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A PATHOPHYSIOLOGICAL STUDY OF THE VISCERAL ORGANISM

A Thesis
Presented to the Faculty of the Graduate School of the
University of Toronto in Partial Fulfilment of the
Requirements for the Degree of

Master of Veterinary Science

by

David Cartwright Secord

May, 1961
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ABSTRACT

A pathophysiological study of the visceral organism.

A technique is described using the cat or dog for the isolation of the thoracic and abdominal viscera with an intact functioning circulatory system (visceral organism).

The preparation requires artificial respiration and is attached to a blood reservoir while maintained in a constant temperature bath.

In a series of preparations maintained at a mean blood pressure range of 40-45 mm. Hg. the lifespan was $6.3 \pm 1.3$ hours. The gross and microscopic appearance of the viscera at necropsy were similar to the pathological changes reported in a variety of intact animals exposed to prolonged hypotension. Bacteriological examination showed a marked proliferation of common pathogens in the gastrointestinal tract and their invasion of the tissues. Preparations pretreated with antibiotics had a lifespan of $17 \pm 5.8$ hours but showed greater tissue damage terminally.

Increasing the mean blood pressure range to 80-85 mm. Hg. prevented the tissue damage but contributed to the production of pulmonary edema and death of the visceral organisms at $7.7 \pm 1.8$ hours.

The use of antibiotic pretreatment failed to alter the end results.

A marked leukopenia characterized by a neutropenia was
observed to occur rapidly in all preparations. The leukopenia was maintained at a level of 1500 to 2500 W.B.C. per cu.mm. from the first hour after the preparation was made until its death. A rising blood urea nitrogen was observed as was a hyperglycemia followed by a slow decline in blood glucose levels to values approaching normal values at the time of death.
I INTRODUCTION

In 1913 Carrel successfully isolated the intact, functional thoracic and abdominal organs of the dog and the cat. This symbiosis of organs was called a visceral organism. The lifespan of the preparations varied from three to 12 hours during which a progressive degeneration of the viscera occurred. Carrel felt that the death of the visceral organism was due to septicemia caused by bacterial invasion from the portal blood and peritonitis.

In 1930 Markowitz and Essex repeated the isolation of the visceral organism and reported a similar lifespan, degree of activity and syndrome of visceral degeneration. The most significant alteration observed was the marked autolysis of the gastrointestinal tract. Markowitz, Archibald and Downie (1959) suggested that the protective mechanism which normally prevents the digestive enzymes from attacking the intestinal mucosa in the intact living animal was lacking in the visceral organism and thus gastrointestinal autolysis occurred. They further suggested that this protective mechanism may possibly be associated with either an enzyme in the blood or the leukocytes.

Studies of the maintenance of isolated organs by Carrel and Lindbergh (1938) have demonstrated that the most important factor required for successful preparations was to prevent bacterial invasion and proliferation in the isolated tissues. In addition, studies of traumatic shock by Fine (1954) have demonstrated that antibiotics
supported the experimental animals by preventing bacterial proliferation and invasion of the tissues. Similar gastrointestinal lesions, believed to be of bacterial origin, have been reported in many conditions associated with agranulocytosis and since the visceral organism is lacking bone marrow, the disappearance of granulocytes may be a contributing factor. Consequently, antibiotic pre-treatment of the visceral organism may enhance the lifespan of the preparations and possibly alter the formation of the gastrointestinal erosions.

Similar gastrointestinal lesions have been reported in a variety of conditions in intact animals that resulted in prolonged hypotension and irreversible shock (Wiggers, 1950). The use of constant pressure transfusions in the visceral organism may eliminate periods of ischemia and anoxia produced by other methods of transfusion. In addition, aortic transfusion will allow the establishment of a relatively even blood pressure and observations of the effect on the visceral organism of alterations of blood pressure.

The visceral organism may be considered as the complement of the eviscerated animal. Consequently this complex of isolated organs may yield information that would enhance the visceral organism as a tool for the physiologist and pharmacologist.
II HISTORICAL REVIEW

The concept of maintaining a portion of the body alive by artificial perfusion has intrigued man for many years. LeGallois in 1912 wrote "If one could substitute for the heart a kind of injection of arterial blood, either natural or artificially made, one would succeed easily in maintaining alive indefinitely any part of the body whatsoever." The earliest attempt was recorded by Kay in 1828, whereby the irritability of dying muscle was restored by artificial perfusion with blood. In 1858, Brown-Sequard forced oxygenated blood through the arteries by means of a syringe. In this manner he observed the return of certain cerebral functions in the isolated head. An apparatus whereby blood could be forced under constant pressure from a reservoir into the blood vessels was described in 1868 by Ludwig and Schmidt. Salveoli in 1880 isolated strips of rabbit and dog intestine and observed mucosal edema and sloughing following perfusion. Martin in 1881 isolated the heart and lungs of a dog in situ and mentioned the remarkable ability of the heart to withstand manipulation. An artificial lung which aerated the perfusate without interrupting the flow of blood was reported by von Fry and Gruber in 1885. In 1895, a method to aerate blood perfused through a lung maintained by artificial respiration was described by Jacobj. Bainbridge and Evans used the heart-lung preparation to perfuse the kidneys in 1914. At about this time, Carrel attempted to maintain a symbiosis of isolated organs. This
preparation, the visceral organism, consisted of the isolated heart, lungs and abdominal organs. The beating heart maintained the blood pressure and flow. The lungs were artificially inflated. Cardiac failure followed an average lifespan of ten hours. Carrel concluded because of the cardiac failure that the only practical way to maintain isolated organs was to use a system that included an artificial circulation.

In 1920 Belt, Smith and Whipple published a paper which summarized the work on whole organ perfusion. They pointed out that in no case did any technic maintain an organ in a viable state for more than a few hours and that all isolated organs were observed to undergo a process of destruction. They were able to demonstrate with a variety of preparations that "the use of physiological saline, Locke's solution, or various modified solutions with or without red blood corpuscles does not permit a physiological perfusion of organs."

In 1929, Markowitz and Essex repeated the preparation of the visceral organism and varied the technic of maintenance. They reported a similar syndrome of degeneration, a similar lifespan of the preparation and stressed the appearance of gastrointestinal autolysis at necropsy examination. They then used this preparation the same year to demonstrate the peripheral effects of rattlesnake venom (Essex and Markowitz, 1930).

In 1938 Carrel and Lindbergh described their results of over 25 years' experience in the field of whole organ culture. The Lindbergh pump, the preparation of the sterile tissue, the use and
sterilization of the glassware, the different perfusion solutions used and the results obtained by the perfusion of different organs, were fully described. Unfortunately the perfusion apparatus was of such a size that only small organs such as a cat thyroid gland or functional portions of larger organs were studied.

Work in the field of whole organ perfusion continued with progress occurring in the development of newer mechanical perfusion equipment as summarized by Karlson, Dennis, Sanderson and Culmer in 1951.

In recent years a variety of organs has been perfused successfully and has provided useful information. Hyman and Chambers in 1943 demonstrated the ability of adrenal cortical steroids to reduce the rate of edema formation in perfused frog's legs. In 1951, Brauer, Pessotti and Pizzolato were able to maintain a perfused rat liver for 25 hours. The histological appearance of the cells was unchanged, bromsulfalein was removed from the blood and excreted in the bile, and the hepatic reticuloendothelial cells were able to remove injected particulate matter. The ability of A.C.T.H. to increase the output of isolated bovine adrenal gland was demonstrated by Ganis, Miller and Axelrod in 1955, using homologous blood as a perfusate. Alexander, Huggett, Nixon and Widdas in 1955 used a perfused sheep placenta to demonstrate that fructose is continually produced by the placenta independent of the level of foetal blood glucose. Lillehei in 1957 demonstrated that when the intestinal tract of dogs was vivipерfused by another animal these dogs
did not develop irreversible shock when subjected to prolonged hypotension by controlled hemorrhage. In 1958 Geiger demonstrated a lifespan of one and a half hours when cats’ brains were perfused in situ with defibrinated ox blood. Yet when liver was included in the circulation the lifespan was four hours.

Within the last decade, technical developments have brought about the widespread use of blood pumps and artificial oxygenators capable of supporting the circulation of an entire animal. This equipment has made possible intracardiac surgery under direct vision and has facilitated studies of the circulation, metabolism and function of the normally innervated heart (Ikens, Everson and Meuller, 1959) and lungs (Daly, Elsden, Hebb, von Ludany and Petrovskaja, 1942) in situ. Recent work in the field of extracorporeal circulation has been reviewed by Cloves (1960).

Technics for the perfusion and growth of individual cells or cell groups have been developed in recent years. The true beginning of tissue culture has been accredited to Harrison in 1907, who explanted a small piece of embryonic frog medulla into a hanging drop of lymph and observed the growth of axons from the surviving cells. Several years later Burrows (1910) demonstrated the advantage of a plasma clot which increased the lifespan of the embryonic tissue and simplified subculture. In 1912 Carrel discovered that embryo extract had a strong growth-promoting effect on certain cells. Subsequently the technic of growing tissue cells in plasma clots, supplemented with embryo extract, in a depression slide became standard practice.
The development of tissue culture in a manner suitable for accurate physiological research was promoted largely by Carrel. In 1923, using rigid aseptic technics, he developed a system for maintaining tissues in a condition of uninterrupted reproduction in a specially designed flask. The tissue was imbedded in plasma bathed by a supernatant fluid which could be changed as frequently as the metabolic needs of the tissue required. In this manner Carrel was able to maintain a strain of cells in active multiplication for 34 years (Paul, 1959). Recently Earle (1954) has developed elaborate modifications of the Carrel flask method for the maintenance of large numbers of strains of normal and malignant cells in continuous culture.

An entirely different approach to the maintenance of tissues in vitro was initiated by Fell in 1929 for the maintenance of portions of intact tissues. The tissue was grown on the surface of a plasma clot in a watch-glass, placed on moist cotton inside a Petri plate and the growing tissue was transferred to fresh media as required. Trowell in 1959 reported that a wide variety of tissues have been maintained for six to nine days, in this manner. An exhaustive review of the literature of tissue culture research was made by Murray and Kopek in 1953.

The obvious difficulties involved in maintaining mammalian tissue in an artificial state is readily demonstrated by a comparison of the lifespan from a proliferating strain of cells through to a complete symbiosis of organs. Using Carrel's method individual
cell strains have been maintained for over 34 years. Trowell (1959) has observed a lifespan of six to nine days with portions of various organs. Brauer (1951) has demonstrated the isolation of a complete liver for 25 hours, while Markowitz and Essex (1930) have maintained the intact thoracic and abdominal viscera for 12 hours. Cloves (1961) has reported that under optimum conditions of whole body perfusion only 50 per cent of the subjects have survived after three hours of perfusion. From the evidence uncovered by these workers it is apparent that as the number of cells and organs to be perfused increases and as the number of functions to be performed multiplies the lifespan of the preparation decreases.
III METHODS

1. General Methods and Equipment.

(a) Choice of Subjects.

All subjects used in the production of a visceral organism were selected at random and weighed from three to five kilograms. The cats used were normal, healthy animals on clinical examination, with total leukocyte counts within the normal range. When any gross pathology was evident during surgery or upon the isolation of the viscera, this subject was discarded and the experiment terminated.

No pretreatment was given to any animal prior to surgery except in the groups specifically pretreated with antibiotics.

(b) Blood Collection and Storage.

All blood used for transfusion was collected by cannulation of the carotid artery of normal, healthy cats anesthetized with pentobarbital sodium. The blood was collected into siliconed blood bottles into which 5 mg. of heparin per 100 ml. of blood had been placed. Then an equal volume of Locke’s solution (Todd, Sanford, Wills, 1953) was added and the blood-Locke solution was stored at room temperature for no longer than two hours prior to use. A commercial blood administration set attached to a metal arterial cannula was used for transfusion of the blood-Locke solution.

* Nembutal. Abbott Laboratories, Montreal.
** Empty plasma container, Abbott Laboratories, Montreal.
*** Heparin. Connaught Laboratories, Toronto.
In view of the recognition of definite feline blood groups (Holmes, 1950; Eyquem and Podliachouk, 1954) the sera and red cells of the recipient and the first two prospective donors were cross-matched and any reactors discarded.

(c) General Equipment.

The mineral oil bath was a galvanized metal box 12" long, 9" wide and 5" high, which had been modified by the addition of a slotted clamp at the deep end for the attachment of the tracheal cannula. A metal tube was welded on the wall of the tray beside the clamp for the removal and collection of intestinal fluid and debris.

A perforated metal tray (12" x 9") was placed in the box at an angle of approximately 15 degrees and supported the visceral organism. When the viscera were placed on the tray the angle increased the venous return to the heart.

The mineral oil container was scrubbed with Phisohex* and hot water before and after use.

Immediately prior to use the metal tray was covered with several layers of sterile gauze sponge and the container was filled to within one-half inch of the top with light, white, mineral oil.

The mineral oil was maintained at a temperature of 38 ± 1° C. throughout the experiment with an adjustable heater placed immediately beneath the metal container. The temperature of the mineral oil was measured by means of a mercury laboratory thermometer.

* Phisohex. Winthrop Laboratories, Aurora.
Artificial respiration was maintained during surgery and throughout the experiment at a constant rate of 25 respirations per minute by means of an intermittent positive pressure Harvard pump. The degree of lung expansion was controlled by the use of rubber tubing with an adjustable clamp, attached to one side of the tracheal cannula. The assembled equipment is shown in Figure 1.

(d) Surgical Technic.

The operation was designed for the removal of the intact thoracic and abdominal viscera from an experimental animal. The surgery was performed under aseptic conditions, as rapidly as possible, with minimum loss of blood.

A cat was anesthetized with thiopental sodium* given intravenously to effect, and intrasplenitically thereafter as required.

The animal was placed in the dorsal recumbent position. A roll of paper towels about five centimeters in diameter was placed under the midthoracic region to provide better exposure of the internal thoracic structures. Routine surgical drapes were then applied.

A midline skin incision was made from the level of the episternum to the level of the pubis. Hemostats were applied to all bleeding vessels. The incision was then continued through the linea alba from the episternum to the pubis and the underlying falciiform ligament was severed. The colon was identified, carried

* Pentothal, Abbott Laboratories, Montreal.
Figure 1

A photograph illustrating the basic equipment arrangement for maintaining a visceral organism. From left to right: the blood reservoir, mercury manometer, mineral oil container (diagonal line indicates the slope of inner tray) with heater beneath, collection beaker, and respiratory pump.
into the centre of the operating field and a colectomy performed. The ileo-caeco-colic artery and vein were identified lying within the mesentery of the caecum, in conjunction with the mesenteric lymph nodes. These vessels were isolated and then divided. Routine-silk sutures were tied on all vessels remaining with the viscera and hemostats were applied to the body where necessary. The middle colic and posterior mesenteric vessels were then isolated and sectioned. The avascular portion of the colic mesentery was cut with a pair of scissors. The colon was severed between two pair of forceps at the entrance to the pelvic cavity, and the terminal ileum was severed between a ligature placed proximally and a pair of forceps placed distally. The colon was then removed. The aorta and posterior vena cava were identified about 2 cm. distal to the level of the posterior pole of the left kidney. These vessels were freed of fascia for a distance of one centimeter and divided. The ends of the ligature on the proximal end of the vessels were left ten centimeters long. A ligature was then passed from the area of the aortic-caval ligation to the posterior lateral pole of each kidney and the contained tissues were sectioned. Consequently the right and left ureter and the right and left internal spermatic artery and vein in the male, or the corresponding ovarian vessels in the female, were included in the ligature and severed. The peritoneum was severed at the lateral border of each kidney which were then in turn reflected medially to expose the phrenico-abdominal vessels. These vessels were isolated and sectioned, distal to each adrenal gland. The long
ends of the aortic-caval ligature were grasped and used to retract the aorta and vena cava ventrally in order to expose the underlying lumbar arteries and veins. These vessels were ligated in pairs up to the pillars of the diaphragm. This part of the operation was facilitated by using a Cleveland ligature carrier. Connective tissue surrounding the vessels was included in each ligature. No attempt was made to preserve the cisterna chyli. The abdominal viscera were then replaced in the abdominal cavity.

The abdominal skin incision was extended in an anterior direction along the midline to the level of the larynx. The sternoccephalicus and underlying sternohyoideus muscles were separated in the midline to expose the trachea and associated structures. The left carotid artery was isolated and 15 cc. of the transfusion fluid (blood-Locke’s solution) was injected into the vessel. The trachea was freed of all adjacent tissue, sectioned near the thoracic inlet and a tracheal cannula was inserted and tied in position. The esophagus was isolated and sectioned between ligatures.

The sternum was split longitudinally in the midline with a small pair of bone-cutting forceps and the artificial respiration pump was started. The chest was gently opened and proper lung inflation was obtained by adjusting the outlet from the respiration equipment. The internal thoracic artery and vein were ligated and severed as were the pericardiaphrenico artery and vein. The diaphragm was severed with an electrosurgical scalpel. The incision followed the costal border of the diaphragm from the sternal attachment.
to the pillars of the diaphragm. Stay sutures of No. 3 silk were placed around each half of the split sternum at the eighth intercostal space and the long suture ends were clamped to each side of the surgical table after applying traction to open the chest. This traction, in conjunction with the arched spine caused by the roll of paper towels, was essential for adequate exposure of the dorsal thoracic structures. The vessels at the thoracic inlet were cleared of adjacent connective tissue and a ligature was passed around the left common carotid and subclavian arteries and the left external and internal jugular and subclavian veins. The ligature was tightened and the tissues were severed between a ligature and forceps. This was then repeated on the right side. The trachea and oesophagus were retracted ventrally and the underlying tissues were severed and ligated down to and including the vena azygos. The thoracic viscera were retracted ventrally and to the left and the dorsal intercostal vessels were ligated down to but not including the pillars of the diaphragm. Each pair of vessels were ligated and severed without the use of forceps placed distally. A large pair of forceps were placed across the pillars of the diaphragm, the last remaining tissue joining the viscera to the body and a scalpel was used to sever the tissue on the carcass side of the forceps.

The viscera were then lifted out of the carcass, inverted and placed in a mineral oil bath. The tracheal cannula was placed in a submerged metal holder at the front of the mineral oil bath and the long ends of the aortic-caval ligature were clamped with a pair
of forceps and traction was provided when they were hung over the back wall of the bath. The viscera were now suspended on the sloping metal tray in the bath. The respiratory pump was readjusted to supply adequate ventilation of the lungs. The aorta was isolated posterior to the renal arteries and cannulated for the attachment of the blood administration tubing. The clamp was released from the tubing and arterial transfusion was started.

The diaphragmatic pedicle was ligated and the forceps released. The ileum was cannulated with rubber tubing which was used to collect the intestinal contents. The left common carotid artery was cannulated with a metal cannula attached to a short piece of blind rubber tubing which was subsequently used to collect blood samples.

The viscera were then gently examined, any bleeding points were ligated and the positioning of the viscera within the mineral oil was checked and any obstructions were relieved. A single layer of cotton gauze was placed over the dorsal portion of the lungs to protect and moisten them when they protruded from the mineral oil.

(e) Experimental Recordings.

During the preparation and maintenance of a visceral organism the following observations were recorded.

1. Sex, weight, condition and approximate age of the subject.
2. Room temperature.
3. Anesthetic dosage.
4. Time required for surgery and the condition of the viscera immediately after surgery.
5. The respiratory rate.

6. The volume of transfusion fluid used, the volume of blood lost from the preparation and the volume of fluid lost through the intestinal tract.

7. The lifespan of the preparation.

At regular hourly intervals the following observations were recorded:

1. The temperature of the mineral oil.
2. The heart rate of the visceral organism.
3. The blood pressure.
4. The presence and degree of activity of intestinal motility.
5. The general condition.
6. Any comments regarding significant alterations.

The lifespan of the visceral organism was measured from the time of the isolation of the viscera until the organism was no longer able to maintain the desired blood pressure without excessive blood transfusion. The arterial transfusion was then stopped to avoid overloading the blood vascular system.

(f) Pathological Technics.

After the death of a visceral organism a gross and microscopic pathological examination was made.

Gross changes of the tissues were immediately recorded.

Specimens of the heart (left ventricle), lungs, liver, spleen, kidneys, pancreas, mesenteric lymph node and adrenal gland, were placed in formal saline.
Specimens of the stomach, duodenum, jejunum and ileum were placed in Bouin's solution.

All specimens were sectioned, stained with hematoxylin and eosin, and examined microscopically.

(g) Bacteriological Technics.

A routine series of bacteriological examinations were made following the death of a visceral organism (Dawson and Goldie, 1958). One ml. of gastrointestinal contents was removed, using sterile technic, from the stomach and jejunum. Blood samples (1 ml.) were taken from the left ventricle of the heart and the portal vein. Each specimen was placed in a sterile container and refrigerated for a period not exceeding 24 hours. An inoculum from each specimen was cultured in the following manner:

i. Aerobic - The specimens were cultured in 10% sheep blood agar, MacConkey's agar, and in trypticase-soy broth. The cultures were incubated and examined at 24 hours. The trypticase-soy broth was subcultured on blood agar and MacConkey's agar, aerobically, for 24 hours and then examined.

ii. Anaerobic - The specimens were inoculated into cooked meat media and on 10% sheep blood agar. These were incubated in a Brewer anaerobic jar for 24 hours and then examined.

iii. Bacterial counts of the intestinal contents were determined by making plate counts. They were prepared by taking
one ml. of intestinal contents and placing it in nine ml. of sterile saline. Serial dilutions of this suspension were made to $10^{-9}$. One milliliter of each dilution was mixed with fifteen milliliters of tryptose agar, incubated for 24 hours and counts were made using a Quebec Colony counter.

Each container of mineral oil was examined for sterility prior to use by placing one ml. of mineral oil into ten ml. of tryptase-soy broth, incubated for 48 hours and then examined visually.

Bacterial types were determined on the basis of colony characteristics on blood and MacConkey's agar, gram stain and microscopic examination.

(h) Hematological Technics.

In the preparation and maintenance of a visceral organism a series of hematological examinations were made (Coffin, 1953).

Prior to surgery blood was taken directly from the ear vein of the subject and used immediately.

One ml. of blood-Locke solution was removed from the reservoir prior to use.

One ml. of blood was removed from the subject at the midpoint of surgery, at 30 minutes, one hour and then hourly intervals up to and including the death of the visceral organism.

The following examinations were performed on each sample of blood:
1. Packed cell volume.
2. Total white blood cells.
3. Differential distribution of white cell types.

The differential counts were reported as percentages in three groups only. The immature and mature neutrophils were reported as segmented cells, the lymphocytes and monocytes were reported as mononuclear cells and the eosinophiles were reported as such.

(i) Analytical Technics.

Clinical examinations of the blood were done on the patient prior to surgery, as well as on the samples of blood removed at intervals from the visceral organism.

Prior to surgery the blood was removed from an ear vein and examined immediately. All blood removed from a visceral organism or from a reservoir was added to potassium oxalate, refrigerated and used within 12 to 24 hours. Intestinal contents and blood lost through hemorrhage were also added to potassium oxalate and refrigerated for 12 to 24 hours prior to use.

I. Urea Nitrogen (Blood) - The blood urea nitrogen was determined by the micro method of King (1951).

II. Blood Glucose - The blood glucose was determined by the modified micro method of Folin and Wu (Todd, Sanford and Wells, 1953).

In cases where the blood glucose was found to be elevated over 300 mg. %, the filtrate was diluted with distilled water, directly as to the range of elevation.
(j) Statistical Methods.

The results of the various examinations of the groups were reported as the mean of the group ± the standard deviation of the mean.

arkin and Colton's (1955) standard method was used to determine the significance of the difference between means of the experimental data. Fisher's table of 't' was used to obtain the P values.

The significance of differences between means was considered as follows:

P > .05, not significant.
P < .05 to .02, probably significant.
P < .02 to .001, significant.
P < .001, highly significant.

2. Experimental Methods.

(a) Antibiotic Treatment.

In a select series of animals each individual received two grams of neomycin sulphate* and 125 mg. of phenoxymethyl penicillin** every day, for three days, prior to surgery. Within two hours of surgery a priming dose of the two antibiotics was administered.

The regimen of dosage was one gram of neomycin and 125 mg. of penicillin, orally, in the morning and one gram of neomycin, orally, in the late afternoon. The priming dose consisted of one gram of neomycin and 125 mg. of penicillin.

* Mycifradin, The Upjohn Co., Toronto.
** Compocillin VK, Abbott Laboratories, Montreal.
The occasional cat was unable to tolerate this dosage of antibiotics. In these cases, the antibiotic treatment was discontinued and the individuals were removed from the experiment.

(b) Blood Pressure Measurement and Control.

The blood pressure was controlled with the aid of a blood reservoir directly connected to the lower abdominal aorta of the visceral organism. The height of the blood reservoir above the level of the heart of the visceral organism aided in controlling the blood pressure of the system. The height was set to establish a mean blood pressure of 40 to 45 mm. Hg. in one series of experiments and to establish a blood pressure of 80 to 85 mm. Hg. in another series of experiments.

The blood pressure was measured by the insertion of a needle, directly connected to a mercury U-tube manometer, into the rubber tubing of the blood administration set at its connection with the arterial cannula.

The desired blood pressure was maintained throughout the experiment by raising or lowering the blood reservoir, when necessary, thus increasing or decreasing the blood volume of the viscera.

(c) Plasma Infusion.

Fifty ml. of blood was collected at death from three different visceral organisms all pretreated with antibiotics. The blood was refrigerated for a period no longer than 48 hours. Then 20 ml. of plasma was removed from each bottle and ten ml. were given intravenously to six anaesthetized cats. A sample of blood was
obtained from the ear vein prior to plasma administration. Blood samples were then removed at five-minute, 30-minute, one-hour and hourly intervals up to five hours.

Each blood sample was examined immediately for the total number of white blood cells, a smear was prepared and a differential count made.

A control series of six cats was handled in the same manner using normal feline plasma, collected into heparinized, siliconed blood bottles.

The results of the differential smears were reported in absolute terms in the three classes previously mentioned.
IV OBSERVATIONS

1. Low Blood Pressure, Untreated Preparations (Group I)

A total of 12 cats and three immature dogs were used in the development of the technic of preparation and maintenance of the visceral organism. During these experiments when the technic of blood administration described by Markowitz and Essex (1930) was used, the mean blood pressure of the preparation appeared to remain near 40 mm. Hg. It was discovered that when several preparations were maintained by arterial transfusion at a similar blood pressure the activities and degenerative processes described by Carrel (1913) and Markowitz and Essex (1930) were reproduced. Consequently a series of ten visceral organisms were prepared from cats and maintained at a mean blood pressure range of 40 to 45 mm. Hg. In these experiments the activities, lifespan, pathology, bacteriology, hematology and blood chemistry were studied.

After the isolation of the viscera and the transfusion of fluid, the heart rate became established at a constant rate of 140 to 160 beats per minute and remained so until the later stages of the experiment (Table I). The spleen was enlarged in the majority of cases but was otherwise normal in colour and appearance. When manual pressure was applied to the organ, a transient decrease in size and elevation of blood pressure resulted. The liver was initially normal in appearance and its alterations in colour proved to be the most accurate indicator of the general condition of the preparation.
The lungs were normal in appearance as were the kidneys, pancreas and mesenteric lymph nodes.

The stomach had an increased tone and at no time was any motility seen. The small intestines were normal in appearance and non-motile when first placed in the bath. Fifteen minutes after surgery they became strongly motile and a variety of intestinal movements were seen (Fig. 2). These movements primarily were pendular movements and areas of systole and diastole. The motility continued with decreasing intensity for several hours. On two occasions when the intestines contained small quantities of ingesta, chyle appeared within the intestinal lymphatic ducts and spilled out of the ruptured cisterna chyli.

The volume of fluid entering the viscera was constant over long periods of time but occasionally five or ten ml. of blood-Locke's solution passed from the reservoir to the visceral organism in a period of seconds only to be returned to the reservoir shortly afterwards. Strong pulsations were observed in the aorta, renal artery, anterior mesenteric artery and all of its branches. The intestinal wall was covered with a network of small vessels which gave it an over-all pink colour.

A bile stained viscid fluid containing mucus was discharged from the intestinal tract, 90 minutes after the start of the experiments. Blood appeared in the discharge within 15 minutes.

One to three hours before death a progressive deterioration of the viscera became evident. The liver became progressively
cyanotic and engorged starting at the periphery after 4.8 ± 1.4 hours. The edges of the organ appeared swollen and rounded. Usually one or more ischemic infarcts appeared at the borders terminally. A thin layer of fibrin appeared on the ventral surface of the liver where it contacted the gauze sponges. External hemorrhage became apparent and increased as time passed. The dorsal surface of the lungs became red and small strands of fibrin collected within the mesh of the gauze covering. The mesenteric lymph nodes became markedly enlarged.

The intestinal motility decreased until at 4.3 ± 0.8 hours it was completely absent. The loss of tone commenced in the duodenum and progressed in both directions. During this period the stomach and intestines slowly filled with fluid. The peritoneal surface became increasingly hyperemic and the network of blood vessels increased in number. The hyperemia was also seen in the mesentery and pericardium. Toward the end of each experiment the intestines and mesentery became cyanotic (Fig. 3). The intestinal discharge became progressively bloody, increased in volume and had a putrid odor.

The heart rate decreased to 104 ± 18 beats per minute (Table I). Terminally, sporadic periods of irregular heart beats occurred, accompanied by a fall in blood pressure. The end point of the experiment was characterized by a sudden and unpredicted cessation of cardiac function, not necessarily occurring during a period of irregularity.
Figure 2

A dorsal view of the visceral organism (Group 1), one hour after isolation. The small intestine was normal in appearance and was motile.

Figure 3

A terminal view of the same preparation showed that the small intestine was congested, cyanotic, dilated, and non-motile.
The lifespan of the group was 6.3 ± 2.3 hours. Variations were observed in the volume of blood-Locke’s solution used, hemorrhage, intestinal fluid collected and fluid volume retained by the preparation. The changes are shown in Table 2.

No gross changes were seen in the heart. Microscopically the myocardium contained focal areas of hemorrhage.

The dorsal surface of the lungs was congested and covered with strands of fibrin. Microscopically the bronchioles contained mucus and cellular debris. The alveoli were generally congested and emphysematous at the peripheral portions of the lobules. Interlobular leukocytic invasion, perivascular edema and dorsal subpleural hemorrhage were common findings.

The liver was dark red, the ventral surface was covered with fibrin and occasional small ischemic infarcts were observed at the periphery of the lobes (Fig. 4). The cut surface oozed blood and the tissue was soft and friable. The gall-bladder was filled with bile. Microscopically, there was a general loss of architecture and generalized congestion and edema with leukocytes scattered throughout the parenchyma. Generalized central lobular necrosis grading outward to cloudy swelling was observed (Fig. 9). Occasionally focal areas of hemorrhage were seen and on several occasions hemorrhagic infarcts were demonstrated.

Varying degrees of enlargement were observed in the spleen and the tissue was usually friable on cut section. Microscopic examination demonstrated mild to marked congestion.
Figure 4

An example of an ischemic infarct at the periphery of a liver lobule is shown.
Grossly the renal cortex was grey-yellow and friable. The corticomedullary junction was congested and oozed blood when sectioned. The renal pelvis was enlarged and filled with blood-stained urine. Microscopically the convoluted tubules were necrotic with areas of focal hemorrhage scattered throughout the cortex (Fig. 5). Many of the glomeruli were necrotic while others were relatively unchanged. There was generalized hemorrhage and congestion of the corticomedullary region. The lumen of the collecting and connecting tubules were dilated. The epithelium was flattened.

The pancreas was edematous, soft and pale in colour. Microscopically the only significant alteration was edema of the connective tissue elements.

The mesenteric nodes were enlarged, soft and edematous. Microscopic examination indicated that active lymph follicles were present in all nodes examined. The medulla was edematous and usually congested.

On cross-section the cortex of the adrenal glands appeared unaltered, the medulla was friable and congested. Microscopically, the cortex contained focal areas of hemorrhage and necrosis throughout all three zones and on several occasions this was generalized (Fig. 6). The medulla was edematous and congested with mildly to moderately necrotic areas.

Grossly, the stomach was dilated and filled with bile tinged, bloody, viscid fluid. The fundic area was congested with many areas of ecchymosis and contained varying numbers of small
Figure 5

Congestion and necrosis of the renal cortex (Group 1).

Hematoxylin and Eosin. Mag. X200.

Figure 6

Hemorrhagic necrosis of the adrenal cortex (Group 1).

Hematoxylin and Eosin. Mag. X200.
erosions (Fig. 7). These lesions were most severe at the junction of the fundic and pyloric antra. At no time were lesions observed in the pyloric antrum proper and rarely did they occur in the cardiac region. Microscopically varying degrees of hemorrhage and necrosis were observed at the surface of the mucosa (Fig. 11) and on several occasions the mucosa was eroded to the basement membrane (Fig. 8). No polymorphonuclear cells were observed in or near the necrotic areas. Characteristically necrosis of the chief cells was observed while the parietal cells were unchanged. Submucosal and mucosal edema was common.

Grossly the lumen of the small intestines was filled with bile tinged, bloody, viscid fluid. Hemorrhagic necrosis, erosions, ecchymosis, and mucosal congestion were observed in varying degrees throughout the complete tract and were particularly severe in the jejunum (Fig. 10). The lesions appeared to follow a line along the antimesenteric border of the intestines. On several occasions the lesions only occurred in focal areas within the jejunum. Microscopically, necrosis, tissue debris, hemorrhage and congestion of the distal areas of the villi was a common finding (Fig. 12). At no time were any polymorphonuclear cells observed within the mucosa. Edema of the submucosa was a common finding.

Hematological examination of five visceral organisms indicated that the packed cell volume prior to surgery was 36 ± 3% and at death it was 30 ± 8%. The packed cell volume of the blood-Locke’s solution was 17 ± 2%. The total white blood cells prior to
Figure 7

A gastric erosion in the fundic area (Group 1). Magnified from 3X.

Figure 8

Figure 9

Central lobular necrosis of the liver (Group 1). Hema-toxylin and Eosin. Mag. X125.

Figure 10

Hemorrhagic necrosis and erosions of the gastrointestinal mucosa (Group 1).
Destruction of the gastric mucosa and congestion of the submucosal blood vessels (Group 1). Hematoxylin and Eosin. Mag. X125.
surgery were 13,000 ± 700 per cu.mm. but at death a marked reduction to 2000 ± 400 cells per cu.mm. was observed. The total white cells of the blood-Locke's solution were 3,500 ± 500 per cu.mm. Prior to surgery the white cell types were normal but at death a change had occurred with the neutrophils totalling 11 ± 5%, the mononuclear cells were 79 ± 4% and the eosinophils were 9 ± 8%.

Bacteriological examination of the viscera and body tissues at the death of five untreated visceral organisms indicated a predominance of Escherichia coli, clostridial species and streptococcal species. In every case each of these bacteria were demonstrated in the stomach and intestinal contents and in the majority of cases they were also demonstrated in the portal and heart blood (Table 6). Total plate counts of the intestinal contents indicated that more than $3 \times 10^8$ bacteria per ml. were present.

Examination of the blood urea nitrogen levels of five visceral organisms at death indicated that the urea nitrogen levels were increased (Table 7).

2. **Low Blood Pressure, Antibiotic Pretreated Preparations (Group II)**

The results of the previous series of experiments indicated that possibly death of the preparations was associated with enterotoxemia or septicemia. Fine (1954) reported that dogs subject to experimental hemorrhagic shock were protected by the use of antibiotics. The decline of the visceral organisms in Group I and final pathology resembled the changes that occurred in the intact animal following the production of shock (Wiggers, 1950); consequently it
Figure 12

Destruction of the intestinal mucosa, midjejunal area (Group 1). Hematoxylin and Eosin. Mag. X125.
was decided to prepare a series of visceral organisms from cats pre-
treated with antibiotics. A significant alteration of lifespan and
final pathology was anticipated.

Neomycin as recommended by Cohn and Longacre (1956, 1957,
1959) was the antibiotic of choice for sterilization of the intesti-
 nal tract. Unsatisfactory results were obtained in the reduction
of clostridial species in preliminary experiments; therefore, oral
penicillin was included in the treatment.

Ten visceral organisms were prepared and blood was remov-
ed from each one at death. Blood was removed from five of the ten
at hourly intervals, in addition, to study the pattern of change of
the leukocytes. The various changes recorded and their relationship
to time are shown in Figure 13.

In this group the same syndrome of progressive visceral
degeneration was observed but it occurred slower, and the prepara-
tion remained viable for a longer period of time. The lesions ob-
served were ultimately more extensive than in the unprotected group.

In the early stages, these preparations were similar in
appearance to those in Group I. The intestinal motility, however,
was very weak during the first two hours and then disappeared.
Hyperemia, loss of muscle tone and subsequent ballooning of the
stomach and bowel occurred at $5.5 \pm 1.2$ hours. The intestinal dis-
charge became bloody at the same time as in Group I but the dis-
charge was odorless. The livers became congested after the prepara-
tion had survived $9.4 \pm 2.6$ hours. The heart rate decreased at the
Figure 13

A composite figure to demonstrate the pattern of alterations observed in total white blood cells, packed blood cell volume, heart rate, blood urea nitrogen, and blood glucose, and the quantity of blood transfused into a visceral organism. This has been prepared from one preparation only (Group II).
same time as in the untreated group but the preparations survived for a longer period so that the terminal heart rate was lower than that of Group I (Table 1). During the terminal half of the lifespan, irregular heart beats and coincident drops in blood pressure occurred but recovery followed almost immediately. Death was characterized by a sudden, unpredictable heart failure.

The lifespan of the group was 17.2 ± 5.8 hours. The volume of blood-Locke's solution, hemorrhage, intestinal contents collected and fluid volume retained by the preparation are shown in Table 2.

The gross pathological changes observed in the heart, lungs, liver, kidneys, spleen, pancreas, adrenal glands and mesenteric lymph nodes were similar to those seen in Group I. The gastric and intestinal changes were similar in location and appearance but the severity of the changes and extent of the areas involved were greater. In every case the congestion, hemorrhage and necrosis extended throughout the duodenum, jejunum and ileum. In several instances the mucosa had the appearance of raw liver (Fig. 14).

Microscopically, a similar but more severe pattern of alterations was observed in all organs. Generalized massive areas of necrosis were present in the liver. The majority of kidney glomeruli were congested and necrotic. Focal hemorrhage and necrosis of the adrenal cortex occurred in every case. The stomach showed severe congestion of the mucosal and submucosal blood vessels. All areas of the intestinal mucosa demonstrated some degree of necrosis and
in many instances it was difficult to distinguish the remaining mucosa from the tissue debris (Fig. 15). The mucosal and submucosal vessels were congested.

Hematological examination of five visceral organisms indicated that the packed cell volume prior to surgery was $34 \pm 5\%$ and at death it was increased to $56 \pm 12\%$. The packed cell volume of the blood-Locke's solution was $15 \pm 3\%$. The total white blood cells prior to surgery were $11,500 \pm 600$ per cu.mm. and at death were $2,200 \pm 500$ per cu.mm. The total white blood cells of the blood-Locke's solution were $3,200 \pm 400$ per cu.mm. Prior to surgery the white blood cell types were within the normal ranges but at death the neutrophils were $12 \pm 8\%$, the mononuclear cells were $76 \pm 6\%$, and the eosinophils were $12 \pm 9\%$. During the period of survival of five visceral organisms examinations showed that the packed cell volume was normal prior to surgery, had declined by 30 minutes and remained so for one to two hours postsurgically and then slowly increased until death (Table 4). The packed cell volume of the blood-Locke's solution was $18 \pm 3\%$. The total white blood cells were within normal ranges prior to surgery and during surgery, decreased at 30 minutes to $3,400 \pm 350$ cells per cu.mm. and at one hour and hourly intervals thereafter were within the range of 1500 to 2500 (Table 3). The total white blood cells in the blood-Locke's solution were $3,500 \pm 500$ cells per cu.mm. The differential counts indicated a decrease of segmented cells and a relative increase of the percentage of mononuclear from the presurgical to one hour sample.
Figure 14

Severe hemorrhagic necrosis of the jejunum (middle third) of a 27 hour visceral organism (Group II).

Figure 15

and then they remained constant for each cell type until death. The percentage of eosinophils were unchanged (Table 3). The differential counts of the blood-Locke's solution were within normal limits.

No bacteria were cultured from the tissues or intestinal contents of four visceral organisms at death. In one preparation Aerobacter species were demonstrated in the portal and heart blood only (Table 7).

Examination of the blood removed from ten visceral organisms at death indicated that the urea nitrogen levels were elevated. This level was slightly higher than the terminal blood urea nitrogen levels found in Group I. In five preparations examined, the blood urea nitrogen levels were normal during surgery and slightly elevated at 50% of the lifespan (Table 8).

Examination of the blood removed from five preparations prior to surgery indicated that the blood glucose levels were 88 ± 13 mg. % which is within the normal range of 77 to 118 mg. % as reported by Bloom (1957). During surgery a marked hyperglycemia was demonstrated (246 ± 110) and was increased to 692 ± 268 mg. % at 30 minutes after isolation. The elevated glucose levels then slowly declined at a rate of 50 to 75 mg. per hour until at death the mean glucose level was 149 ± 110 mg. per 100 ml. At no time were any glucose levels found below the normal range. The blood glucose levels of the blood-Locke's solutions (108 ± 18) were slightly higher than normal. The glucose levels recorded in the hemorrhaged blood
and the intestinal contents appeared to vary directly as to the ultimate glucose level of the individual preparation and indirectly as to the fluid volume lost from the preparation. The results of the blood glucose examinations of this group are summarized in Table 9.

3. **High Blood Pressure, Untreated Preparations (Group III)**

The intestinal lesions, observed terminally in the visceral organism, were similar to the lesions reported in a variety of conditions all associated with intestinal ischemia. Lillehei (1957) has reported that when dogs were subjected to prolonged hypotension while the intestinal tract was perfused at normal pressure by a donor animal, the development of characteristic intestinal necrosis was prevented. It was, therefore, decided to increase the blood pressure in a series of visceral organisms in an attempt to prevent the intestinal ischemia, which apparently contributed to the death of the preparation. Wiggers, Glaser, Canavarro and Treat (1943) have reported a mean blood pressure range of 75 to 80 mm. Hg. in the decerebrate cat and Phemister, Eichelberger and Laester (1945) have reported a mean blood pressure range of 60 to 80 mm. Hg. in the decerebrate dog. It was thus decided to increase the mean blood pressure range of the visceral organism to 80 to 85 mm. Hg. A total of six cats were included in the series.

Shortly after the establishment of the visceral organism in its bath, there was marked intestinal motility which continued for two to three hours. The intestinal discharge was bile tinged,
clear and viscid, and remained so throughout the lifespan of each preparation. The intestinal motility gradually decreased in intensity and after four hours the intestines appeared hyperemic and slightly ballooned. A mild degree of intestinal motility persisted until death. The heart rate remained within the range of 140 to 160 beats per minute throughout the lifespan and no irregular heart beats were observed (Table 1).

A blood tinged fluid appeared in the tracheal cannula after four hours and the lungs became edematous. In order to maintain adequate tidal air volume, the pressure applied to the lungs had to be slowly increased. The lungs were finally so distended that severe hemorrhage occurred from the surface and into the tracheal cannula. The blood pressure was reduced as a result and the experiments were terminated. At this time, the liver and spleen were normal in appearance, the heart rate was unchanged and intestinal motility was still present.

The mean lifespan of the group was 7.4 ± 2.3 hours. The volumes of blood-Locke's solution, the hemorrhage and the intestinal discharge were similar to those in Group II. The fluid volume retained by the preparations was greater than in Group I or II (Table 2).

At necropsy examination, the heart was unaltered in gross appearance. Microscopically, focal areas of hemorrhage were present in the myocardium.

All lobes of the lungs were extremely enlarged and did not
collapse when the air pressure was removed (Fig. 16). The dorsal surface was congested and covered with fibrinous strands. The trachea was filled with bloody froth. The cut surface of the lungs exuded large quantities of bloody froth. Microscopically, the bronchioles contained cellular debris and pink staining exudate. Marked alveolar emphysema was present throughout the lungs. Interlobular leukocytic invasion, generalized edema, and hemorrhage, were common findings.

The liver was unaltered in gross appearance. Microscopically, generalized congestion was present with many erythrocytes contained within the venous sinusoids. The central veins, hepatic sinusoids and hepatic veins were dilated (Fig. 17). Cloudy swelling of the parenchyma surrounding the central veins and edema of the hepatic triad were common findings.

Varying degrees of enlargement were observed in the spleen, and the organ was usually friable. Microscopically the spleen showed congestion of the red pulp.

Grossly the renal cortex was pale and friable on cut section. The cortico-medullary area was congested. The renal pelvis was enlarged and filled with blood tinged urine. Microscopically the convoluted tubules exhibited varying degrees of cloudy swelling to necrosis. The lumen of the tubules were swollen and occasionally contained eosinophilic material. The majority of the glomeruli were normal, some were congested and others necrotic. The cortico-medullary area was congested with focal areas of hemorrhage. The
lumen of the collecting and connecting tubules were enlarged and the epithelial cells were flattened.

The pancreas was soft, edematous and pale in colour. Microscopically the connective tissue stroma was edematous.

The mesenteric lymph nodes were enlarged, soft and edematous. Microscopically the lymphoid follicles showed signs of activity and the medulla was edematous and congested.

The adrenal cortex was soft, friable and hemorrhagic. The medulla was congested and friable. Microscopically the cortex contained focal areas of hemorrhage and necrosis in all zones, while other sections contained generalized hemorrhage, necrosis and tissue debris. The adrenal medulla was edematous and congested with focal areas of hemorrhage and necrosis.

The stomach was filled with clear, bile tinged, viscid fluid. No significant alterations were observed in the mucous membranes (Fig. 18) except in one case where an area of congestion one centimetre in diameter was observed in the cardiac region. Microscopically the only alteration was congestion of blood vessels in the mucosa. The submucosa was edematous and the submucosal vessels were congested.

The intestines were filled with clear, bile tinged viscid fluid. The mucous membrane was unaltered. Occasionally focal necrosis of the distal epithelial cells was observed microscopically. The blood vessels of the mucosa were congested. The submucosa was edematous and the submucosal vessels were congested.
Figure 16

At the left, marked pulmonary edema and congestion (Group III). To the right, normal, collapsed lungs.

Figure 17

Edema of the hepatic sinusoids (Group III). Hematoxylin and Eosin. Mag. X200.
Figure 18

Unaltered gastric mucosa (Group III). Hematoxylin and Eosin. Mag. X125.
Hematological examination of the six visceral organisms showed that the packed cell volume decreased immediately after surgery and then slowly increased to the presurgical levels at the midpoint of lifespan. The packed cell volume continued to increase until at death it was significantly higher than presurgical values (Table 4). A similar leukopenia occurred in Group III as in Group II. The total white cell count rapidly declined for one hour and then remained constant until the end of the experiment. There was a similar change in segmented and mononuclear cells as seen in Group II. The percentage of eosinophils was unchanged.

Examination of the blood-Locke's solution indicated a packed cell volume of 50% normal, a total white cell count of 3,700 ± 800 per cu.mm. and a distribution of cell types within accepted normal limits.

Bacteriological examination of the viscera and intestinal contents following the death of five untreated visceral organisms indicated a predominance of *Escherichia coli*, clostridial species and streptococcal species. In every case each of these bacteria was demonstrated in the intestinal contents while in three cases they were demonstrated in the heart and portal blood (Table 6). The total plate counts of the intestinal contents indicated that more than $3 \times 10^8$ bacteria per ml. were present.

Examination of the blood urea nitrogen levels of five preparations indicated that during surgery the levels were within normal limits. The urea nitrogen levels were found to be elevated at the
Examination of the blood glucose levels of three preparations in this group indicated that prior to surgery the subjects had normal glucose levels (90 ± 8). During surgery the blood glucose rose to 252 ± 95 mg.%. The highest blood glucose levels recorded were at 30 minutes (419 ± 145). The glucose levels proceeded to decline at a relatively even rate of 25 to 75 mg. per hour until termination of the experiment. In one case the blood glucose level remained within the normal range for the last three hours of lifespan. In no case were hypoglycemic glucose levels recorded. Varying degrees of glucose were observed in the blood lost from hemorrhage and in the intestinal contents. The blood-glucose levels of the blood-Locke's solution were 107 ± 22 mg.%. The results of the blood glucose examinations are summarized in Table 4.

4. High Blood Pressure, Antibiotic Pretreated Preparations (Group IV)

Three visceral organisms were prepared from antibiotic pretreated cats in an attempt to determine the effect of antibiotics on the development of the pulmonary edema and lifespan.

There was no apparent difference in the activities of the preparations from those in Group III which were not treated with antibiotics (Tables 1 and 2). The gross and microscopic tissue changes were similar to the observations reported in Group III (Fig. 19 and 20). The packed cell volume, total leukocyte counts and differential examinations did not significantly vary from the changes observed in
Group III (Table 3).

Bacteriological examination of the viscera and intestinal contents at the death of three visceral organisms indicated that no bacteria were present in two preparations. In the remaining preparation, *Escherichia coli* were demonstrated in the portal blood and heart blood and streptococcal species were present in the portal blood (Table 7).

Examination of the blood urea nitrogen levels of three visceral organisms indicated that during surgery the urea levels were normal. A progressive elevation of the urea nitrogen levels was observed at the midpoint and termination of the experiment (Table 8).

Examination of the glucose levels in the blood, intestinal contents and transfusion solution showed that the results were similar to the findings in Group III. The results of the glucose examinations in the two groups were combined and summarized, in Table 9.

5. Plasma Infusion

Schweinburg, Smiddy and Fine (1958) described a severe granulocytopenia in dogs and rabbits in irreversible shock and following *E. coli* endotoxin injection. The authors also reported that when plasma from a rabbit in irreversible shock was injected intravenously into normal rabbits, severe granulocytopenia occurred. The visceral organism at a blood pressure range of 40-45 mm. Hg. resembles an irreversibly shocked animal. In order to see if a granulocytopenic agent was present, plasma from three antibiotic pretreated preparations (Group II) was injected into normal cats. It was presumed
Figure 19

Unaltered gastrointestinal mucosa (Group IV).

Figure 20

Unaltered intestinal mucosa (jejunum) and congestion of the submucosal blood vessels (Group IV). Hematoxylin and Eosin. Mag. X125.
that a similar granulocytopenia would occur in the recipient as had occurred in the visceral organism and thus offer a possible explanation for the loss of leukocytes observed in the preparations.

Hematological examination of six normal cats that received plasma from three antibiotic pretreated visceral organisms (Group II) demonstrated that granulocytosis had occurred. Five minutes after the administration of plasma, there was a neutropenia. This was rapidly followed by a marked leukocytosis at two hours, characterized by a probably significant neutrophilia. The mononuclear cells and eosinophils remained unchanged (Table 10). In the control group of six which received normal plasma there was no apparent change in total leukocytes or in cellular types during the five hour period (Table 11).
TABLE 1

Visceral Organism: Heart Rate (Beats Per Minute)

<table>
<thead>
<tr>
<th>No. of Preparations</th>
<th>Group I 10</th>
<th>Group II 10</th>
<th>Group III 6</th>
<th>Group IV 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifespan (hr.)</td>
<td>6.3 ± 2.3*</td>
<td>17.5 ± 5.8</td>
<td>7.4 ± 2.3</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td>5% Lifespan</td>
<td>147 ± 9</td>
<td>155 ± 9</td>
<td>148 ± 11</td>
<td>152 ± 7</td>
</tr>
<tr>
<td>25% Lifespan</td>
<td>150 ± 5</td>
<td>145 ± 2</td>
<td>157 ± 10</td>
<td>161 ± 7</td>
</tr>
<tr>
<td>50% Lifespan</td>
<td>146 ± 9</td>
<td>119 ± 16</td>
<td>158 ± 7</td>
<td>162 ± 7</td>
</tr>
<tr>
<td>75% Lifespan</td>
<td>127 ± 9</td>
<td>95 ± 19</td>
<td>152 ± 12</td>
<td>151 ± 4</td>
</tr>
<tr>
<td>95% Lifespan</td>
<td>104 ± 9</td>
<td>79 ± 13</td>
<td>148 ± 13</td>
<td>145 ± 8</td>
</tr>
</tbody>
</table>

* Standard Deviation of the Mean.
TABLE 2

Visceral Organism: Fluid Volumes (ml.)

<table>
<thead>
<tr>
<th>No. of Preparations</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion</td>
<td>313 ± 167*</td>
<td>750 ± 390</td>
<td>707 ± 346</td>
<td>419 ± 224</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>127 ± 134</td>
<td>302 ± 214</td>
<td>239 ± 230</td>
<td>62 ± 39</td>
</tr>
<tr>
<td>Retained Fluid</td>
<td>185 ± 74</td>
<td>448 ± 203</td>
<td>457 ± 141</td>
<td>353 ± 200</td>
</tr>
<tr>
<td>Intestinal Loss</td>
<td>99 ± 64</td>
<td>335 ± 185</td>
<td>257 ± 128</td>
<td>225 ± 187</td>
</tr>
<tr>
<td>Fluid Vol. Incr.</td>
<td>86 ± 28</td>
<td>113 ± 29</td>
<td>200 ± 77</td>
<td>162 ± 32</td>
</tr>
</tbody>
</table>

* Standard Deviation of the Mean.
### TABLE 3

**Visceral Organism: Hematology**

<table>
<thead>
<tr>
<th>Time</th>
<th>No. of Observations</th>
<th>No. of Preparations</th>
<th>W.B.C.</th>
<th>Seg.</th>
<th>Mono.</th>
<th>Eosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presurgery and Surgery</td>
<td>36</td>
<td>24</td>
<td>11,000 ± 4,100*</td>
<td>61 ± 7</td>
<td>34 ± 6</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>½ hr. 1 hr. 2 hr.</td>
<td>39</td>
<td>13</td>
<td>2,500 ± 400</td>
<td>25 ± 12</td>
<td>70 ± 12</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>3 hr. and 4 hr.</td>
<td>28</td>
<td>15</td>
<td>1,900 ± 300</td>
<td>17 ± 7</td>
<td>76 ± 7</td>
<td>7 ± 7</td>
</tr>
<tr>
<td>5 hr. and 6 hr.</td>
<td>27</td>
<td>14</td>
<td>1,800 ± 600</td>
<td>15 ± 3</td>
<td>80 ± 4</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>7 hr. and 8 hr.</td>
<td>26</td>
<td>14</td>
<td>1,800 ± 400</td>
<td>16 ± 5</td>
<td>78 ± 5</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>9 hr. and 10 hr.</td>
<td>14</td>
<td>9</td>
<td>1,800 ± 500</td>
<td>17 ± 7</td>
<td>78 ± 8</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>10 hr.</td>
<td>20</td>
<td>10</td>
<td>1,700 ± 400</td>
<td>12 ± 5</td>
<td>82 ± 5</td>
<td>6 ± 5</td>
</tr>
</tbody>
</table>

* Standard Deviation of the Mean.
### TABLE 4

Visceral Organism:  Packed Cell Volume (ml. per 100 ml.)

<table>
<thead>
<tr>
<th></th>
<th>No. of Prep.</th>
<th>P.C.V.</th>
<th>'P' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presurgery (a)</td>
<td>24</td>
<td>35 ± 3*</td>
<td>-</td>
</tr>
<tr>
<td>10% Lifespan (b)</td>
<td>14</td>
<td>24 ± 5</td>
<td>a:b &lt; .001</td>
</tr>
<tr>
<td>50% Lifespan (c)</td>
<td>14</td>
<td>34 ± 5</td>
<td>a:c &gt; .5</td>
</tr>
<tr>
<td>100% Lifespan (d)</td>
<td>24</td>
<td>45 ± 13</td>
<td>a:d &lt; .001</td>
</tr>
</tbody>
</table>

* Standard Deviation of the Mean.
TABLE 5

Blood-Locke's Solutions: Hematology

<table>
<thead>
<tr>
<th>Solutions</th>
<th>P.C.V.</th>
<th>W.B.C.</th>
<th>Seg.</th>
<th>Mono.</th>
<th>Eosin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>57</td>
<td>17 ± 13*</td>
<td>3,900 ± 1,200</td>
<td>59 ± 7</td>
<td>35 ± 7</td>
</tr>
</tbody>
</table>

* Standard Deviation of the Mean.

TABLE 6

Bacteriology: Untreated Visceral Organisms

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>Cl. spp.</th>
<th>Strep. spp.</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>8/10</td>
<td>5/10</td>
<td>6/10</td>
<td>staph. spp. 1/10</td>
</tr>
<tr>
<td>Portal</td>
<td>7/10</td>
<td>6/10</td>
<td>5/10</td>
<td>staph. spp. 1/10</td>
</tr>
<tr>
<td>Stomach</td>
<td>5/5</td>
<td>4/5</td>
<td>4/5</td>
<td>staph. spp. 1/5</td>
</tr>
<tr>
<td>Jejunum</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>staph. spp. 1/10</td>
</tr>
<tr>
<td>Colon</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>bacillus spp. 5/5</td>
</tr>
</tbody>
</table>
TABLE 7

Bacteriology: Antibiotic Treated Visceral Organisms

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>Cl. spp.</th>
<th>Strep. spp.</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1/8</td>
<td>0/8</td>
<td>0/8</td>
<td>aerobacter spp. 1/8*</td>
</tr>
<tr>
<td>Portal</td>
<td>1/8</td>
<td>0/8</td>
<td>1/8</td>
<td>aerobacter spp. 1/8*</td>
</tr>
<tr>
<td>Stomach</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>-</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1/8</td>
<td>0/8</td>
<td>1/8</td>
<td>-</td>
</tr>
<tr>
<td>Colon</td>
<td>0/5</td>
<td>1/5</td>
<td>1/5</td>
<td>Yeast 2/5</td>
</tr>
</tbody>
</table>

* Probable contaminant.
TABLE 8

Visceral Organism: Blood Urea Nitrogen (mg. per 100 ml.)

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Preparations</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td>22 ± 5</td>
<td>18 ± 2</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>50% Lifespan</td>
<td></td>
<td>57 ± 3</td>
<td>30 ± 4</td>
<td>49 ± 9</td>
</tr>
<tr>
<td>100% Lifespan</td>
<td>33 ± 9*</td>
<td>60 ± 23**</td>
<td>33 ± 3</td>
<td>62 ± 2</td>
</tr>
</tbody>
</table>

* Standard Deviation of the Mean.
** Ten preparations.
<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>40-45 mm. Hg.</th>
<th>80-85 mm. Hg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Preparations</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**Sampling Time**

<table>
<thead>
<tr>
<th></th>
<th>40-45 mm. Hg.</th>
<th>80-85 mm. Hg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presurgery</td>
<td>88 ± 13*</td>
<td>91 ± 13</td>
</tr>
<tr>
<td>Surgery</td>
<td>246 ± 110</td>
<td>199 ± 30</td>
</tr>
<tr>
<td>½ hr.</td>
<td>692 ± 268</td>
<td>425 ± 137</td>
</tr>
<tr>
<td>1 hr.</td>
<td>636 ± 266</td>
<td>381 ± 114</td>
</tr>
<tr>
<td>2 hr.</td>
<td>526 ± 214</td>
<td>326 ± 117</td>
</tr>
<tr>
<td>3 hr.</td>
<td>467 ± 178</td>
<td>301 ± 134</td>
</tr>
<tr>
<td>4 hr.</td>
<td>425 ± 151</td>
<td>279 ± 121</td>
</tr>
<tr>
<td>5 hr.</td>
<td>353 ± 129</td>
<td>218 ± 110</td>
</tr>
<tr>
<td>6 hr.</td>
<td>300 ± 121</td>
<td>198 ± 100</td>
</tr>
<tr>
<td>7 hr.</td>
<td>255 ± 110</td>
<td>167 ± 63</td>
</tr>
<tr>
<td>8 hr.</td>
<td>232 ± 89</td>
<td>-</td>
</tr>
<tr>
<td>9 hr.</td>
<td>200 ± 86</td>
<td>-</td>
</tr>
<tr>
<td>10 hr.</td>
<td>166 ± 47</td>
<td>-</td>
</tr>
<tr>
<td>Death</td>
<td>149 ± 110</td>
<td>149 ± 58</td>
</tr>
<tr>
<td>Blood-Locke’s Solution</td>
<td>108 ± 18</td>
<td>108 ± 25</td>
</tr>
<tr>
<td>Hemorrhage**</td>
<td>150 ± 11</td>
<td>192 ± 23</td>
</tr>
<tr>
<td>Intestinal Discharge**</td>
<td>73 ± 35</td>
<td>332 ± 483</td>
</tr>
</tbody>
</table>

*Standard Deviation of the Mean.

**Mg. of glucose.
TABLE 10

Hematology: Visceral Organism Plasma Infusion (6 Cats)

<table>
<thead>
<tr>
<th>Time</th>
<th>W.B.C.</th>
<th>Seg.</th>
<th>Mono.</th>
<th>Eosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr.</td>
<td>8,000 ± 3,100*</td>
<td>4,200 ± 1,700</td>
<td>3,200 ± 1,600</td>
<td>530 ± 540</td>
</tr>
<tr>
<td>5 min.</td>
<td>7,000 ± 2,800</td>
<td>2,800 ± 1,400</td>
<td>3,900 ± 1,500</td>
<td>370 ± 150</td>
</tr>
<tr>
<td>30 min.</td>
<td>7,500 ± 3,600</td>
<td>3,500 ± 2,300</td>
<td>3,500 ± 2,300</td>
<td>440 ± 360</td>
</tr>
<tr>
<td>1 hr.</td>
<td>9,000 ± 4,500</td>
<td>4,700 ± 2,700</td>
<td>3,600 ± 2,300</td>
<td>510 ± 480</td>
</tr>
<tr>
<td>2 hr.</td>
<td>14,600 ± 7,500</td>
<td>9,900 ± 3,500</td>
<td>3,700 ± 1,800</td>
<td>800 ± 1,000</td>
</tr>
<tr>
<td>3 hr.</td>
<td>16,200 ± 10,900</td>
<td>10,600 ± 5,300</td>
<td>4,200 ± 2,000</td>
<td>450 ± 210</td>
</tr>
<tr>
<td>4 hr.</td>
<td>16,600 ± 10,300</td>
<td>12,200 ± 9,200</td>
<td>3,700 ± 1,800</td>
<td>620 ± 490</td>
</tr>
<tr>
<td>5 hr.</td>
<td>16,100 ± 9,400</td>
<td>12,200 ± 8,300</td>
<td>3,300 ± 1,800</td>
<td>510 ± 480</td>
</tr>
</tbody>
</table>

0 hr. W.B.C. 8,000 ± 3,100  \( ^*P^ \) value < .05
5 hr. W.B.C. 16,100 ± 9,400

0 hr. Seg. 4,200 ± 1,700  \( ^*P^ \) value < .05
5 hr. Seg. 12,200 ± 8,300

* Standard Deviation of the Mean.
TABLE II

Hematology: Normal Plasma Infusion (6 Cats)

<table>
<thead>
<tr>
<th>Time</th>
<th>W.B.C.</th>
<th>Seg.</th>
<th>Mono.</th>
<th>Eosin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10,400 ± 4,900*</td>
<td>5,700 ± 3,700</td>
<td>4,300 ± 2,200</td>
<td>370 ± 340</td>
</tr>
<tr>
<td>5 min.</td>
<td>10,100 ± 5,800</td>
<td>5,600 ± 3,600</td>
<td>4,100 ± 2,100</td>
<td>390 ± 280</td>
</tr>
<tr>
<td>30 min.</td>
<td>10,100 ± 5,800</td>
<td>5,800 ± 4,100</td>
<td>3,700 ± 2,100</td>
<td>380 ± 230</td>
</tr>
<tr>
<td>1 hr.</td>
<td>10,400 ± 6,000</td>
<td>5,900 ± 4,000</td>
<td>4,600 ± 2,100</td>
<td>470 ± 340</td>
</tr>
<tr>
<td>2 hr.</td>
<td>11,000 ± 4,600</td>
<td>6,300 ± 3,500</td>
<td>3,900 ± 2,200</td>
<td>560 ± 570</td>
</tr>
<tr>
<td>3 hr.</td>
<td>10,900 ± 4,700</td>
<td>6,300 ± 3,600</td>
<td>4,100 ± 1,900</td>
<td>510 ± 400</td>
</tr>
<tr>
<td>4 hr.</td>
<td>10,600 ± 4,800</td>
<td>6,100 ± 3,500</td>
<td>3,900 ± 1,700</td>
<td>480 ± 410</td>
</tr>
<tr>
<td>5 hr.</td>
<td>10,600 ± 4,800</td>
<td>6,100 ± 3,300</td>
<td>3,800 ± 1,800</td>
<td>340 ± 210</td>
</tr>
</tbody>
</table>

* Standard Deviation of the Mean.
Both Carrel (1913) and Markowitz and Essex (1930) prepared visceral organisms from dogs and cats. Carrel used cats more often than dogs while Markowitz (1959) has reported that the technic was never successfully employed with adult dogs, only with pups. In the present study a visceral organism was prepared from a young dog (31/2 months), and maintained at a mean blood pressure range of 40 to 45 mm Hg. The lifespan was only two hours and a similar degenerative process was observed as occurred in the cats in Group I. The liver became more enlarged than those observed in cats. Similar observations were reported by Markowitz and Essex (1930). The congestion and necrosis of the gastrointestinal tract, even at two hours, was quite severe. The dog was discarded as the experimental animal because of the difficulties involved in obtaining immature dogs of a standard size. Adult cats could be obtained at a standard size throughout the year. The cat proved to be a very hardy experimental animal as only two out of 44 preparations failed after the establishment of the technic. Difficulties arose, however, in obtaining an adequate compatible blood supply to use as a transfusion during the experiments.

The continuous blood supply was necessary for the visceral organism because of the vasodilatation and reduced vascular tone as well as an inevitable continuous hemorrhage and loss of gastrointestinal fluid. Carrel (1913) reported that the first transfusion was
given in his series immediately after surgery. The blood was removed from the carotid artery of an anesthetized cat and transfused into the aorta or vena cava of the preparation. No mention was made of the blood pressure of the organism, whether or not transfusion was continued throughout the lifespan or how much blood was used during the transfusion. Markowitz and Essex (1930) reported that the visceral organism was transfused immediately after surgery and at given intervals of time throughout the experiment, using a paraffined syringe and blood removed from an anesthetized cat. The volume of blood injected was four ml. per kilogram of body weight. Transfusion was performed when pulsations were no longer observed in the small branches of the mesenteric arteries. These intervals of time averaged two hours. Markowitz and Essex (1930) have reported that immediately following transfusion the blood pressure was 70 mm. Hg. and yet at a blood pressure of 40 mm. Hg. in Group I pulsations were observed in the mesenteric artery and its branches. In view of this, it would appear that the visceral organism of Markowitz and Essex would have a blood pressure varying from 70 mm. Hg. to below 40 mm. Hg.

The adaption of a blood reservoir attached to the posterior aorta of the visceral organism proved to be a satisfactory innovation in the technic of maintaining the visceral organism. This gave the preparation a continuous supply of blood with an adjustable pressure head which could be raised or lowered to provide the desired blood pressure. The reservoir was connected immediately following
surgery. The results of the experiments where from 65 to 400 ml. of transfusion fluid were used during an eight-hour period clearly demonstrated the inadequacy of transfusing given quantities at given intervals of time. The aortic transfusion also established a coronary pressure immediately following isolation of the viscera and revived the heart. In addition, the continuous arterial transfusion enabled the visceral organism to maintain an even blood pressure throughout the experiment.

The technic of surgery was essentially the same as that used by Markowitz and Essex (1930). The use of an electrosurgical scalpel to sever the diaphragm saved time and avoided additional manipulation of the viscera. Markowitz and Essex freed the dorsal thoracic vessels by first severing all the dorsal arteries and then the intercostal veins, thus hoping to conserve blood within the viscera. Modification of this method so that the arteries and veins were ligated and severed together increased the surgical speed without deleterious effects. The time required for surgery was 35 to 45 minutes, if the surgical period was prolonged much beyond this time the preparation usually failed. The only portion of the surgery in which any difficulty was encountered was when the anterior thoracic vessels were ligated and severed; care had to be shown in the ligation to prevent the production of a large hematoma in the area.

Carrel (1913) used Ringer's solution in the visceral organism's bath. The viscera were placed dorsal side upward in a metal tray filled with the solution and the container was placed in an incubator to maintain the desired temperature. The exchange of various
metabolites from the viscera to the Ringer's solution would probably preclude the measurement of any increase in concentration of the metabolites in the viscera. The prospect of placing the preparation, solution and container, all in an incubator, seemed impractical. Markowitz and Essex (1930) placed the visceral organism ventral side uppermost in a wooden box heated with an inner light bulb and covered with a piece of glass. The viscera were placed on a sloping tray and humidity was maintained by the addition of cotton gauze moistened in saline and placed in the box. Unfortunately, once the preparation was placed in the box, the glass covering became covered with condensation and prevented accurate observation of the viscera. Any time that the glass covering was removed to examine, or if necessary, manipulate the viscera, the temperature within the box would rapidly fall.

Mineral oil proved to be the ideal environmental medium. It was clear and provided an excellent view of the preparation, as well as preventing dehydration. It was easily maintained at a constant temperature with an adjustable heater. The mineral oil provided buoyancy to the viscera and yet did not interfere with any natural movement. The possibility of diffusion of substances from the viscera was avoided. Any fluid lost from the preparation was heavier than the mineral oil, collected on the bottom of the container and was available for measurement.

The slope of the tray and the depth of the mineral oil were important. If the slope of the tray was too great or the depth
of the mineral oil too shallow, then the dorsal portion of the viscera would be exposed to the air. Conversely, if the level of the mineral oil was deep enough to completely cover the lungs during inflation, mechanical damage to the viscera resulted.

Although the Locke's solution contained a reasonable balance of electrolytes and the necessary quantity of glucose, the final blood-Locke's solution only contained approximately one-half the desired osmotic pressure due to the dilution of the plasma proteins. As a result the greater the lifespan of the visceral organism, the greater the quantity of blood-Locke's solution entered the circulation, this probably resulted in a progressive decrease of the osmotic pressure within the blood in the visceral organism. The Locke's solution, as judged by the packed cell volume, remained in the circulation for a brief period of time. It is not known to what extent the Locke's solution was involved in the formation of the generalized edema and loss of fluid from the intestinal tract in all preparations, or the development of pulmonary edema which resulted in the death of the preparations maintained at the higher blood pressure, but it is reasonable to conclude that it did. Markowitz and Essex (1930), however, described the formation of a generalized edema in the visceral organism transfused with whole blood. Daly (1946) discussed the problem of pulmonary edema in perfusion systems and emphasizes that it is primarily due to the abnormal nature of the perfusate in addition to the removal of the nervous control of the lungs.
The various lesions observed in the visceral organisms maintained at the lower blood pressure range apparently were not influenced by the surgical preparation as the lesions did not appear until several hours after the viscera were placed in the bath. A visceral organism, sacrificed immediately after surgery, failed to reveal any significant gross or microscopic alterations of the tissues. The lesions in the gastrointestinal tract developed slowly during the lifespan. Various untreated and antibiotic pretreated preparations, arbitrarily sacrificed at intervals of 2 to ten hours after isolation showed that the lesions were present. These lesions did not appear to be influenced by the reaction of the gastrointestinal contents. In all groups the reaction of these contents was close to neutral and ranged from 7.1 to 7.6.

The bacteria demonstrated in the blood of the untreated visceral organisms were the same as those demonstrated in the intestinal tract. The same types of bacteria have been observed by Harmin and Hawkins (1937) in their studies of experimental peritonitis in dogs. Antibiotic pretreatment of the low blood pressure visceral organism reduced the number of bacteria in the bowel and the tissues. This resulted in a significant increase in lifespan. Consequently the bacterial invasion and probable toxemia in the untreated preparations contributed to their death.

Antibiotic pretreatment while lengthening the lifespan of the low pressure group, did not appear to alter the gastrointestinal lesions. Apparently the bacteria had no effect upon the formation of
these lesions. Similar findings have been reported by Hardy, Morris, Yow and Haynes (1954), Downie (1956), Zweifach, Gordon, Wagner and Reynier (1958) and McNulty and Linares (1960), who showed that pretreatment with various antibiotics or in disease-free rats following the production of irreversible shock that gastrointestinal necrosis and hemorrhage were constant and consistent findings. In the visceral organism the increased lifespan prolonged the period of intestinal ischemia and on many occasions the mucosal lesions were more severe. Similar findings have been reported by Werle, Cosby and Wiggers (1942) who reported that the primary factors involved in the gastrointestinal lesions of a shocked animal were a reduced blood pressure maintained for a prolonged period of time.

Since similar bacteriological results were observed in the untreated visceral organisms maintained at the higher blood pressure without intestinal lesions, it would appear that the marked decrease of circulating neutrophils were primarily responsible for the bacterial invasion of the tissues (Cronkite and Brecher, 1955). No difference in observations occurred between the two high pressure groups. Since the death of these preparations was due to the development of pulmonary edema, it is impossible to compare the ultimate effect of the reduction of bacteria on these preparations. But it is presumed that if the pulmonary edema could be prevented, the bacteria would have a similar effect as in the low pressure groups.

Similar gastrointestinal lesions have been reported in a variety of conditions in intact animals. They have been reported
in experimental hemorrhagic shock (Werle, Cosby and Wiggers, 1942; Lillehei, 1957), experimental septic shock and tourniquet shock (Schweinburg and Fine, 1960), temporary arterial and venous occlusions (Medins and Laufman, 1958; Turner, Neely and Barnett, 1959), following injection of bacterial endotoxin (Weil, MacLean, Spink and Visscher, 1956), spinal shock (Phemister et al., 1945), intravascular thrombi formation (Hardaway and McKay, 1959), experimental elevation of portal pressure (Selkurt, Alexander and Patterson, 1947), and long-term injection of adrenaline (Penner and Bernheim, 1939). The common factor in all of these various experiments is prolonged intestinal ischemia. In addition, similar lesions, loss of motility, ballooning of the intestines and a bloody mucous discharge have been reported in attempts to maintain the isolated intestine (Salveoli, 1881; Roese, 1931; and Ohnell, 1939). It has been reported that when the intestines have been protected from ischemia during prolonged hypotension by the use of viviperfusion (Lillehei, 1957), the use of adrenolytic compounds (Shapiro, Bronsther, Frank and Fine, 1958; Inglis, Hamson and Gurd, 1959; Lillehei and MacLean, 1959), or intestinal hypothermia (Lillehei, Goot and Miller, 1959), that these lesions have been prevented.

In view of these results and since similar lesions occurred in the visceral organism at a blood pressure of 40 mm. Hg. and were prevented by an elevation of the blood pressure to 80 mm. Hg. it would appear that these gastrointestinal lesions resulted from prolonged ischemia and were not due to the lack of a protective
mechanism in the visceral organism as suggested by Markowitz et al. (1959).

The normal lifespan of leukocytes has not yet been accurately determined. White (1954) has suggested a lifespan of 30 to 90 minutes, Weisberger, Heinle, Storaasli, and Hannah (1950) 30 minutes, Farr (1951) 90 minutes, Carddock, Perry and Lawrence (1960) four to eight hours, Lawrence, Ervin, and Wetrich (1945) 18 hours, Lala, Bhattocharjee, and Gupta (1960) 2.7 days, Bierman, Kelly, Marshall and Baluda (1959) 8.6 days, Kline and Clifton (1952) 9.2 days and Perry, Craddock and Lawrence (1958) 12 to 14 days. The marked differences in results appeared to depend upon the technics used by the authors.

Hematological examination of the visceral organisms indicated that a severe leukopenia characterized by a neutropenia developed 30 minutes to one hour after the isolation of the viscera. This was unaltered by variations of blood pressure or administration of antibiotics. The leukopenia apparently develops as the result of excess destruction of the white blood cells or by their sequestration in the tissues of the visceral organism.

Histopathological examination of the tissues of the visceral organism indicated that leukocytes were sequestered in the lungs and the liver. But the visceral organism contained both homologous and autogenous leukocytes and it was impossible to differentiate the one population from the other, microscopically. Weisberger et al. (1950); Weisberger, Guyton, Heinle and Storaasli (1951);
Bierman, Kelly and Cordes (1955); and Ambrus and Ambrus (1959) have demonstrated that transfused homologous leukocytes are sequestered in the lungs, liver, and spleen of intact animals shortly after transfusion. In a single experiment, the pooled leukocytes from 100 ml. of blood labelled in vivo with diisopropylfluorophosphate were collected by dextran precipitation and centrifugation (Athens, Mauer, Ashenbrucker, Cartwright and Wintrobe, 1959) then transfused into a visceral organism one hour after its isolation. The preparation was sacrificed 30 minutes later. Examination of the various tissues indicated the highest radioactivity in the lungs, liver and spleen. Microscopic examination of the tissues confirmed the sequestration of leukocytes in these tissues. On the other hand, it has been demonstrated by Bierman, Byron, Kelly and Petraki (1951); Bierman et al. (1955); and Ambrus and Ambrus (1959) that autogenous leukocytes are normally removed from the circulation by the lungs, liver and spleen. In a single experiment a visceral organism was sacrificed at one hour and the tissue sections were examined. Large quantities of leukocytes were observed in the lungs and liver. In view of the short lifespan of the preparation, these leukocytes were presumed to be predominantly autogenous in nature. Bierman, Kelly, Cordes, Byron, Polyhemus and Rappaport (1952) have demonstrated that leukocytes were released from the lungs of intact animals one minute after the intravenous administration of epinephrine and norepinephrine. The resulting leukocytosis lasted for five minutes. In a single visceral organism, one hour after isolation, epinephrine was administered
intravenously. The circulating leukocytes increased from 2,200 to 4,900 cells per cu.mm. and the neutrophils were primarily affected. The increased number of leukocytes lasted for five minutes. Thus it appears that the autogenous leukocytes, as well as the homologous leukocytes, are sequestered in the lungs, liver and spleen of the visceral organism and may be stimulated to reappear in the circulation.

Leukocyte counts made two hours after the isolation of the viscera had a range of 1,500 to 2,500 cells per cu.mm. The total leukocytes and differential distribution of cells then remained at a constant level until death of the preparation. This constant level did not appear to be influenced by the total number of leukocytes in the intact animal or by the total number of leukocytes in the transfusion fluid. Ambrus and Ambrus (1959) reported that in isolated heart-lung, liver and spleen preparations, irrespective of the number of leukocytes introduced into the preparation, the count was rapidly reduced to a constant level of 500 to 1500 cells per cu.mm. Neutrophil granulocytes were chiefly affected. No changes were reported in leukocyte counts of blood circulated through isolated, hind-limb preparations. These findings are in agreement with Craddock, Perry and Lawrence (1956) who reported that it was impossible to lower the leukocyte count below 1000 cells per cu.mm. in intact animals, even with the most extensive leukopheretic procedures. Ambrus and Ambrus have suggested that in the intact animal a double regulatory mechanism controls the leukocyte level. Whereby the
production regulator regulates to levels higher than normal and the elimination regulator regulates to levels lower than the normal circulating leukocyte levels. By means of these two regulators, the leukocyte count is maintained within normal ranges. Possibly this is an explanation of the results observed in the visceral organisms, which does not have any bone marrow.

The leukopenia was likely augmented by the mineral oil media in which the organs were suspended and by their contact with the gauze sponge. These materials were both foreign to the viscera and likely stimulated the leukocytes to migrate to the visceral pleura, peritoneum and tissues. The mineral oil bath possibly contributed to a leukopheresis with a constant level of leukocytes being maintained in the circulation (Craddock et al., 1956). Numerous unsuccessful attempts were made to demonstrate leukocytes in the mineral oil, gauze sponge or on the surface of the viscera. But the fibrin strands that slowly formed on the lung and liver surfaces were evidence that there was considerable damage and protein seepage. Such conditions would normally stimulate the invasion of leukocytes from the circulation.

The transfusion of visceral organism plasma into normal cats indicated that a factor capable of stimulating an increase in circulating leukocytes was present rather than a factor that resulted in leukopenia.

Rodbard (1945) reported that in dogs following bilateral nephrectomy the average lifespan was 85 hours while Houck (1954)
maintained dogs with nonprotein nitrogen blood levels of 100 to 135 mg. % for 35 days. In the visceral organism the terminal blood urea levels were increased in all of the preparations which were examined. The average level recorded was 43 ± 15 mg. % and was not considered excessively high. These urea levels were not the true accumulations of urea produced as there was probably varying amounts of urea lost in the hemorrhage and in the intestinal discharge. It was presumed that ligation of the ureters did not contribute to the death of the preparations. An apparent difference in terminal urea nitrogen levels was observed between the untreated and antibiotic pretreated groups but this difference was not statistically significant. The blood urea levels were apparently unaffected by alterations of blood pressure. Comparison of the blood urea levels recorded during surgery, at 50 per cent and 100 per cent of the lifespan indicated rising urea levels, demonstrating continued liver function in the visceral organism.

Determination of the blood glucose levels of the visceral organisms indicated that a marked hyperglycemia occurred during surgery and at 30 minutes after isolation. Blood glucose levels of over 900 mg. % occurred in several instances. This increase of glucose was probably of hepatic origin due to anoxia, release of adrenalin, and stimulation of the splanchnic nerves (Soskin, 1927) with a smaller portion resulting from the release of glucogenic corticosteroids from the adrenal cortex (Long, Katzin and Fry, 1940). All of these stimuli probably occurred during the surgery involved in the isolation of the viscera. The extremely high glucose levels
were likely due to the progressive decrease of the parietal tissue during surgery, which would normally dilute the blood glucose.

Beginning at one hour and continuing until the death of the visceral organism, the blood glucose levels were observed to decline at a slow even rate. This decline was partially the result of the dilution of the total glucose in the preparation by the progressive addition of blood-Locke's solution, concurrent with the loss of glucose through hemorrhage and intestinal fluid. In several instances the loss of fluid from the preparation and the addition of blood-Locke's solution were relatively quite small, yet the decline of the blood glucose was unaltered. The utilization of glucose by the extrahepatic tissues and the formation of glycogen by the liver were considered as possible factors contributing to the decrease of blood glucose. Glycogen determinations of the liver, however, were not performed. In no case were hypoglycemic levels observed and in two instances the glucose levels fell to and remained within the normal range until the death of the organisms.

A visceral organism, pretreated with antibiotics, was maintained at a mean blood pressure range of 40 to 45 mm Hg. After two hours the preparation was given an intra-arterial injection of insulin* (0.8 units). This injection reduced the blood pressure momentarily and was then followed by a period of hypertension which lasted for 10 to 15 minutes. The heart rate increased from 138 to over 175 beats per minute and remained accelerated for one hour.

* Insulin - Toronto, Connaught Laboratories, Toronto.
All signs of intestinal motility ceased. The visceral organism slowly returned to its preinjection state after a period of two hours, whereupon insulin (1.6 units) was again injected and similar observations were made. Examination of the blood glucose levels following the administration of insulin indicated an apparent increase in the rate of glucose decline.

In the irreversibly shocked animal the blood glucose levels show an initial prolonged hyperglycemia with a terminal hypoglycemia (Westerfeld, Weisiger, Ferris and Hastings, 1944). While in the isolated liver, glycogenolysis and a prolonged hyperglycemia are observed (Markowitz and Essex, 1930; Ambrus and Ambrus, 1959). The visceral organism has relatively little striated muscle and an intact liver. In this study a preliminary examination of the carbohydrate metabolism has been made and the possibility of the effects of insulin have been suggested. In view of these findings and considering the tissues involved, the visceral organism appears to be an unique preparation for the study of carbohydrate metabolism.
VI SUMMARY AND CONCLUSIONS

A technic is described, using the dog or the cat, for the isolation and maintenance of the thoracic and abdominal viscera with an intact functioning circulatory system. The preparation (visceral organism) requires artificial respiration, is attached to a blood reservoir and is maintained in a constant temperature mineral oil bath. The technic of maintaining the visceral organism proved to be a more satisfactory method than those previously described.

In a series of preparations maintained at a mean blood pressure range of 40 to 45 mm. Hg, the lifespan was $6.3 \pm 1.3$ hours. The viscera were active and normal in appearance when isolated but slowly degenerated. Death was characterized by an unpredictable cardiovascular failure. The gross and microscopic appearance of the viscera were similar to the pathological changes associated with prolonged hypotension and ischemia in a variety of intact animals. Bacteriological examination showed a marked proliferation of common pathogens in the gastrointestinal tract and their invasion of the tissues. Similar preparations pretreated with oral neomycin and penicillin underwent the same process of degeneration. The lifespan of the series was increased to $17.5 \pm 5.2$ hours and similar but more severe pathological changes were observed. The bacteria while shortening the life of the low pressure preparations did not influence the gastrointestinal lesions.

In a series of visceral organisms maintained at a mean
blood pressure range of 80 to 85 mm Hg., the syndrome of visceral degeneration was prevented. The lifespan of the series was 7.7 ± 1.8 hours and death was due to the progressive development of pulmonary edema. The gross and microscopic appearance of the viscera were relatively unaltered. A similar pattern of bacterial proliferation and tissue invasion was observed. Antibiotic pretreatment did not significantly alter the activities, the lifespan of the preparations or the appearance of the tissues at necropsy. The bacteria and their toxins do not affect the higher pressure visceral organisms prior to the development of pulmonary edema.

A marked leukopenia, characterized by a predominant neutropenia, was observed. The circulating leukocytes were maintained at a constant level of 1500 to 2500 cells per cu.mm. from the first hour after isolation until death. These observations were unaltered by the total circulating leukocytes prior to surgery, the total number of leukocytes in the transfusion fluid or changes in the blood pressure and antibiotic pretreatment of the preparations.

Elevated blood urea nitrogen levels were observed in all preparations. These levels were not elevated to those associated with uremic manifestations in intact animals.

A marked hyperglycemia was observed during surgery and reached a peak at 30 minutes after isolation of the preparations. The blood glucose levels slowly declined and approached normal values at the time of death. Hypoglycemic levels were never observed.
Further study of the carbohydrate metabolism in the visceral organism may increase the general knowledge of carbohydrate metabolism in the intact animal.
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VITA

NAME: David Cartwright Secord

BORN: Guelph, Ontario, June 6, 1933

EDUCATED:

Primary: Toronto Public Schools, 1939-47

Secondary: Upper Canada College,
Toronto, 1947-52
Forest Hill Village Collegiate,
Toronto, 1952-53

University: University of Toronto, 1953-58

Course and Degree: Veterinary Medicine, D.V.M., 1958
THESIS

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