 60.5 | | 45.0 | |
| 22 (Splenect.) | C | | 43.0 | | 51.6 * | 4.50 |
| | T10 | | 42.0 | | | 4.40 |
| | 30 | | 42.0 | | | 4.15 |
| | RT70 | | 43.0 | | 52.0 | |
| | 85 | | 43.0 | | | 4.28 |
| 24 (Splenect.) | C | 126 | 37.5 | | 36.1 | 3.85 |
| | T15 | 46 | 33.0 | | | |
| | 35 | | 36.0 | | 35.2 # | 3.62 |
| | 50 | 40 | 37.0 | | 35.2 | |
| | RT85 | 80 | 40.0 | | | |
| | 110 | 80 | 39.0 | | | 3.80 |
| 25 (Splenect.) | C | 116 | 30.5 | | 16.2 * | 4.15 |
| | T20 | 66 | 27.5 | | | |
| | 45 | | 29.5 | | | 4.00 |
| | 60 | 60 | 28.5 | | 16.7 | |
| | 70 | | 28.0 | | | |
| | RT95 | 88 | 26.5 | | | |
| | 140 | | 25.0 | | | |

* 30 min. initial mixing period for tagged cells
# reinjection of tagged cells

| Dog No. | Time in Min. | MAP mm. Hg. | Hcrt. | T-1824 Space cc/Kg. | $Cr^{51}$ RBC vol. cc/Kg | Total Protein Gm. % |
|---|---|---|---|---|---|---|
| 26 (Splenect.) | C | 114 | 39.5 | | 28.0 | 4.33 |
| | T20 | 40 | 37.5 | | 28.5 | |
| | 45 | | 37.0 | | 25.5 # | 3.64 |
| | 65 | | 35.0 | | | 3.50 |
| | RF115 | 76 | 36.5 | | | 3.65 |
| 31 (Splenect.) | C | 104 | 50.5 | 40.3 | 29.6 | 5.00 |
| | T20 | 62 | 49.0 | | | 4.60 |
| | 45 | | 48.0 | 39.6 | 29.0 # | 4.38 |
| | 55 | | 49.0 | | | |
| | 65 | | 46.5 | | | |
| 29 (Splenect.) | C | 114 | 40.0 | | (see table 4) | 4.80 |
| | T25 | 50 | 38.0 | | | 4.40 |
| 30 (Splenect.) | (see table 4) | | | | | |

# reinjection of tagged cells