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Histological Changes in the Rat's Spleen Induced by Selected Anesthetic Agents

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HISTOLOGICAL CHANGES IN THE RAT'S

SPLEEN INDUCED BY SELECTED

ANESTHETIC AGENTS

by
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This Thesis submitted by Donald R. Fowler in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota is hereby approved by the Faculty Advisory Committee under whom the work has been done.

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   <u>By Selected Anesthetic Agents</u>

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ABSTRACT

The purpose of this study was to determine what histological parameters of the rat's spleen were altered as a result of the anesthetic agent used. It also was desired to determine which anesthetic agent might be the most suitable to use in a transillumination study of the microcirculation of the living rat's spleen.

It was found that sodium Amytal administered intraperitoneally results in splenic dilatation. Chloral hydrate and sodium thiopental, given intraperitoneally, resulted in splenic contraction. Penthrane, ether, and chloroform administered by inhalation technique also resulted in splenic contraction. Penthrane and chloral hydrate while producing splenic contraction appear to do so to a lesser extent than the other contracting anesthetic used.

In the contracted spleen the organ decreased in size, especially in length; the capsule was thicker and the red pulp sinuses were smaller in diameter. It was also noted that the ratio of the white pulp nodule in respect to the marginal zone varied with contraction and dilatation of the spleen. In the contracted spleen the marginal zone decreased in size and in the number of erythrocytes found within the zone. In the dilatated spleen the marginal zone increased in size with respect to the white pulp nodule, and the marginal zone appeared flooded with erythrocytes.

To investigate the microcirculation through the marginal zone sodium Amytal appears to be the best anesthetic because it was during the use of this anesthetic that the marginal zone was richly supplied with erythrocytes.

It is apparent that to determine the entire microcirculation of the spleen one must also use an anesthetic that will produce splenic contraction and of those tested Pentothal should be satisfactory.

CHAPTER I

INTRODUCTION

It appears quite possible that the divergent opinions on
splenic circulation arrived at by investigators using the method of
transillumination of the organ in the living anesthetized animal
might be attributed to the fact that the anesthetic agents themselves
might produce profound differences in the observed circulatory patterns.
For this reason it was decided to see if in fact certain selected
anesthetic agents would by themselves elicit demonstratable
histological changes in the spleen. Knowledge of such factors would
make subsequent transillumination studies more meaningful and less
subject to erroneous interpretations by the observer.

The introduction of this paper will be divided into three
subdivisions: (1) General History of Anesthetics, (2) History of
Splenic Physiology and the Effects of Anesthetics upon that Organ,
and (3) Purposes of this Study.

General History of Anesthetics

Anesthetic agents have been utilized since ancient times to
relieve or prevent pain during surgical procedures. It is thought
that the Egyptians used some types of narcotics and that the Chinese
made use of the analgesic properties of hashish for that purpose.

Pliny and Dioscorides (Cohen and Dripps, 1970) advocated the use of mandragora (belladonna alkaloids) prior to surgery. Many bizarre methods were employed to render a person unconscious temporarily so an operation could be performed. One method used was to place a wooden bowl upon the patient's head and strike it with a heavy object. Another practice was employed by the Assyrians who asphyxiated children by strangulation prior to circumcision. This method was also used in Italy as late as the seventeenth century. Hemp and alcohol were also used to diminish pain (Cohen and Dripps, 1970).

The ability to perform painless surgery upon man and experimental animals became a reality and a general practice 125 years ago. The first three agents used to produce general anesthesia, ether, chloroform, and nitrous oxide, are still used as general anesthetics for both human and animal surgery.

Ether was first discovered in the thirteenth century by a chemist, Raymond Lully, who named his discovery "sweet vitriol." Unfortunately, his formula was lost for two centuries before it was rediscovered by Paracelsus who wrote in the year 1540, "of all the extracts of vitriol...it has an agreeable taste so that even chickens take it gladly and thereafter fall asleep for a long time, awakening undamaged." Paracelsus further proposed that it might be used to alleviate pain during illness. Unforturnately, the substance, which was renamed ether by Frobenius in 1792, was not used for relieving pain but was instead used for the treatment of asthma and other respiratory diseases. C. Long, a physician in a rural Georgia

village, was the first to use ether as a general anesthetic in 1842.
Adverse local public opinion forced him to abandon his process of
painless surgery, and William T.G. Morton has been credited with
administering the first general anesthetic to a surgical patient
at Massachusetts General Hospital, November 7, 1846 (Kemp, 1948).

Chloroform was discovered in 1831 simultaneously by three
investigators: Guthrie in the United States, Soubieron in France,
and Leipzig in Germany. Flourens demonstrated in 1847 that chloroform
produced narcosis in animals, and that same year, James Simpson, an
Edinburgh surgeon, began to use it as an anesthetic for his patients
during childbirth. The practice of using chloroform to relieve the
pain associated with childbirth was attacked by the clergy who
maintained that the relief of pain of woman during delivery was
against the teachings of Holy Writ. The controversy ended in the
year 1853 when John Snow administered chloroform to Queen Victoria
during the delivery of Prince Leopold (Kemp, 1948). Ultimately,
the popularity of chloroform declined as a controversy arose
as to whether it was the cause of a higher death rate during surgery
than that found when ether was used as the anesthetic (Orth, 1958).

Nitrous oxide was discovered by Joseph Priestly in 1776 and
its anesthetic properties were noted by Humphry Davy who named
it laughing gas. Henry Hinckman performed surgery on small animals
using nitrous oxide as an anesthetic, and he tried to convince his
fellow surgeons of the gas's value, but they scoffed at his suggestions.
Finally, he offered his findings to the French Academy of Science on

December 28, 1828. The Academy failed to find any merit in
Hinckman's proposals just as they were to do fifty years later
when they rejected C. Bernard's incandescent light bulb. As with
ether, nitrous oxide was used by the medical profession as a drug
for the treatment of upper respiratory diseases. In 1863 C. Q.
Colton popularized the administration of nitrous oxide for painless
dentistry. It was not until 1910, after the invention of improved
gas delivery machines, that nitrous oxide with oxygen became relatively
safe for surgical anesthesia (Kemp, 1948).

One of the first reports of the effects of anesthetics upon
the blood was submitted in 1861 to the Royal Medico-Chirurgical
Society by Sampson who felt that anesthetics destroyed blood
corpuscles. He based his claim upon the fact, that when he added
an anesthetic drug to whole blood, it resulted in destruction and
damage to blood cells. Eight years later McQuillen (Hamburger and
Euring, 1911), after examination of patient's blood before and after
anesthesia, disputed Sampson's theory. Hamburger and Euring (1911)
noted that ether caused a ten per cent increase in the hematocrit
of dogs. At that time a satisfactory explanation as to how ether
could elicit such a response was not offered. The answer to the
mystery began to unfold, in part, as a result of a sea voyage.

History of Splenic Physiology and the Effects
of Anesthetics upon that Organ

In 1921 Barcroft and his associates were journeying to Peru
to investigate the effects of high altitude upon hemoglobin. While

aboard the steamship R.M.S. Victoria, they recorded daily samples of their own hemoglobin to establish a base line at sea level. As they entered the Caribbean Sea they noticed an increase in their hemoglobin and blood volume. They were at a loss to explain the change and later, when they reached a cooler climate, the hemoglobin and blood volume returned to normal. They speculated as to where in the body hemoglobin could be stored that was relatively free of carbon dioxide that could be released to compensate for temperature change. They agreed that the spleen was the most logical organ (Barcroft, 1925). Gray had suggested in 1854 that the spleen was a reservoir for red blood cells (Izquierdo and Cannon, 1928). After a number of unique experiments where metal clips were attached to the splenic capsule and the animals observed with x-ray, Barcroft and his associates concluded that: (1) the normal spleen was larger in life than after death, (2) during hemorrhage, splenic contraction contributed blood to the circulation that approximately equaled that lost, (3) during exercise the spleen contracted and added additional blood to the circulation according to the needs of the animal, (4) that, in some cases, the anesthetic urethrane seemed to cause splenic contraction (Barcroft, et al., 1925). Prior to this time there was no known function attributed to the spleen.

Cruickshank (1926) reported that the blood which came from the spleen of a cat upon contraction was richer in hemoglobin than that found in the peripherial circulation and that the amount of blood added to the general circulation amounted to about 2.5 to 5.6

per cent of the total blood volume of the animal. J. Barcroft and L.T. Poole (1927) concurred with Cruickshank's observation that the blood released from the spleen during contraction was richer in hemoglobin than that found in the peripherial circulation.

Barcroft and Stephens (1927) estimated that the amount of blood contributed by a contracting dog's spleen was equal to twenty per cent of the total blood volume. They further noted that for the spleen to exert maximum contraction, the splenic nerves must be intact. They suggested that psychological processes that culminated in violence could produce splenic contraction. Actually, Ferrari in 1897 had noted that emotional excitement had an influence upon the blood count. Cannon and de la Paz (Izquierdo and Cannon, 1928) demonstrated that excitement resulted in the secretion of adrenalin, and they proposed that the increase of erythrocytes resulted from emotional excitement mediated by the adrenal glands.

Strausser and Wolf in 1905 reported that adrenalin in small amounts resulted in splenic contraction. Izquierdo and Cannon (1928) proposed that when the normal spleen contracts under emotional stress, two factors were involved: (1) the concentration of adrenalin in the blood and (2) sympathatic nerve impulses.

Barcroft and Florey (1928) proposed two methods by which the spleen could concentrate the erythrocytes. They felt that the splenic veins possessed a marked capacity to constrict, and, as a result of that constriction, the plasma would be drained off by the lymphatic vessels. The second method proposed was a special form

of plasma skimming whereby the plasma would be removed by the blood
stream. These investigators demonstrated their first theory by
simply ligating the splenic vein of cats.

The bulk of research that deals with the effects produced by
different anesthetics has been performed primarily upon three species:
man, dog, and cats. Caution must be used in applying knowledge gained
from one species of animal to a different species. A few examples
will suffice to illustrate this point. C.L. Conley (1911) observed
that while ether caused an increase in the hematocrit and a decrease
in the plasma volume in dogs, these same responses were absent when
ether was used upon the cat. G.M. Gruber (1941) reported that
Pentothal will act as an anesthetic when given in sufficient amounts
to most experimental animals, but the same drug used upon rats will
produce a deep hypnosis but only when given in amounts resulting
in the death of fifty per cent of the animals. When a rat is in
deep hypnosis, it will respond to sharp pain by squeaking. One cannot
expect identical responses from drugs of the same class; for example,
in man the spleen dilatates and the erythrocyte count increases when
most barbiturates are used, but these responses are in exactly the
opposite direction when the ultra-short acting barbiturates such
as sodium thiopental are used (Adrenai, 1970).

E. Hausner and his associates (1937), using the method developed
by Barcroft to visualize the spleen, investigated the effects four
different anesthetic drugs had upon the organ. They found that the use
of ether resulted in splenic contraction. Sodium Amytal and

pentobarbital both caused the spleen to dilatate; however, the effects of the anesthetics continued to influence that organ's size for approximately 24 hours in the case of sodium Amytal and for 6 hours and 30 minutes in the case of pentobarbital. The results observed in the case of sodium Pentothal were not consistent. In most cases its use resulted in splenic dilatation, but in some cases it resulted in contraction of that organ.

Anesthetic influences upon blood values have been reported by a number of investigators. They seem to agree as to the type and direction of the changes, but they report a variety of values in respect to the magnitude of each change. A brief summary of some of the reported effects that different anesthetics have produced will be listed for some of the agents.

Ether has been reported to produce the following changes in the blood values of man and dogs (Hamburger and Euring, 1908; Searles and Essex, 1936; Hausner, 1937; Bollman, et al., 1938; Vanderveen, 1962): the hematocrit, hemoglobin, erythrocyte count, cell volume, and platelet counts all increase. The clotting time in man shows a slight increase, but it is slightly decreased in the dog. In man there is a slight increase in the amount of epinephrine in the peripherial blood. Plasma volume is decreased in both species.

Sodium Amytal has been reported to produce the following changes in the blood values of man, dogs, and cats (Bourne, 1930; Searles and Essex, 1936; Hausner, et al., 1937; Bollman, et al., 1938): the hematocrit, cell volume, hemoglobin, and erythrocyte

count all decrease, but the plasma volume and total blood volume are increased.

Nembutal has been reported to produce the following changes in the blood values of dogs: the hematocrit, plasma protein, hemoglobin, and erythrocyte count all show a marked decrease.

Sodium pentobarbital and Pentothal produce changes in blood values similar to sodium Amytal in man and dogs. Chloroform produces changes that are similar to ether, and methoxyflurane has been reported to result in metabolic acidosis in dogs. Thiopental produces similar changes in the blood values of both man and dogs, increased hematocrit, but no change in clotting time has been observed.

All of the changes noted in the concentration of blood in animals under the influence of anesthetics cannot be attributed to the spleen entirely. Investigation has shown that a part of the change produced is due to the increase or decrease of plasma volume. In the absence of the spleen, for example, in dogs under the influence of ether, fewer erythrocytes are added to the circulation, but there is a reduction in the amount of plasma. The reduction of the plasma seems to be independent of the presence or absence of the spleen (Bollman, et al., 1938). General anesthesia in man is accompanied by a plasma volume change which is in the opposite direction to a simultaneous change in arterial and venous pressures (Price, et al., 1956).

It must not be assumed that anesthetics influence only the blood concentration, blood pH, blood pressure, etc., or that the spleen

is the only portion of the viscera that undergoes transitory changes.
For example, it has been reported that, without the use of
sleep-producing drugs that the measured capacity of the colon of the
rat ranged from 3 to 7 cc, but when sodium Amytal was administered,
the capacity increased to a range of 6 to 15 cc (Pendergrass and
Griffith, 1949). Neither must it be assumed that all of the effects
produced by an anesthetic are transitory. Okamoto (Johnson, et al.,
1971) demonstrated that in rats the prolonged administration of nitrous
oxide resulted in severe leukopenia with a relative lymphocytosis.
Johnson, et al. (1971) found evidence that nitrous oxide exerted its
influence upon the stem cell or an alternate progenitor cell. Nitrous
oxide has been shown to be teratogenic in rats (Fink, et al., 1967),
and the combination of Halothane with ionization produces an increase
in mortality, changes in liver cells, and occasionally patches of
splenic lymphoid hyperplasia (Bruce and Koepke, 1969).

Purpose of this Study

A number of investigators have attempted to discover how
blood circulates through the spleen by using the transillumination
technique. Rather than solve the problem, it has resulted in a
controversy that rages today. Three types of circulation have been
ascribed to the spleen, an open system (MacKenzie, et al., 1941),
a closed system (Knisely, 1935) and an intermediary system (Peck
and Hoerr, 1951). Some of the controversy no doubt has arisen because
of the wide variety of animals that have been used by investigators.
MacKenzie and his associates used mice, rats, rabbits, guinea pigs, and

cats, and Knisely used mice, cats, and rats. Snook (1950) pointed
out the danger in trying to form a composite picture of an organ
using multiple species. In the transillumination studies performed
a wide variety of anesthetics and their routes of administration were
used. Peck and Hoerr (1951) used sodium Amytal, barbital, pentobarbital,
and Dial given subcutaneously. Gall (1948) administered nembutal
intraperitonelly, MacKenzie, et al. (1941) used sodium Amytal given
subcutaneously and ether prior to removal of the spleen on the larger
animals, Knisely (1935) administered sodium Amytal subcutaneously,
and Parpart, et al. (1955) gave a solution of ethyl urethane subcutaneously.

It is the purpose of this paper to attempt to determine what
histological effects different anesthetics have upon the rat's spleen
before attempting a transillumination study of the microcirculation
of that organ. It is felt that without some knowledge of the
anesthetics' effect upon the spleen it would be difficult to interpret
accurately splenic circulation, especially when attempting to observe
vascular connections of the living, functioning organ. At best,
transillumination studies are difficult to carry out and the results
are quite subjective, and open to interpretation. It is believed
that by knowing ahead of time what effects various anesthetics
will have on the spleen one anesthetic may be selected for use which
will enhance the visualization of the particular segment of the
vasculature under study.

CHAPTER II

MATERIALS AND METHODS

General Procedures

Fifty-four Sprague-Dawley albino rats, 26 males and 28 females,
were used in this study. Their ages varied from 2 to 13 months and
their weights ranged from 226 grams to 450 grams. The animals were
housed in individual cages and maintained on a diet of "Purina Rat
Chow" and water ad libitum.

The animals were separated into two major groups which were
designated as Groups A and B. Group A, composed of the 16 oldest
animals, was divided into two equal subdivisions which were designated
as subdivision AI and AII. Each subdivision contained an equal
number of male and female animals. The A group animals were used
in a pilot study to note the differences between a dilatated and a
contracted spleen. To obtain maximum splenic contraction, in addition
to the anesthetic, an intravenous injection of epinephrine was
administered to the AI subdivision animals just prior to clamping
the vascular supply of the spleen. To obtain maximum dilatation of
the spleen the AII subdivision animals were given an injection of
Dibenzyline·HCl, an alpha adrenergic blocking agent, two hours before
the anesthetic was administered. A minimum of 30 minutes was allowed

to pass after the anesthetic was administered before surgery was started to allow the spleen to reach maximum dilatation. The group B animals, the experimental animals, were divided into six unequal subdivisions each of which contained animals of both sexes. They were designated as subdivisions BI, BII, BIII, BIV, BV, and BVI. These animals received only an anesthetic agent prior to surgery (See Table 1, Treatment of Animals of Various Groups and Subdivisions). Other than the above mentioned treatments all of the animals were handled identically.

Each animal was weighed at least once every four days throughout its confinement, and the rat's weight was recorded every two days two weeks prior to surgery. The above was performed for two basic reasons: (1) to monitor the general health of the animal and (2) to accustom the rat to being handled so it would not become unduly alarmed prior to surgery. The day prior to scheduled surgery, those animals selected were taken to the area where the operations were to be performed, which allowed them time to become assustomed to their new location. Eighteen hours before surgery all food was removed from the cages so all the animals would respond uniformly to similar dosages of anesthetic drugs.

Initial blood work was performed upon each animal before any drug was administered. To obtain blood samples the animals were wrapped in terry-cloth towels with only the tails exposed. This method did not appear to unduly frighten the rats as had an earlier method in which a wire restraining cage was used. The tail was cleaned

TABLE 1--Continued

Group and Sub-division	Dose in mg/kg	Strength mg/cc	Additional Drugs Administered	Route	Dose in mg/kg	Strength mg/cc	Remarks
A-I	55	10	Epinephrine (Harvey Lab., Inc.)	Intra-venous	0.1	1	Epinephrine was administered just prior to the clamping of the vascular supply of spleen.
A-II	100	10	Dibenzyline ˙HCl pheno-xybenzamine hydrochloride (S.K. & F.)	Intra-peritoneal	8	10	Dibenzyline˙HCl was given a minimum of 2 hours prior to the administration of the anesthetic agent
B-I	100	10	none				A minimum of 30 minutes was allowed to pass before surgery was started after anesthetic was administered.
B-II	350	10	none				A minimum of 15 minutes was allowed to elapse before surgery was started after anesthetic was administered.
B-III	55	10	none				See remarks subdivision B-II

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TABLE 1--Continued

Group and Sub-division	Dose in mg/kg	Strength mg/cc	Additional Drugs Administered	Route	Dose in mg/kg	Strength mg/cc	Remarks
B-IV							Cotton saturated with the anesthetic was placed in the bottom of a covered glass container. The animal was put into the container and was removed only after it became limp. Further anesthesia was maintained using a nose cone (Hoar, 1964).
B-V							See remarks, subdivision B-IV
B-VI							See remarks, subdivision B-IV

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and blood samples were obtained by docking the tip of the tail,
using a sharp knife. After collecting the blood samples, a
tourniquet was applied to the rat's tail to prevent excessive blood
loss and the rat was returned to its cage. Duplicate hematocrit
samples were collected in heparinized capillary tubes (Biological
Research, Inc.), sealed with plastic Critocaps (Sherwood Medical
Industries, Inc.), spun down in a micro-centrifuge, and the results
were read directly from a Critocap Micro-hemtocrit Tube Reader
(Sherwood Medical Industries, Inc.). Duplicate red blood cell counts
were collected in red cell diluting pipettes diluted to the proper
level using Hayem's solution (Cambridge Chemical Products, Inc.),
thoroughly agitated by an electric pipette shaker, and the cells
counted using a Bright Line hemocytometer (A.O. Spencer). The method
of counting and the calculations used were similar to that described
by Wintrobe (1967). If a large discrepancy was noted in the
duplicate blood work performed upon an animal, it was returned to
the animal room and no further work was performed upon it for at
least one month. If the discrepancy was minor, then the mean value
was determined and recorded for the blood values.

After the animal reached a surgical plane, the hair of the
abdomen was clipped and a midline incision, followed by a long
paracostal incision, was made. The spleen was exteriorized, care
being exercised not to add excessive pressure upon the organ, and
a hemostate of sufficient size to clamp all the vascular supply
simultaneously was applied near the hilus of the spleen. An additional

hemostat was placed near the first and the organ was removed by cutting between the instruments.

The spleen was placed in a (5°C) solution of physiological saline while hematocrits and red blood cell counts were again performed by methods that have been previously described. The blood was obtained from the tip of the tail in the same manner as previously mentioned.

Volume Determination

Fat and extraneous tissue was removed and the blood vessels were cauterized using a common pencil type soldering iron with a fine tip. A vernier caliper was used to measure the length, width of convex surface and the height of the organ. The latter two were measured from the center of the spleen. All measurements were made in triplicate and the mean was recorded to the nearest 0.1 mm. Volumes were determined by a method based upon Archimedes' principle employing the following formula (Anderson and Medin, 1962):

$$\text{Vol. in cc} = \frac{\text{Gland + Apparatus}}{\text{Wt in air-wt in } H_2O} - \frac{\text{Apparatus}}{\text{Wt in air-wt in } H_2O}$$

Fixation

The entire spleen was placed in 10 per cent neutral formalin solution and removed after 30 minutes had elapsed. It was necessary to do this to prevent excessive blood loss when cutting the organ into smaller 5 mm pieces. One-half of the smaller pieces were then fixed in 10 per cent neutral formalin and one-half in Carnoy's fluid for the recommended times (Preece, 1959).

Dehydration, Clearing and Paraffin Embedding

That tissue fixed in 10 per cent neutral formalin was dehydrated through a graded series of alcohols and cleared in cedar wood oil (Fisher Scientific Co.). The tissue fixed in Carnoy's fluid was dehydrated in absolute alcohol and cleared in chloroform. All material was embedded in paraffin and the tissue was oriented in such a manner that when it was sectioned one-half of the sections would be viewed in longitudinal section and one-half the tissue viewed in cross-section. Sections were cut 9 micra in thickness.

Staining

Sections of tissue fixed in both fixatives were stained using the following stains: Weigert's iron hematoxylin and eosin, Masson's trichrome stain (Goldner-Foot modification), and periodic acid Schiff reaction (McManus).

Methods used to make Observations

1. Capsule thickness

With the aid of an ocular micrometer previously calibrated using a stage micrometer #58810 (American Optical Co.), a minimum of six capsular measurements were recorded per spleen, three from a cross-section and three from a longitudinal section. The cross-sections were oriented in such a way that the hilus faced the clock position of nine. Measurements were taken from positions one, four, and eight. On the longitudinal sections two measurements were taken, one at each

end of one side, and one measurement was recorded from the center
of the opposite side. If the capsule at the above-mentioned
locations appeared to be either a tangential cut or a trabecula was
at that location, then the measurement was recorded at the nearest
point where these conditions did not exist. All capsular measurements
were taken using a 43 power objective and 15 power ocular.

Sinus Measurements

A minimum of 12 measurements were recorded from each spleen,
six from longitudinal sections and six from cross-sections. All
measurements were recorded from locations where the sinus appeared
to be entering a red pulp collecting vein, and measurements were
recorded using a 43 power objective and a 15 power ocular.

Marginal Zone and White Pulp Nodule Measurements

A minimum of six measurements, three from longitudinal sections
and three from cross-sections were recorded. Nodules were selected
that appeared to be circular and the radius was determined. From the
same nodule the marginal zone was measured by simply continuing the
line from the nodule and extending it to the observer's left so that
the measurement was made from the marginal sinus to the edge of the
marginal zone. Calculations were made to determine what per cent
of the line was occupied by the marginal zone and what per cent
was occupied by the white pulp nodule. All measurements were made
using a 10 power objective and a 15 power ocular.

Density of Erythrocytes in Marginal Zone

A minimum of 16 different counts were recorded for each spleen, eight from cross-sections and eight from longitudinal sections. With the aid of an ocular grid the number of red blood cells occupying one square of the grid when it was located at the clock positions 12, 3, 6, and 9, was counted and the mean was determined for the animal.

CHAPTER III

OBSERVATIONS

Introduction

The effects of numerous drugs and anesthetics upon the
spleen in respect to contraction and dilatation are well-known
in man and dogs. The anesthetics and drugs used in this study
which resulted in relaxation of the capsular and trabecular
smooth muscle, thus allowing dilatation, were sodium Amytal and
the alpha-adrenergic blocking agent Dibenzyline·HCl. The drugs
and anesthetics used, which resulted in splenic contraction, were
epinephrine, ether, chloroform, and sodium thiopental. The effects
produced by Penthrane and chloral hydrate appear to be similar
with respect to the spleen to those of the known constricting
anesthetics.

Capsular Measurements

Individual capsular measurements, excluding the pilot groups of
animals AI and AII, ranged from a minimum of 3.6 micra to a maximum
of 14.4 micra. Subdivision BI animals, which received only an
anesthetic that produces splenic dilatation, capsular thickness
averaged 7.41 micra. Subdivision BV animals, which received only
an anesthetic that produces splenic contraction, the capsule averaged

11.4 micra. In the remaining subdivisions capsule thickness ranged
from 9.01 micra to 11.2 micra (See Table 2, Selected Morphological
Parameters of the Rat Spleen with Various Treatments).

Sinus Measurements

Individual sinus measurements, excluding the pilot animal
groups, ranged from a minimum of 4.8 micra to a maximum of 24 micra.
Subdivision BI animals (dilatated spleens) sinus measurements averaged
12.1 micra while those animals of subdivision BVI, (contracted
spleens) sinus measurements averaged 8.73 micra. The remainder of the
subdivisions varied from averages of 9.6 micra to 9.19 micra (See Table
2).

White Pulp Nodule and Marginal Zone Measurements

The percentage of the white pulp nodule that occupied a linear
line that extended from the radius of the nodule and continued through
the thickness of the marginal zone excluding the diameter of the
marginal sinus (Figure 1) ranged from a minimum of 51.59 per cent
to a maximum of 72.34 per cent. In subdivision BI animals dilatated
spleens, the white pulp nodule occupied an average of 58.80 per cent
while those of subdivision BII, contracted spleen, occupied an
average of 64.79 per cent. The white pulp nodules of the remaining
subdivisions ranged from an average of 61.39 per cent to 64.88 per
cent. The greatest difference in the percentages seemed to result
from the increase and decrease of the marginal zone measurements
(See Table 2).

TABLE 2

SELECTED MORPHOLOGICAL PARAMETERS OF THE
RAT SPLEEN WITH VARIOUS TREATMENTS

Subdivision[a] and Treatment Received	Anesthetic Drug + Drug				Anesthetic Only			
	A-I Pento-thal + Epine-phrine	A-II Sodium Amytal + Dibenzy-line·HCl	B-I Sodium Amytal	B-II Chloral Hydrate	B-III Pento-thal	B-IV Penth-rane	B-V Ethyl Ether	B-VI Chloro-form
Capsule Thickness (in microns)								
Average[b]	12.58	8.30	7.41	9.01	11.20	9.62	11.40	10.70
Range: high	19.20	12.00	10.80	14.40	12.00	14.40	14.40	14.40
Range: low	6.80	4.80	3.60	4.30	7.20	6.00	8.40	7.20
Number of measurements	42 ea.	42 ea.	60 ea.	72 ea.	12 ea.	42 ea.	30 ea.	18 ea.
Number of animals	7 ea.	7 ea.	10 ea.	12 ea.	2 ea.	7 ea.	5 ea.	3 ea.
Sinus Diameter (in microns)								
Average[b]	11.08	12.24	12.10	9.19	9.33	9.60	9.55	8.73
Range: high	16.80	16.80	19.20	16.80	16.80	24.00	14.40	15.60
Range: low	4.80	4.80	4.80	4.80	4.80	4.80	4.80	4.80
Number of measurements	60 ea.	60 ea.	120 ea.	155 ea.	136 ea.	107 ea.	60 ea.	44 ea.
White Pulp Nodule and Marginal Measurements								
Average $\frac{WPN}{WPN+MZ-MS}$ (100)	61.24%	55.62%	58.80%	63.80%	64.79%	61.39%	64.98%	62.03%
Range: high	68.33%	62.29%	66.41%	72.34%	66.17%	65.10%	71.25%	67.80%

24

TABLE 2--Continued

| Subdivision[a] and Treatment Received | Anesthetic Drug + Drug | | Anesthetic Only | | | | | |
	A-I Pento-thal + Epine-phrine	A-II Sodium Amytal + Dibenzy-line·HCl	B-I Sodium Amytal	B-II Chloral Hydrate	B-III Pento-thal	B-IV Penth-rane	B-V Ethyl Ether	B-VI Chloro-form
White Pulp Nodule and Range: low	53.15%	47.79%	51.59%	58.78%	61.22%	53.80%	60.15%	59.50%
Average[b] Radius WPN[c] (in microns)	126	102.8	131.6	136.8	130.7	127.7	133.8	122.7
Average[b] Thickness MZ (in microns)	79.4	82.0	92.2	78.2	71.0	80.3	72.1	75.1
Number of measurements	30 ea.	30 ea.	60 ea.	77 ea.	15 ea.	54 ea.	30 ea.	39 ea.

[a]Subdivisions A-I and B-II received an anesthetic that produced splenic dilatation while subdivisions A-II, B-I, B-III, B-IV, B-V, and B-VI received an anesthetic that produced contraction.

[b]Difference between the means of B-I and the means of the remaining B subdivisions were highly significant (P=.01) and there was no significance (P=.05) among the contracting anesthetics B-II-B-VI.

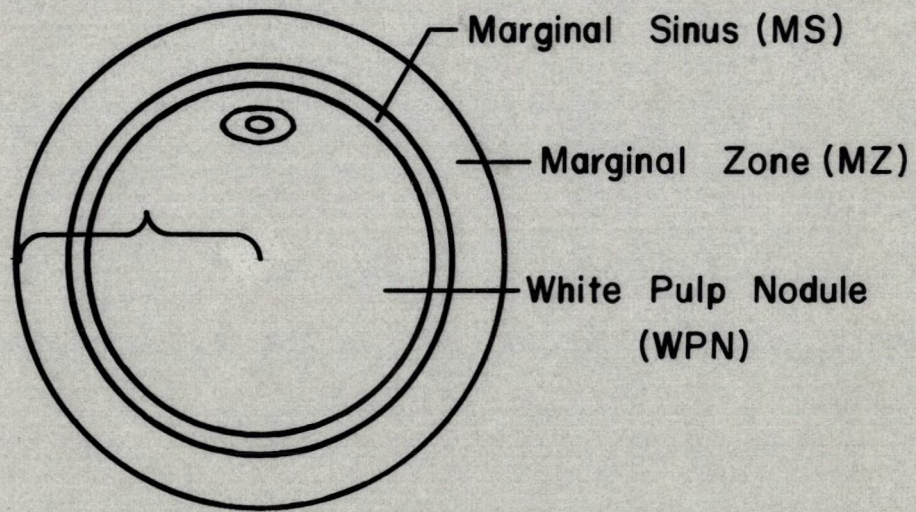[c]WPN = White Pulp Nodule   MZ = Marginal Zone   MS = Marginal Sinus

Marginal Sinus (MS)

Marginal Zone (MZ)

White Pulp Nodule (WPN)

Fig. I ... Percentage WPN = $\dfrac{\text{WPN}}{\text{WPN} + \text{MZ} - \text{MS}}$ (100)

Density of Erythrocytes in Marginal Zone

The average number of erythrocytes, per unit, in the marginal zone of subdivision AI animals, contracted spleen, was 7.81 RBC/unit while the average found in AII subdivision animals, dilatated spleen, was 24.04 RBC/unit (See Table 3 Marginal Zone Erythrocyte Density).

TABLE 3

MARGINAL ZONE ERYTHROCYTE DENSITY

Subdivisions	AI Pentothal + Epinephrine		AII Sodium Amytal + Dibenzyline°HCl	
Average RBC/Unit	7.81	each	24.04	each
Range: High	19	each	40	each
Range: Low	0	each	3	each
Number of Measurements	112	each	112	each
Number of Animals	7	each	7	each

Gross Physical Changes

Examples of gross physical differences between a contracted and dilatated spleen can be observed by comparing the data of subdivision AI and AII (See Table 4 Selected Gross Physical and Calculated Measurements of the Rat Spleen Treated with Various Anesthetics and Drugs). The animals of these two groups were nearly identical in both age and weights. The best examples of the effects

TABLE 4

SELECTED GROSS PHYSICAL AND CALCULATED MEASUREMENTS OF THE RAT SPLEEN TREATED WITH VARIOUS ANESTHETICS AND DRUGS

Subdivisions and Treatment Received	A-I Pento-thal + Epine-phrine	A-II[b] Sodium Amytal + Dibenzy-line·HCl	B-I[b] Sodium Amytal	B-II Chloral Hydrate	B-III Pento-thal	B-IV Penth-rane	B-V Ethyl Ether	B-VI Chloro-form
Average weight in grams								
Total Spleens	0.6653	0.9207	1.0640	0.8497	1.1604	0.9537	0.9537	0.8295
Male Spleens	0.7000	0.9696	1.1671	0.9900	1.1604	1.025	1.1680	1.2000
Female Spleens	0.6393	0.8718	0.9609	0.7328		0.9002	0.6939	0.6443
Spleen Per cent of body wt.								
Total animals	0.20%	0.27%	0.42%	0.31%	0.35%	0.33%	0.25%	0.30%
Male Animals	0.18%	0.25%	0.43%	0.31%	0.34%	0.28%	0.29%	0.38%
Female Animals	0.22%	0.30%	0.40%	0.30%		0.36%	0.22%	0.25%
Calculated organ volume[a] in milliliter	0.6158	0.8684	0.9769	0.7199				
Average length Spleen in centimeters								
Total	3.62	3.79	4.18	3.92	4.54	3.98	4.05	4.00
Male	3.61	3.89	4.28	4.13	4.54	3.89	4.53	4.55
Female	3.63	3.70	4.08	3.73		4.05	3.73	3.73
Average width convex								
surface-Total Spleens	0.89	0.98	1.00	0.95	1.05	0.95	0.98	0.92
(in centimeters)-Male	0.90	0.97	1.00	0.98	1.05	0.99	0.96	0.98
Female	0.89	0.98	0.99	0.91		0.93	0.91	0.89

TABLE 4--Continued

Subdivisions and Treatment Received	A-I Pento-thal + Epine-phrine	A-II[b] Sodium Amytal + Dibenzy-line·HCl	B-I[b] Sodium Amytal	B-II Chloral Hydrate	B-III Pento-thal	B-IV Penth-rane	B-V Ethyl Ether	B-VI Chloro-form
Average height in centimeters								
Total	0.48	0.53	0.50	0.48	0.47	0.50	0.50	0.46
Male	0.49	0.55	0.50	0.58	0.47	0.50	0.54	0.50
Female	0.48	0.50	0.50	0.45		0.50	0.48	0.43
Number animals								
Average weight in grams								
Total	334	337	251	279	327	296	310	269
Male	377	385	267	325	327	359	400	310
Female	290	290	235	250		250	250	249

[a]It was found after 47 measurements that organ volume could be determined by using the following ratio

$$\frac{1 \text{ gm. Spleen weight}}{\text{weight spleen}} . \frac{.9\text{ml.}}{x\text{ml.}} \quad \text{accuracy answer} \pm .056 \text{ ml.}$$

[b]These subdivisioned animals' spleens dilated

of anesthetics alone upon the physical measurements of the spleen

may be observed by comparing subdivisions BI and BII as the animals

of these subdivisions were approximately of the same ages and

weights.

Blood Values Before and After Treatment

The blood values obtained prior to and after treatment are to

be found on Table 5, Blood Values of Rats Before and after Administration

of various Drugs and Anesthetics.

TABLE 5

BLOOD VALUES OF RATS BEFORE AND AFTER ADMINISTRATION
OF VARIOUS DRUGS AND ANESTHETICS

Subdivision and Treatment Received	A-I Pento-thal + Epine-phrine	A-II Sodium Amytal + Dibenzy-line·HCl	B-I Sodium Amytal	B-II Chloral Hydrate	B-III Pento-thal	B-IV Penth-rane	B-V Ethyl Ether	B-VI Chloro-form
Average Hematocrit prior to treatment	48.62%	50.0%	47.5%	46.8%	48.0%	48.0%	48.0%	49.0%
Average Hematocrit after treatment	50.6%	47.0%	42.4%	45.1%	47.0%	44.6%	44.8%	45.0%
Average Change in Hematocrit	+1.98%	-3.0%	-5.1%	-1.7%	-1.0%	-3.5%	-3.2%	-4.0%
Average RBC count in millions	9.01	10.98	8.53	8.90		9.46	9.7	10.6

CHAPTER IV

DISCUSSION

Macroscopic Changes

Linear changes cannot be seen with the naked eye, but they can be appraised by carefully measuring the organ (See Table 4). By comparing subdivision BI animals with subdivision BII animals, it may be noted that the dilatated spleens of the BI animals average 0.26 cm greater length, 0.05 cm greater width, and 0.02 cm greater thickness than the contracted spleens of the animals in subdivision BII. The animals used in the pilot study, subdivisions AI and AII, which averaged only 3 grams difference in total body weight also present a similar picture (See Table 4). The greatest difference in the physical macroscopic measurements are noted with respect to the length of the spleen. This seems to imply that when a rat's spleen dilates it does so in a manner that is similar to the changes one observes when he inflates a child's sausage-type balloon.

Comparing the dilatated spleens of subdivision BI animals with the remaining subdivisions of the B group (See Table 4) it may be noted that in all cases the subdivision BI animals' spleens weighed more than those found in the remaining subdivisions. It should also be noted that this subdivision contains the animals which possessed the least average body weight.

After calculating the total organ volume of 37 rats' spleens
using a method based upon Archimedes' principle (Anderson and Medin,
1962), it was found that one can estimate the volume of the rat's
spleen within plus or minus 0.05 ml by simply setting up a ratio in
which 1 gram of splenic weight equals 0.9 ml. It should be noted
in relation to volume that the spleens of all of the subdivisions of
the B group except BI possesses a smaller volume than the dilatated
spleens of the latter subdivision.

One observation with respect to the spleen's physical
macroscopic measurements that is not related to the effects of
anesthetics, but appears to be related to the age of the animal,
is the overall size difference in the spleens of the older animals
compared to the younger animals. By comparing the two major groups
of animals, A and B, it may be noted that the younger animals possess
larger spleens than those found in the older animals. Blaustein
and Diggs (1963) stated that the architecture of an atrophied spleen
is normal, but the total mass of splenic tissue is reduced in older
human individuals. It seems possible that a similar situation also
exists in the rat's spleen.

Microscopic Observations

As one might expect, the capsule of the contracted spleen
is thicker than that found in the dilatated organ. In the experimental
animals, B group subdivisions, the capsule of the dilatated spleen
averaged 7.41 micra thick while the contracted splenic capsules of
the remaining subdivisions averaged between 9.01 to 11.7 micra

(See Table 2).   In the older animals of A group subdivisions splenic

capsules show similar changes, but the capsules of the spleens are

thicker. This might indicate that the capsule of the rat's spleen

increases in thickness with age.

Comparison of the various experimental animal subdivisions show

that in the dilatated spleen, the sinus diameter is increased and

conversely it decreases in the contracted spleen (Table 2).

An inverse relationship seems to exist between the marginal

zone and the white pulp nodule. If one draws a line from the center

of the nodule and extends that line through the marginal zone (See

Figure 1), it is noted that in the dilatated spleen the white pulp

nodule occupies 58.8 per cent of the line with the marginal zone

occupying the remainder. However, in the contracted spleen the white

pulp nodule occupies a greater percentage of the line (See Table 2).

In the experimental subdivisions of animals with contracted spleens

the increase of the white pulp nodules ranges from a minimum of 2.59

per cent to 6.18 per cent. Three possible explanations for this change

are: (1) the marginal zone decreases in size with splenic contraction,

(2) the white pulp nodule increases in size when the spleen contracts,

and (3) both the marginal zone and the white pulp nodule change in

size, one becoming larger while the other becomes smaller. The

evidence seems to indicate that the marginal zone varies in thickness

with splenic contraction and dilatation.

A proposed mechanism to explain how the marginal zone could

increase in size during dilatation and decrease during contraction

is as follows. During splenic dilatation, a greater amount of blood flows from the central artery through the follicular capillaries and enters the marginal sinus. The erythrocytes then enter the meshes of the marginal zone in the manner described by Snook (1964) and migrate through the region. The movement of the erythrocytes through the zone increases the erythrocyte concentration in the marginal zone and results in the zone's increase in size, thus changing the ratio of white pulp nodule to marginal zone. During contraction the erythrocytes are literally squeezed out of the zone which results in its decrease in size. To substantiate the above theory, it may be noted that the number of erythrocytes in the marginal zone of the rat increase three-fold in the dilatated spleen with respect to the same region in the contracted organ (See Table 3 and Plates 1 and 2). This response might be due to the anesthetic's effect upon the nodular and penicelli arteries.

Anesthetics

An analysis of the effects of anesthetics on the spleen of the rat indicates that sodium amytal results in splenic dilatation. Chloral hydrate, methoxyflurane, thiopental, ether and chloroform all produce splenic contraction. Chloral hydrate and methoxyflurane, while they produce splenic contractions, appear to do so to a lesser degree than the remaining three drugs of the contracting group. It must be kept in mind that these changes only describe the effects of the anesthetics named when they are administered by the same route and at the same strength used in this study. The author feels that

PLATE I

Fig. 2.--<u>Contracted Rat Spleen, Pentothal anesthesia</u>. Relatively few erythrocytes may be seen in the marginal zone (MZ) and the marginal sinus (MS). WPN, white pulp nodule. Masson's trichrome stain. 378X.

Fig. 3.--<u>Dilatated Rat Spleen, sodium Amytal anesthesia</u>. The marginal zone (MZ) and the marginal sinus (MS) are engorged with erythrocytes. WPN, white pulp nodule. Masson's trichrome stain. 378X.
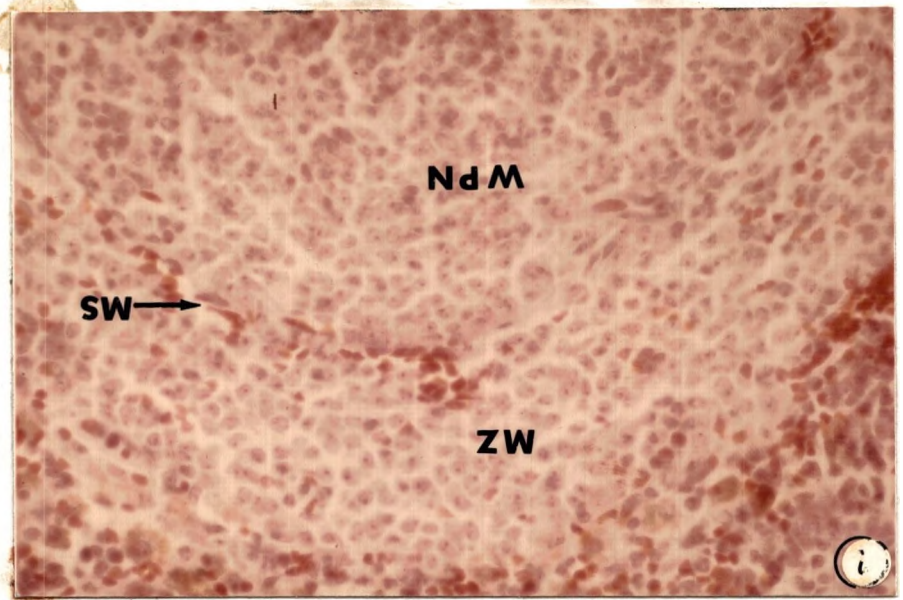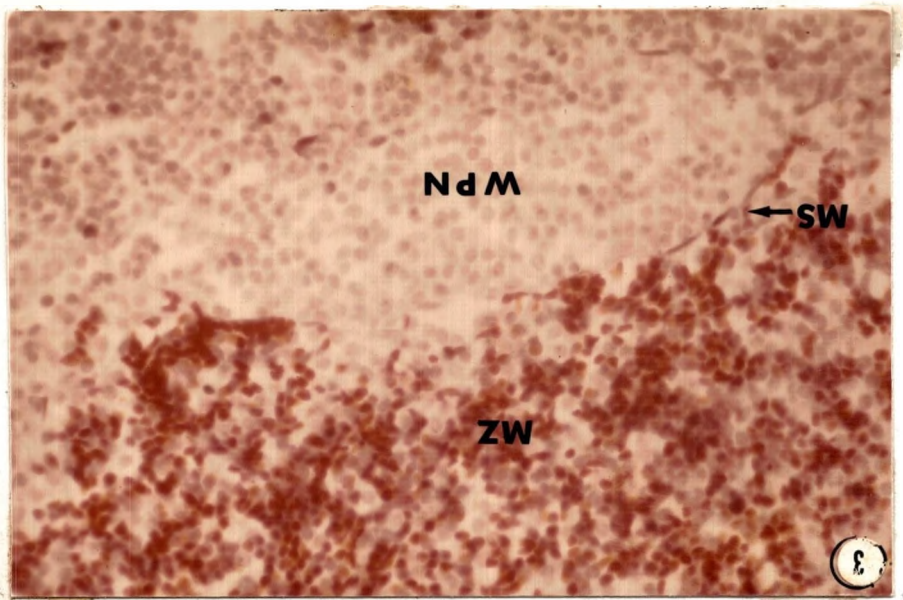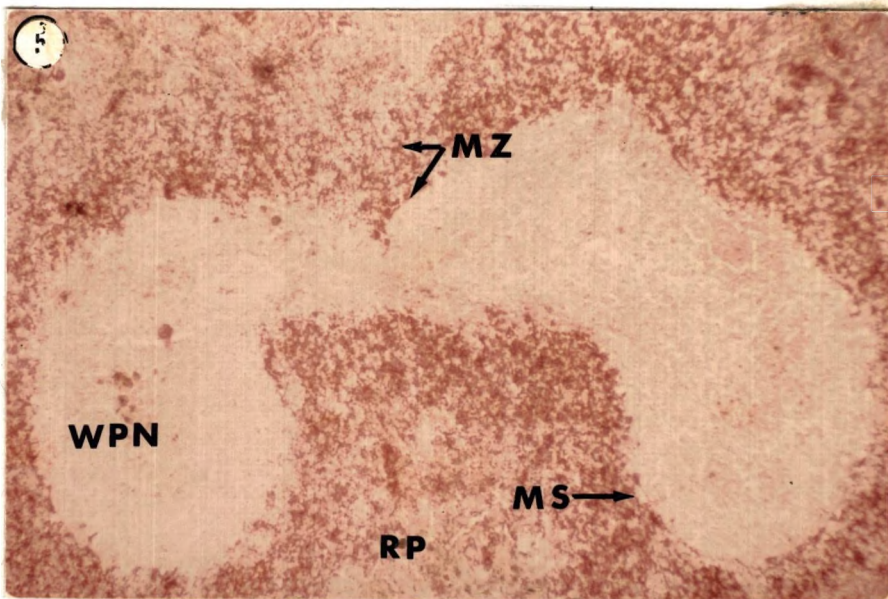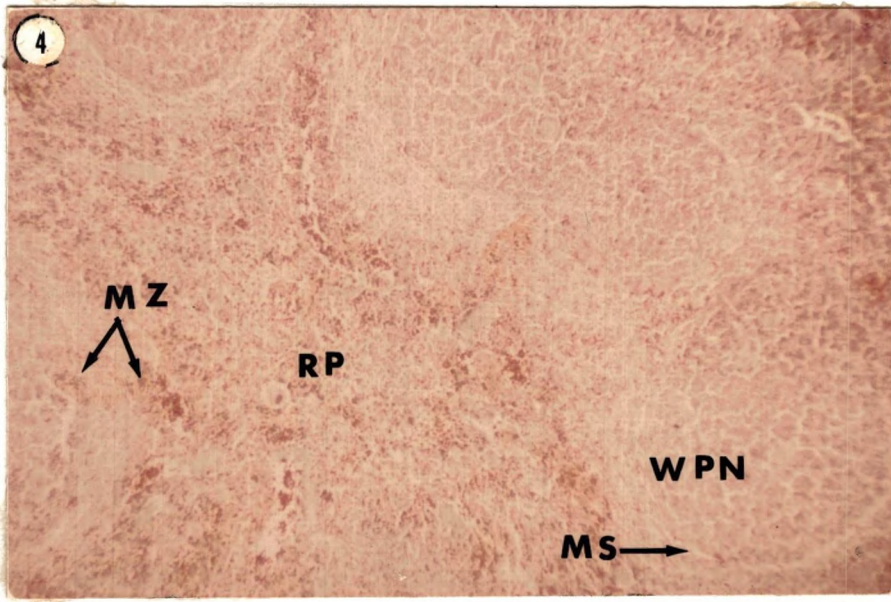
PLATE I

PLATE II

Fig. 4.--<u>Contracted Rat Spleen, Pentothal anesthesia</u>. One may observe in this low power photomicrograph that few erythrocytes occupy the marginal zone (MZ) or the marginal sinus (MS), but are primarily confined to the red pulp (RP). WPN, white pulp nodule. Masson's trichrome stain. 84X.

Fig. 5.--<u>Dilatated Rat Spleen, sodium Amytal anesthesia</u>. This low power photomicrograph illustrates how the white pulp nodule (WPN) is surrounded by erythrocytes that completely fill the marginal sinus (MS), the marginal zone (MZ), RP, red pulp. Masson's trichrome stain. 84X.

PLATE II

the strength and the route administered might produce different results with the same anesthetic if either is varied. One basis for this opinion is the pH of the anesthetic. For example, the chloral hydrate used in this study was found to have a pH of 5. When this is injected into the abdominal cavity, the acidity could produce pain which might result in splenic contraction. If this same anesthetic was administered orally, it would be doubtful that the pH would have any effect upon the spleen. The sodium Amytal used in this study had a pH of 9.2 and the thiopental a pH of 10.25.

Gruber (1941) noted that thiopental did not produce true anesthesia in rats but instead produced a state of deep hypnosis. This effect was found in the animals used in this study. Gruber further stated that in order to produce deep hypnosis by intraperitoneal injection, it was necessary to use the drug in such strength that it resulted in the death of 50 per cent of the animals treated (LD_{50}). The present author did not find this to be the case in either this study or when doing a preliminary study. Approximately 25 animals received this drug and not a single animal died as a result of overdose of thiopental. Barnes and Eltherington (1964) reported that an intraperitoneal injection of 120mg/kg results in a LD_{50} in laboratory rats. The author found that 55mg/kg of thiopental was sufficient to produce deep hypnosis in the rat when given intraperitoneally. It was noted in our preliminary study of the anesthetics used that if one failed to fast the animals before the administration of anesthetics, depending upon the animal's individual eating habits,

a wide range of anesthetic dosages were required to produce hypnosis. It is possible that Gruber failed to fast his animals in attempting to find a standard dosage which would produce deep hypnosis in all the animals, thus obtaining LD_{50}.

Blood Values

Originally the author felt that splenic dilatation and contraction could be monitored by simply observing the change in the hematocrit and the erythrocyte count before and after the administration of the anesthetics. This idea was found to be erroneus as such factors as the change in plasma volume were not taken into consideration. It may be noted that the average hematocrit prior to anesthesia was 47.6 per cent (See Table 5), and after anesthetics were given, the hematocrit decreased 5.1 per cent in subdivision BI and 1 per cent in subdivision BIII. The average decrease in the animals which received anesthetics that produce splenic contraction was 2.8 per cent. The erythrocyte count of the rats before an anesthetic was administered was 9.18 million per cubic millimeter. This contrasts with the values reported by Andrew (1965) of 6.18 mil/mm^3 Creshoff, et al. (1962) of 9.35 mil/mm^3 and those found in the Handbook of Biological Data (1965) of 8.19 mil/mm^3. After the administration of anesthetics which produce splenic contraction, the average count was 8.39 mil/mm^3.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to attempt to determine what
histological effects selected anesthetics have upon the rat's spleen
before attempting a transillumination study of the microcirculation
of that organ. It was felt that without some knowledge of an
anesthetic's effect upon the spleen it would be difficult to interpret
accurately splenic circulation.

It was found that in the contracted spleen the organ decreased
in size, especially in length; the capsule was thicker and the red
pulp sinuses were smaller in diameter. It was also noted that the
ratio of the white pulp nodule in respect to the marginal zone varied
with contraction and dilatation of the spleen. In the contracted
spleen the marginal zone decreased in size and the number of
erythrocytes within the zone. In the dilatated spleen the marginal
zone increased in size with respect to the white pulp nodule, and
the marginal zone appeared flooded with erythrocytes.

Sodium Amytal administered intraperitoneally results in splenic
dilatation. Chloral hydrate and sodium thiopental, given intraperitoneally,
resulted in splenic contraction. Penthrane, ether, and chloroform
administered by inhalation technique also resulted in splenic contraction.

Penthrane and chloral hydrate while producing splenic contraction appear to do so to a lesser extent than the other contracting anesthetics used.

The author feels that it might be best to avoid using intraperitoneal injections to administer anesthetics due to the possible effects of the pH osmoiality, and the effect of the extra volume in the peritoneal cavity. Again it must be stressed that the results apply only to the anesthetics with identical strengths and administered by the same route as used in this study.

To investigate the microcirculation through the marginal zone, sodium Amytal appears to be the best anesthetic because it was seen during the use of this anesthetic that the marginal zone was richly supplied with erythrocytes.

An interesting possibility exists in that the contracting anesthetic drugs might "shut down" the marginal zone circulation, but that the penicillar system might still be fully operative. By using both types of anesthetic drugs one might determine if there are two circulations acting within the spleen, penicillar-sinus system versus a white pulp marginal zone system.

It is also possible that one could observe the effects of splenic contraction and dilatation in the same animal using the same field. If the investigator first administered an anesthetic that resulted in splenic dilatation, sodium Amytal, and later gave an intravenous injection of epinephrine, it should be possible to note the changes as the spleen went from a dilatated to a contracted and possibly back to the dilatated condition.

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