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A METABOLIC STUDY OF THE ISOLATED PERFUSED SNAKE LIVER

by

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ABSTRACT

A metabolic study was made on the isolated, perfused liver of Natrix fasciata fasciata (Linnaeus, 1776). Uric acid production, perfusate protein levels, serum protein patterns, perfusate glucose levels, and bile production were evaluated in this study as criteria of liver function.

The perfused liver was found to produce uric acid up through the fourth hour of perfusion. No significant differences (between the perfusions) were found in uric acid production / gm of liver tissue.

Perfusate protein and glucose levels were found to be of a fluctuating or cyclic nature. There was found to be an inverse relationship between these two levels: that is, a rise in the protein level was accompanied by a characteristic decrease in the glucose level, the reverse also holding true. Also, both levels were found to be inversely related to liver weight.

Plasma protein pattern studies showed that definite changes were produced within certain specific bands which corresponded to the fluctuations found in the total protein levels. The chemical nature of these bands was also determined.

Bile production was recorded in only two of the five perfusions because of the difficulty encountered in cannulating the minute bile duct.

Histological studies revealed some necrosis of the perfused liver tissue.

It was concluded that the isolated liver of N. fasciata fasciata could be perfused in this system from 4 to 5 hours without significant decreases in the functional activities that were examined.

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INTRODUCTION

The technique of *in vitro* liver perfusion was conceived almost a hundred years ago by such early physiologists as Bernard, Schroeder, and Ludwig (Miller, 1959) and has very recently lent itself to a direct approach in the answering of many questions in hepatic physiology.

Because liver metabolism is so intimately related to other metabolic phenomena and is easily influenced, directly or indirectly, by other body processes such as hormone action, variations in nutrition and many other factors, it is extremely difficult to perform studies of its individual phases in intact animals. Many techniques, such as pancreatectomy and adrenalectomy, have evolved in an attempt to circumvent these obstacles. However, they introduce additional complications which might produce undesirable results. Many classical experiments have been performed with liver homogenates; however, liver metabolism is best studied when there is cellular and tissue integrity. In recent years Buchanan (1949) has worked with surviving liver slices incubated in various media, but here again results did not reach *in vivo* proportions.

In effect, studies in liver metabolism are greatly facilitated by an experimental system which would permit more satisfactory duplication of *in vivo* metabolic processes. Theoretically, a desirable system for liver studies should be capable of: (a) general metabolic activity simulating an accepted physiological range (b) response to hormones comparable in rate and extent to that observed in *vitro* (c) synthesis of glycogen, plasma proteins and other products under normal physiological conditions and (d) maintenance of a circulation comparable to that *in vivo*.

It has recently proven possible to meet these criteria by utilizing an improved apparatus for the perfusion of isolated, surviving whole

livers of rats, described by Miller (1951). Subsequently, with the development of this new technique, Miller and his associates presented direct experimental evidence of the dominant role of the liver in the biosynthesis of plasma proteins, utilizing the isolated rat liver. Since this early work, further studies have been carried out by Miller (1951, 1959, 1965) with rats, Axelrod (1956) with dogs, Sokal (1958) with rats, Haft (1958) with rats, Craig (1958, 1959) with frogs and cats, Green (1960) with rats, and Leftwich and Tipton (1963) with rats.

However, with the complex arterial and venous hook-up in the mammalian and amphibian livers it has proven to be a rather arduous task in removing them for in vivo studies. In addition, the slightest temperature variance within the perfused mammalian liver cannot be tolerated which again makes it a difficult organ with which to work. Thus, our attention was focused on looking for a liver that (a) was easily accessible, (b) could be cannulated with a minimum amount of effort, (c) involved a minimum amount of ligation of the associated vessels before removal, and (d) would not be susceptible to metabolic variances with slight changes in temperatures. Preliminary investigations and exploratory operations on such reptilian specimens as Coluber constrictor constrictor (Linnaeus, 1758), Lampropeltis getulus getulus (Linnaeus, 1776), and Natrix sipedon sipedon (Linnaeus, 1758) showed that the snake liver meets these requirements. This liver is a very compact, unilobed, retilinial structure (Fig. 16) and is easily perfused.

It was the purpose of this study to make a detailed analysis of the metabolism of the liver of N. fasciata fasciata. The function of the perfused liver was studied by running several analyses on samples drawn from the perfusate at certain intervals during the perfusion run. These analyses, which included the determination of: (a) perfusate uric acid

concentration; (b) perfusate protein levels; (c) chemical nature and percent composition of plasma protein bands; (d) perfusate glucose levels; and (e) bile production, along with histological studies projected a very good picture of perfused liver function.

MATERIALS AND METHODS

A. THE PERFUSION APPARATUS:

The perfusion apparatus used in this work was similar to that described by Miller (1950) with modifications by Leftwich and Tipton (1963). Basically, the apparatus consisted of: (a) a blood reservoir; (b) a gas humidifier; (c) a multilobed "lung"; (d) a liver chamber; (e) two clot-filters; (f) a gas tank (95% O_2 + 5% CO_2); and (g) a peristaltic pump (Monostat Veristalic Model 72-590-60) which were connected by tubing (rubber Al-Si-Co, amber-1/8 in O. D. and 1/4 in I. D.) forming a closed system (Fig. 17).

The blood reservoir was a modified flask having four openings: (a) an outlet leading to the pump; (b) another outlet leading to the multilobed "lung"; (c) an inlet receiving gas from the humidifier and; (d) another inlet receiving blood from the postcaval vein.

The oxygenated blood was accumulated in the small upper reservoir located just below the bulbed portion of the "lung". Here, the level of perfusion pressure was maintained constant (4.5 cm of perfusate) by the use of three adjustable screw clamps: (a) one located just below the first clot filter; (b) a second located just before the perfusate enters the "lung"; and (c) a third just below the second clot filter. From the "lung", the perfusate passed into the cannulated portal vein of the liver and out the venacava back into the blood reservoir. The oxygen inlet of the "lung" also served as an overflow bypass, although, caution was taken not to allow such an overflow because of unwanted foaming of the perfusate.

To prevent the accumulation of minute clots within the system, two filters were located along the line of circulation as mentioned previously (Fig. 17), the first along the line just above the pump, and the second

between the "lung" and the liver chamber. These filters consisted of two leucite discs, about 2 in in diameter, machined to fit closely with an interposed disc of white silk between them which measured 100 by 150 mesh per inch (Fig. 2).

Preliminary studies on several species of snakes showed that the livers were from 6 to 6 1/2 in long and 1/2 to 1 in wide. A special chamber to accommodate these livers was made of 1/8 in thick leucite which measured 7 1/2 in x 1 1/2 x 1 in (Figs. 2 & 18). Three holes, one at one end and two at the other, 1/16 in in diameter, were drilled at the ends approximately 1/8 in from the bottom to accommodate the three cannulae. The ends of the chamber were constructed so that they could be removed when the liver was placed it it.

The entire apparatus with the exception of the pump and the gas tank was housed in a chamber measuring 3 1/2 ft x 2 ft x 2 ft and was constructed of 1/4 in plywood (Figs. 3 & 19). In order to provide a secure and permanent attachment for the perfusion apparatus, a 1/4 in diameter metal rod was installed within the chamber, centered 5 1/2 in from the back wall (Fig. 17). This provided ample area for the spacial arrangement of the apparatus. To facilitate observation through the chamber window, a 15 watt light was installed in the chamber on the inside just above the door. The wattage of the bulb was such that it did not appreciably alter the temperature of the chamber.

B. SURGICAL TECHNIQUE AND PERFUSATE PREPARATION:

Snakes (N. fasciata fasciata) measuring from 2 to 3 1/2 ft in length, were obtained from the Tote-Em-In Zoo, Wilmington, North Carolina. Upon arrival at our laboratory, several days were allowed for the snakes to adjust to their new environment (screen covered terraria) and

to recover from any dehydration suffered during shipment.

Before surgery could be attempted, several problems in design had to be solved. One involved the types of cannulae to be used. Polyethylene tubing (Clay-Adams Intramedic tubing) was found to be best suited for this purpose. Three different cannula sizes were used: (a) a large size (0.034 in I.D. x 0.050 in O.D.) for the postcava; (b) an intermediate size (0.023 in I.D. x 0.038 in O.D.) for the portal vein; and (c) a smaller size (0.011 in I.D. x 0.023 in O.D.) for the bile duct. Each cannula was provided with a small collar to prevent them from slipping past their ties and out of the vessels (Fig. 20).

To facilitate surgical procedures an operating platform to fit the snake anatomy was constructed. The operating board consisted of a rectangular base (2 2/5 ft x 5 1/2 in x 5 in) which supported a long operating platform (3 3/4 ft x 1 1/2 ft x 3/4 in) raised 3/4 in above the base by two supports allowing for most of the area between the platform and base to be free (Fig. 21). This design proved to have many advantages several of which were: (a) it allowed for the snake to be easily strapped and secured to the board; (b) it allowed for easier manipulation and freedom of movement when operating; and (c) the skin and muscle flaps resulting from the midventral incision could easily be pinned back with hemostats to expose the body cavity.

Twelve hours before surgery and perfusion were to take place, the perfusate was prepared. Blood was drawn from two or three donor snakes by cardiac puncture (using a 10 cc syringe with a 20 gauge needle which contained a small amount of heparinized saline to prevent clotting) and diluted with Krebs-Ringers bicarbonate saline solution which was prepared as suggested by Umbreit, Burris and Stauffer (1959) as follows:

NaCl	0.154 M	20.00 ml
KCl	0.154 M	0.80 ml
$CaCl_2$	0.110 M	0.60 ml
KH_2PO_4	0.154 M	0.20 ml
$NaHCO_3$	0.154 M	5.25 ml

The final volume was diluted to 130 ml with distilled water. The final perfusate was prepared by adding 54 cc of blood to 36 cc of Ringers to make a 60% perfusate. The perfusate was stored overnight (12 hours) under refrigeration ($7^{o}C$). Preparations for surgery were also made at this time (Fig. 22).

The snakes were anesthetized by placing them in a container with a cotton swab soaked with ether (anesthesia, Mallenckrodt Chemical Works) for approximately 10 to 15 minutes. It was found that light anesthetization insured adequate cardiac function. After anesthetization, the snakes were secured to the operating platform with adhesive tape just below the head and at the tail. A midventral incision was made from a point 2 in below the heart and extended posteriorly for about 10 inches. The skin and muscle flaps were secured with hemostats in order to fully expose the liver (Fig. 23). The exposed area was then sprayed with heparinized saline. The mesenteries covering the vessels to be cannulated were quickly removed and thread (Belding-Cortecelli, size no. 50) was passed under the vessels and through the underlying mesenteries by a sewing needle (Fig. 23). These threads were used to secure the cannulae in their respective vessels.

Prior to cannulation, two precautions were taken in order to minimize clot formation: (a) a small needle was inserted into the postcava just below the liver and a small amount of heparinized saline was injected and (b) each cannula was filled with the same saline solution to prevent introduction of air into the liver.

The bile duct was then cannulated by making a small V-shaped cut

in the vessel about 1/2 in below the liver. Into this vessel the cannula was inserted and a tie made in back of the collar (Fig. 24A).

Next, the portal vein was cannulated in the same manner and secured to its vessel by a similar tie. Finally, both cannulae (bile duct and portal vein) were tied together for greater ease in handling (Fig. 24B). A suction apparatus was used to remove the excess blood from the surgical area (Fig. 22).

The postcava was then cannulated in the manner just described at a point about 1/2 in above the posterior end of the liver (Fig. 24C & D). It should be noted here that at the anterior end of the liver, the postcava, bile duct, and portal vein were all located closely within the same mesenteric sheath and were easily confused if care was not taken to keep from twisting the liver or its vessels before cannulation (Fig. 16).

After cannulation, a small amount of heparinized saline was passed into the liver by way of the portal vein cannula to prevent clot formation. The liver was then quickly cut free from the surrounding mesenteries and placed in the liver chamber which held a small quantity of saline solution to facilitate movement and to keep the tissue moist. The entire operation from the initial incision to placement of the liver in the chamber took from 15 to 20 minutes. The liver was without blood supply for about 10 minutes.

C. PERFUSION:

The perfusate was circulated through the system for one-half hour prior to introduction of the liver in order to insure proper oxygenation and temperature equilibrium.

Immediately after the liver was placed in the chamber, the portal vein cannula was connected to the flow of perfusate, the bile duct

cannula placed in the collection tube, and the postcaval vein cannula inserted into the blood reservoir. The perfusate was at first regulated to a slight flow through the liver until the vessels were again dilated. Then the flow was adjusted to 72 to 195 ml/hr which was maintained approximately constant. The volume of perfusate passing through the liver was measured periodically by counting the number of drops (1 drop = 0.1 ml) from the postcaval cannula.

It was found necessary at this time to remove any excess mesenteries that may have remained attached to the liver, since these had a tendency to shrink causing unwanted bending and constriction of the liver and its vessels.

One ml perfusate samples for assay were taken every other half hour for 5 hours. The first sample was taken just before the liver was introduced into the system. The perfusate samples were removed by a small needle (5 cc syringe with a 20 gauge needle) inserted through a rubber dam and into the postcaval inlet. A small hole in the reservoir neck was provided for this purpose (Fig. 2). At the end of the perfusion the liver was removed and tissue samples were fixed for histological studies.

D. PERFUSATE ANALYSES:

1. Uric Acid

The uric acid concentration of the perfusate was determined by the method of Caraway (1955). The sensitivity of this assay was found to be $\pm$ 0.07 mg %.

The reagents for this assay were prepared as follows:

a. Stock Uric Acid Standard, 40 mg %.

Pure uric acid (0.04 gm) was dissolved in 0.46 gm of anhydrous disodium phosphate in 60 ml of distilled water. The

contents were cooled and 0.18 ml of glacial acetic acid was added. The contents were brought to a volume of 100 ml with distilled water. The solution was stored in brown 30 ml polyethylene bottles.

b. Working Uric Acid Standard.

The stock standard was diluted to a desired concentration with distilled water.

c. Sodium Tungstate, 3.2%.

d. Sodium Carbonate, 10%.

e. Sulfuric Acid, 0.2 N.

f. Phosphotungstic Acid.

Molybdate-free sodium tungstate (10 gm) was dissolved in 80 ml of distilled water. To this, 8 ml of 85% phosphotungstic acid was added and refluxed gently for 2 hours. The solution was cooled and diluted to 100 ml and stored in 30 ml brown polyethylene bottles.

g. Dilute Phosphotungstic Acid, 10%.

Perfusate samples were withdrawn at the designated intervals and centrifuged at 1, 500 RPM's for 10 minutes to remove the formed blood elements. Samples of plasma (0.4 ml) were then mixed with 1.6 ml of sodium tungstate and with 1.6 ml of 0.2 N sulfuric acid and centrifuged for 10 min at 3,000 RPM's. A portion of the supernatant (3.2 ml) was mixed with 0.4 ml of 10% sodium carbonate and 0.4 ml of dilute phosphotungstic acid and allowed to stand 30 min for development of a blue color. The absorbance of this mixture was then read at 650 mu on a Bausch & Lomb Spectronic 20 colorimeter.

Prior to the use of the Caraway technique in this study, a standard curve was prepared by measuring the optical densities of

known concentrations of uric acid standards (0.2, 0.5, 1.0, 1.5 mg %) as shown in Figure 4. This test was found to be linear between concentrations of 0.2 and 1.5 mg %. Thus, the uric acid concentrations of the perfusate samples were diluted to bring the concentrations within range of the linearity.

2. Total Protein

The method of Lowery (1951) was used for the measurement of total protein. The sensitivity of this assay was found to be between ± 0.05 gm %.

The reagents used in this assay were prepared as follows:

a. Sodium Carbonate.

2% Na_2CO_3 in 0.1 N NaOH. Made fresh daily.

b. Copper Sulfate.

0.5% $CuSO_4 \cdot 5H_2O$ in 1% sodium tartrate. Made fresh daily.

c. Alkaline Copper Solution.

Reagent a. (50 ml) was mixed with 1 ml of reagent b.

d. Dilute Phenol Reagent.

One ml of phenol reagent (Folin & Ciocalteu, Will Scientific, Inc.) was diluted with 9.8 ml of 0.1 N NaOH.

e. Protein Standard.

Sixty mg of powdered bovine Albumin (Fraction V, Nutritional Biochemicals Corporation) was diluted to make 100 ml with distilled water. To prepare working standards, the stock standard was diluted to desired concentrations.

Prior to the use of the Lowery technique in this study, a standard curve was prepared as in the uric acid analysis. Standard concentrations of proteins (1, 10, 15, and 20 gm %) were used (Fig. 5). This test was found to be linear between concentrations of 1 to 15 gm %. Since the

protein content of the perfusate was above this concentration, the samples were diluted to bring the concentrations within range of the linearity.

Next, 1 ml of these dilutions was added to 5 ml of the alkaline copper solution and mixed thoroughly by shaking. After standing for 10 min, 0.5 ml of reagent d. was added and was thoroughly mixed. The optical densities were read 5 min later on a Bausch & Lomb Spectronic 20 colorimeter.

3. Plasma Protein Patterns

a. Electrophoresis.

The plasma proteins were electrophoresed on 1 in x 6 3/4 in polyacetate strips (Gelman Sepraphore III) by the method of Briere (1964).

The reagents used for electrophoresis were prepared as follows:

(1) Barbital-Barbituric Acid Buffer, pH 8.6, ionic strength 0.05.

A 10.3 gm of diethylbarbituric acid were diluted to I l in distilled water.

(2) Stain, Ponceau S.

Ponceau S (0.5 gm, Allied Chemical) was dissolved in 100 ml of 5% trichloroacetic acid. For washing, 5% glacial acetic acid was used.

(3) Clearing.

The strips were dehydrated in two washings of methanol and cleared in a 10% acetic acid in methanol solution.

Before the strips were ready for electrophoresis, they were first soaked in the buffer solution. In this procedure the strips

were first wetted by floating them on top of the buffer, then they were completely immersed.

Next, the electrophoresis chamber (Gelman Rapid Electrophoresis Chamber No. 51101) was filled with approximately 500 ml of buffer. Heat build-up and evaporation was reduced by starting with a cold buffer (7°C). The acetate strips were blotted gently on filter paper to remove all surface buffer. Approximately 3 lambda of the plasma was applied to the strips by a Gelman Electrophoresis Sample Applicator (No. 51220). This application was made 2 in from one end of the strip at a right angle to the margin making sure to leave 1/8 in on each side of the application free of sample to prevent tailing on the edges of the strip. The strips were then placed in the electrophoretic chamber with the point of sample application about 1/2 in from the cathode support bridge and electrophoresed for 35 min at 400 volts and 6 ma. The potential across the strips was provided by a Precision-Model RS-25 power supply. At the end of the time allowed for separation, the strips were removed and placed in the stain for 5 to 10 min, and afterwards placed in 5% glacial acetic acid (3 washings) to remove excess and background stain.

At this point, the strips were prepared for quantitative analysis by dehydration in two rinses of methanol and clearing in 10% acetic acid in methanol for 60 sec. The cleared strips were analyzed by a Beckman Model RB Analytrol densitometer which scans and evaluates the strips with two traces: (a) one is a curve of dye density versus distance along the strip; and (b) the other is an integration of the area under the curve with

a series of saw teeth, allowing for accurate quantitation.

b. Plasma Protein Band Analysis.

(1) Lipoprotein Identification.

This procedure involved lipid oxidation and staining with a modified Schiff method (Gelman, 1966).

The reagents for this procedure were prepared as follows:

(a) Conc. H_2SO_4.

(b) Barium Peroxide.

(c) HCl (0.001 & 0.1 N).

(d) Schiff's Reagent.

One gm of para-rosaniline (Allied Chemical) was dissolved in 30 ml of 1 N HCl. One gm of potassium meta bisulfite was also dissolved in 170 ml of distilled water. The two solutions were mixed and allowed to stand for 24 hours. Next, a small amount of animal charcoal was added and the solution allowed to stand for 6 more hours. Afterwards, it was filtered and stored under refrigeration (7°C).

(e) 0.5% Nitric Acid.

The plasma samples were electrophoresed at 400 volts, 6 ma, for 35 min in a manner previously described. After electrophoresis the strips were removed from the chamber. The control strips were first stained in Ponceau S and the other strips were placed in a glass chamber which contained barium hydroxide. Sulfuric acid was poured on the hydroxide and the strips were oxidized for 20 min. Next the strips were washed for 1 min in 0.001 N HCl and stained for 15 min in Schiff's reagent. The strips were reduced by washing

3 times (15 min each) in 0.5% nitric acid and once in 0.1 N HCl. The strips were next cleared and quantitated by densitometry.

(2) Glycoprotein Identification.

This procedure involved the Periodic Acid Schiff (PAS) method (Gelman, 1966).

The reagents for this procedure were prepared as follows:

(a) 5% Trichloroacetic Acid.

(b) 0.5% Periodic Acid.

(c) Schiff's Reagent.

One gm of basic fuchsin (Allied Chemical) and 1.9 gm of sodium meta-bisulfite was added to 100 ml of 0.15 N HCl. This was stoppered tightly and kept in the dark overnight and was clear and brownish when removed from the dark. To this solution 500 mg of fresh activated charcoal was added and shaken for 2 min. Next, the solution was filtered into a graduated cylinder and the residue washed to restore the volume to 100 ml. The Schiff reagent was stored under refrigeration (7°C) and discarded when it turned pink.

(d) Sodium Metabisulfite (0.5%).

Prepared fresh daily.

The plasma samples were electrophoresed at 400 volts, 6 ma, for 35 min in a manner previously described. The control strips were stained in Ponceau S. The other strips were fixed in 5% TCA for 2 min and oxidized for 5 min in 0.5% periodic acid. The strips were then washed 2 times with distilled water and placed in the Schiff reagent (brought to room temperature) for 10 min.

The strips were next washed 3 times (2 min for each wash) in 0.5% sodium metabisulfite. After a final washing in distilled water for 5 to 10 min, the strips were dried at room temperature and cleared for quantitation by densitometry.

(3) Albumin Identification.

The albumin fraction was identified as the fastest migrating fraction of the plasma sample, and also by the fact that it reacted negatively with both the glycoprotein and lipoprotein stains.

(4) Gamma Globulin Identification.

The gamma globulins were identified as the slowest migrating portion of the plasma sample.

4. Blood Glucose

The method used to determine blood glucose levels was that of Folin (1929). The sensitivity of this test was found to be ± 0.05 mg %.

The reagents for this assay were prepared as follows:

a. Dilute Tungstic Acid Solution.

A 20 ml portion of 10% sodium tungstate was diluted to 800 ml in a I l flask. To this was added 20 ml of 2/3 N sulfuric acid and the solution was then diluted to the I l mark.

b. Potassium Ferricyanide Solution.

A high grade of potassium ferricyanide (1 gm) was dissolved in distilled water and diluted to 500 ml. This solution was kept away from the light.

c. Cyanide-Carbonate Solution.

Anhydrous sodium carbonate (8 gm) was dissolved in 50 ml of distilled water in a 500 ml volumetric flask by shaking. To this was added 180 ml of freshly prepared 1% sodium cyanide

solution. The final solution was then diluted to the I l mark.

d. Ferric Iron Solution.

A 30 gm portion of gum arabic was transferred to 800 ml of distilled water and heated until all the gum was dissolved. In another flask, 5 gm of ferric sulfate was added to 75 ml of 85% phosphoric acid and 100 ml of distilled water. This solution was heated until the sulfate dissolved. Both solutions (when cooled to room temperature) were added together and diluted to I l.

e. Standard Glucose Solution.

A standard glucose solution (1% Stock Solution, Folin-Wu, Aloe Scientific) corresponding to 200 mg %. (1 ml in 500 ml of distilled water) was used and diluted to the desired concentrations.

A 0.01 ml perfusate sample, from which the formed lelments were removed, was added to 10 ml of the dilute tungstic acid solution. The contents were vigorously shaken and allowed to stand for 5 min followed by centrifugation at 3,000 RPM's for 10 min. Next, 4 ml of the supernatant was transferred to a 25 ml Folin-Wu blood sugar tube and mixed with 1 ml of the potassium ferricyanide solution and 1 ml of the cyanide-carbonate solution. The tubes were boiled for 8 min, cooled to room temperature, then 3 ml of the acid ferric iron solution was added. This solution was allowed to stand for 5 min then diluted to the mark and optical densities (650 mu) were read on a Bausch & Lomb Spectronic 20 colorimeter.

Prior to the use of the Folin technique in this study, a standard curve was prepared by measuring the optical densities of the known concentrations of glucose standards (45, 60, 100, and 200 mg %) as shown

in Figure 6. This test was found to be linear between concentrations of 45 to 200 mg %. It was found that the glucose content of the perfusate samples were well within this range.

5. Bile Production

The determination of the volume of bile production by the perfused liver was made by allowing the bile to flow from the cannula into a small graduated tube (0.1 ml divisions). Care was taken to insure that the bile duct remained free of clots.

E. HISTOLOGY:

Immediately after each perfusion, the liver samples were prepared for general histological studies by the use of Bouin's fixative and hematoxylin and eosin stains. Control tissue slices were taken from blood donors for comparison with the tissue of the perfused livers. The tissue slices were prepared from the procedures outlined by McClung (1950) and Conn (1962).

RESULTS

A. PERFUSATE ANALYSES:

1. Uric Acid

A gradual rise in uric acid concentration was observed in each perfusion (Table 1A & Fig. 7). During perfusion 1 there was a continual increase in the uric acid level from 1.5 to 3.3 mg % during the three hour run. The most pronounced increases took place during the first half hour of perfusion (1.2 mg %) and between the second and third hour (0.5 mg %). In perfusion 2 there was a gradual increase from 2.8 to 3.9 mg %. Over a four hour period, the most pronounced increase occurred during the first half hour of perfusion (0.6 mg %). There was a leveling off at 3.9 mg % after the fourth hour of perfusion.

Of the three perfusions that were carried out for the full five hours, number 3 was the only one that did not show a continual rise in the uric acid level through the fourth hour of perfusion. Although there was a continual increase from 2.5 to 4.0 mg % during the first 2.5 hours of perfusion, it was followed by a very slight decline from 4.0 to 3.8 mg % between hours 2.5 and 4.0. As in perfusion 2, there was a plateauing of the uric acid concentration after the fourth hour. The most significant rise in the uric acid level (0.8 mg %) occurred during the first half hour of perfusion.

In perfusion 4 there was a continual increase in the uric acid level from 2.5 to 3.0 mg % for the entire perfusion run of 2 hours, again with the most noticeable rise occurring during the first half hour of perfusion (0.4 mg %). Perfusion 5, like perfusion 2, showed a continual increase in the uric acid level from 2.9 to 4.9 mg % up to the fourth hour of perfusion with the characteristic leveling off after the fourth hour.

It was shown that liver weight and uric acid production are directly related (Table 1B). For example, in perfusion 1 the weight of the liver (20 gm) was twice that of the liver in perfusion 2 (10 gm), and the amount of uric acid produced by the first liver (1.2 mg %) for the first half hour of perfusion was twice that of the second (0.6 mg %). A linear relationship between liver weight and uric acid production was shown to occur through the third hour of all perfusions (Fig. 8). Furthermore, if such a proportional relationship exists, then, the uric acid concentrations when placed on a per gram liver weight basis should show little variation among levels within each time interval. Tables 1C and 2A & B show these values to closely approximate one another. Also, a high positive correlation among these values was found to range from + 0.874 to 0.985 (0.01 to 0.05 confidence levels) for the first 3 hours of perfusion.

2. Total Protein

The total protein levels were found to fluctuate considerably throughout the five perfusions (Table 3A & Fig. 9). In perfusion 1 there was a decline in the protein level from 6.4 to 6.2 gm % during the first half hour of perfusion. This was followed by a rise from 6.2 to 7.8 gm % which continued through the third hour of perfusion.

In perfusion 2 there was also a decline in the initial level from 3.9 to 2.0 gm % during the first two hours of perfusion. This initial decline was followed by a rise from 2.0 to 4.1 gm % between the second and third hours of perfusion. Again there was a decline from 4.9 to 2.0 gm % between the third and fifth hours of perfusion.

There was an initial rise in the protein level of perfusion 3 during the first half hour from 3.9 to 5.0 gm % which was followed by

a decline from 5.0 to 4.0 gm % at the end of the first hour. The decline in the protein level was again followed by a characteristic rise from 4.0 to 6.2 gm % at the end of the second hour of perfusion. This was in turn followed by another decline from 6.2 to 4.8 gm % at the end of 2.5 hours of perfusion. In turn, there was another rise from 4.8 to 6.5 gm % at the end of 4.5 hours which was again characteristically followed by the beginning of another decrease in the protein level.

During the first hour of perfusion 4 there was an initial rise in the protein level from 4.1 to 5.6 gm % followed by a characteristic drop from 5.6 to 5.5 gm % between the first and second (final) hours of perfusion.

In perfusion 5 there was a decline from 3.2 to 2.8 gm % during the first hour of perfusion. Following this decline was a characteristic rise from 2.8 to 4.3 gm % during hours 0.5 through 4 of the perfusion. As before, this rise was followed by a decline from 4.3 to 2.9 gm % during hours 4 through 5 of the perfusion.

In most instances, especially in those perfusions which were carried out for the full five hours (Perf. 2, 3 & 5), the magnitude of increase in the protein levels was usually very closely related to the magnitude of decrease as shown in Table 3D. An average of these similar values along with those from perfusions 1 (0.09 gm %) and 2 (0.19 gm %) showed a high negative correlation value of -0.90 (0.05 conf. level) when compared with liver weight (Table 4).

3. Plasma Protein Patterns

Densitometric analysis of electrophoretograms revealed five bands within the plasma protein patterns of N. fasciata fasciata. A representative electrophoretic pattern is shown in Figure 10. The bands were

assigned numbers 1 through 5 according to their migration rates. Representative values for each band are given in Table 5. Of the total amount of plasma protein, it was found that band 1 composed of 16.2% (0.62 gm %); band 2, 10.7% (0.42 gm %); band 3, 9.6% (0.35 gm %); band 4, 26.7% (0.90 gm %); and band 5, 36.7% (1.45 gm %).

Band 1 (fastest migrating) reacted negatively with both carbohydrate and lipid stains. However, both the trailing and leading portions of this band reacted positively with the carbohydrate stain. Only the trailing portion reacted positively with the lipid stain. This evidence plus studies of the chemical nature of mammalian plasma (White, Handler, and Smith, 1964) suggested that band 1 was the albumin fraction. Bands 2, 3, and 4 reacted positively with both the carbohydrate and lipid stains indicating the presence of both glyco- and lipoproteins in these bands. It is possible that the alpha and beta globulins are located within this area. The slowest migrating fraction (band 5) stained positively for carbohydrates but negatively for lipids. By virtue of its slow rate of migration and by comparison of its chemical nature with that of mammalian plasma, it is believed that this rather broad band contains the gamma globulins.

When the cyclic nature of the total protein levels was related to the plasma band percentages (Fig. 11) several relationships were observed. Most of the plasma protein fluctuation was located within bands 4 and 5. In all cases, band 5 showed the greatest amount of change usually in the form of an increase. The next bands to show the greatest amount of change were 2 and 3. Band 1 exhibited the least amount of change. Furthermore, it became apparent that the electrophoretic bands fell into groupings according to their: (1) relative percentage of composition; and (2) to their fluctuating patterns. The rank order according to percent-

age of composition was grouped as follows; 5 and 4, 1, and 2 and 3. Several types of patterns were found to occur when a comparison of the trends of the bands within each perfusion was made. Bands 4 and 5 and 1 and 5 were found to show inverse trends while bands 1 and 2, 1 and 3, and 1 and 4 showed direct relationships. Bands 3 and 4 showed signs of being both inversely and directly proportional. Thus, it was found that the proposed albumin fraction (band 1) generally followed the patterns of the proposed globulin fractions except for band 5.

4. Glucose

The glucose levels were also found to be of a cyclic nature. Figure 12 and Table 6A show the glucose levels at specific times during each perfusion. In perfusion 1 there was an initial decline in the glucose level from 72 to 65 mg % during the first half hour of perfusion which was followed by a rise from 65 to 77 mg % from hours 0.5 through 1 of the perfusion. This was followed by a decline from 77 to 66 mg % between hours 1 through 2 of the perfusion.

In perfusion 2 there was a rise of from 84 to 118 mg % in the initial level during the first hour of perfusion. This was followed by a decline from 118 to 72 mg % between hours 1 through 2.5. There was a leveling off at 72 mg % from hours 2.5 through 5.

There was an initial drop in perfusion 3 from 87 to 84 mg % during the first half hour of perfusion which was followed by a rise from 84 to 141 mg % between hours 0.5 through 2.5. This rise was again followed by a decline from 141 to 128 mg % during hours 2.5 through 3 of the perfusion. Between hours 3 through 5 there was another rise in the glucose level from 128 to 170 mg %.

In perfusion 4 there was seen an initial drop from 112 to 83 mg %

during the first half hour of perfusion. This was followed by a rise from 83 to 126 mg % between hours 0.5 through 1 which was in turn followed by a decrease in the glucose level from 126 to 100 mg % up through the completion of the perfusion.

There was an initial rise in the glucose level in perfusion 5 from 114 to 157 mg % during the first half hour of perfusion followed by a decrease from 157 to 110 mg % between hours 0.5 through 2. Between hours 2 through 3 there was another rise from 110 to 151 mg % followed again by a decrease from 151 to 102 mg % between hours 3 through 4.5. In turn, this was followed by another rise in the glucose level from 102 to 147 mg % between hours 4.5 through 5.

A very significant negative correlation of -0.96 (0.01 conf. level) was found between these values and liver weight (Table 7). When both total protein and glucose levels were plotted together (Fig. 13) for comparative studies they were found to possess opposite trends. When the protein levels increased, corresponding glucose levels fell, the opposite also holding true.

B. BILE PRODUCTION:

Due to mechanical difficulties involved in cannulating the small bile duct, bile was collected only during the first two perfusions. The accumulation of bile during perfusion 1 amounted to 0.1 ml and in perfusion 2 amounted to 0.15 ml.

C. LIVER HISTOLOGY:

Histologically there were some differences between sections from the perfused tissue and those from the control liver tissue.

The following criteria suggested by Schiff (1956) were used for

determination of necrotic areas within the perfused liver tissue: (1) congested, moderately widened sinusoids; (2) narrowed hepatic cords; (3) hemorrhaging; and (4) vacuolization. Photomicrographs representative of the control and experimental tissues are shown in Figure 25.

Tissue samples from perfusion 1 showed some vacuolization within the cells; however, no hemorrhaging or abnormalities in cell size were observed. Tissue samples from perfusion 2 also revealed some vacuolization along with hemorrhaging and cell shrinkage. Perfusion 3 tissues showed little vacuolization, some hemorrhaging, and a few abnormal cells (shrinkage). The tissues from perfusion 4 revealed some vacuolization, little hemorrhaging, and cells of normal size. In perfusion 5 there was little if any vacuolization, some cell shrinkage, and a slight amount of hemorrhaging.

Overall, it was estimated that necrosis affected from 2 to 5% of the tissues depending upon the duration of the perfusion.

DISCUSSION

A. URIC ACID:

Hutton (1958) found the circulating levels of uric acid in N. sipedon sipedon to range from 2.89 to 7.80 mg %. Since this species is closely related to N. fasciata fasciata, it would appear that the perfusate levels of uric acid in the present study were physiological.

Since neither exogenous amino acids nor glucose were added to the perfusate, endogenous uric acid production was involved in the present study. As no studies involving endogenous uric acid production in isolated perfused snake livers were available, a comparison was made with similar studies by Miller, et al. (1955) involving endogenous urea production in the isolated perfused rat liver. Their studies revealed that: (1) endogenous urea production proceeded in a linear fashion in five four-hour perfusions; (2) if sufficient quantities of glucose were added to the perfusate, a nitrogen-sparing action took place after two hours of perfusion causing a decrease in the level and linearity of the urea production; and (3) if sufficient amounts of amino acids were added along with the glucose, the urea concentration would level off and in some cases decrease between the first and second hours of perfusion.

In comparing these findings with those of the present study, similarities between rat urea and snake uric acid trends were found. The curves in the present study (Fig. 7) followed closely those of Miller's (Fig. 14) in which he suggested that a nitrogen-sparing action had taken place. Like urea production, the uric acid concentration rose sharply during the first half hour of perfusion then adjusted to a more gradual rise and finally in three perfusions leveled off between hours 3 and 4.

Several questions arose in the present study at this point: (1) by what process was uric acid derived and was the initial part of this process any different from that of urea production in the rat perfusion work of Miller? (2) Was the nitrogen-sparing mechanism applicable to the snake liver system as well? (3) And if so, can this mechanism be related to any other process within this system such as total blood protein or glucose turnover?

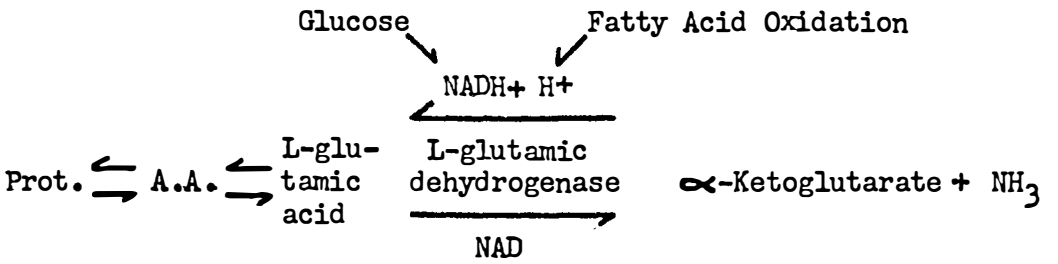
Miller, et al. (1955) found that in urea production the plasma proteins yielded 33% while the liver proteins yielded 58% with 9% coming from catabolism of the hemoglobin released by normal red cell breakdown. Since no exogenous amino acids were added to the snake liver system, the uric acid produced in the present study must have arisen in the same manner, that is, the oxidative deamination of free amino acids from proteins of the blood and liver.

According to Miller (1955), the most common pathway in the oxidative deamination of amino acids in mammals is through the L-glutamic acid dehydrogenase system (plus its coenzyme NAD) which according to Cohen (1954) can control the rate of ammonia production. It is believed that this is also the case with uric acid production in reptiles (Prosser, 1962). Furthermore, it has been shown that both urea and uric acid production are a direct function of ammonia formation. From the glutamic dehydrogenase catalyzed reaction the following mass law expression was derived:

$$\left[NH_3\right] = K \left[\frac{NAD}{NADH + H^+} \cdot \frac{Glutarate}{Ketoglutarate}\right]$$

This expression shows that an increase in NADH + H^+ decreases the $\left[NH_3\right]$ and, thus, urea and uric acid formation. According to Miller (1955), the

nitrogen-sparing mechanism would occur as follows:



This being the case, we would be interested in the metabolic reactions which favor $NADH + H^+$ formation in the snake liver system. Glucose metabolism which favors just such a formation was studied in the present system along with protein metabolism. Both are integral parts of Miller's nitrogen-sparing mechanism, and would serve as indicators of such an action in the snake liver system. If this were the case, then the total protein concentration in the present study would level off along with the uric acid concentration in conjunction with nitrogen sparing. Figure 21 shows this to be true for the total protein in perfusions 2, 3 and 5. In addition, glucose metabolism must be taking place simultaneously. Figure 12 shows a considerable amount of glucose turnover in the snake liver system which would fulfill this criterion. Therefore, on the basis of this evidence plus the similarities in the trends of Figures 12 and 14 that were mentioned earlier, it was concluded that a nitrogen-sparing action occurred in the present studies.

Throughout these studies, uric acid production was expressed in terms of liver weight. This mode of expression gave a very significant inverse relationship between these two factors (Table 20). Furthermore, it was found that body weight, body length, or surface area could just as easily be substituted for liver weight in this expression (Table 8). Miller, et al. (1955) also found that an inverse relationship existed between the surface area of rats and urea production. From the following

expression of Lee (1927) used by Miller (1955):

$$\text{Surface Area } (cm^2) = (0.078) \quad (\text{Body wt gm}) + 148$$

it was seen that surface area was also an expression of the body weight. It would seem from here, as in the present studies, that Miller might have also obtained good negative correlations between urea production and liver weight. However, this was not the case, for although his correlations showed definite signs of an inverse relationship they were not in any way as significant as results from the present studies. This might be expected as the rat liver is a multilobed organ in contrast to the unilobed snake liver, and the volume of perfusate flowing through the rat liver is quite variable from lobe to lobe.

B. TOTAL PROTEIN:

The plasma protein levels of the five perfusions were found to range from 2.0 to 7.8 gm %. These levels were similar to those found by Deutch (1949) in N. sipedon sipedon (7.6 gm %). Therefore, it appears that perfusate protein concentrations in the present study were physiological.

The fluctuating nature of the plasma protein levels within the present study were also found in Miller's rat perfusion work (1965). The rise in the protein levels within the present study is most likely due to protein anabolism by the snake liver. When blood glucose levels were related to protein activity (Fig. 13), both levels showed inverse trends. Perhaps the liver was utilizing glucose as an energy source for protein production, hence, the inverse relationship. The disappearance of the plasma proteins could be attributed to: (1) the formation of

uric acid (2) the formation of free amino acids (3) utilization as an energy source or (4) glyconeogenesis.

As mentioned previously, in perfusions 2, 3, and 5, there was a leveling off of the total protein concentration after the fourth hour of perfusion. This was attributed to the nitrogen-sparing action of the carbohydrates.

Also, the cyclic patterns of perfusions 2, 3, and 5 (Fig. 9) revealed that the amount of plasma protein catabolized during a particular interval was closely equivalent to the amount synthesized by the subsequent interval (Table 3D). Thus, it appears that the snake liver was replacing an amount equivalent to what had been removed from the perfusate.

Furthermore, from the present studies it was revealed that there was a very significant negative correlation between the average amount of protein turnover per gm of liver tissue and total liver weight (Table 4 & Fig. 15A). These results could perhaps be explained on the basis that the larger the liver the greater the amount of non-productive tissue per gm of liver weight.

It was concluded from this aspect of the study that the livers in this system maintained their ability to metabolize the plasma proteins for at least five hours.

C. PLASMA PROTEIN PATTERNS:

Figure 11 shows the percentage composition of the total protein represented by each band as a function of perfusion time. It can be seen that there were definite changes within the various bands of each plasma fraction during the perfusion.

The bands fell into groupings according to their relative percentage of composition and pattern of change. The rank order according to

percentage of composition was grouped as follows; 5, 4, 1, 2, and 3 (Fig. 11). Most of the fluctuation was within bands 4 and 5. In all cases, band 5 showed the greatest amount of change usually in the form of an increase. It is suggested that this increase was due either to an increase within the gamma globulin fraction or to an increased production of some other globulin fraction. The next bands to show the greatest amount of change were 2 and 3. Band 1 exhibited the least amount of change. It was apparent that bands 2 through 5 (the globulins) showed a greater amount of activity than band 1 (albumin). Miller and Bale (1949) found similar results while working with the plasma proteins of dogs. Specifically, they found that the globulin fractions were metabolized at a faster rate than the plasma albumin fractions. Furthermore, Miller and Bale (1949) noted that a high amino acid level of the blood favored albumin production while a low amino acid level favored globulin production. Since exogenous amino acids were not added to the snake liver system, globulin production was favored over the albumin.

Several types of patterns were found to occur within the bands of each perfusion, they were: (1) an inverse trend between bands; (2) a direct relationship (similar trends);and (3) those trends which showed signs of possessing both inverse and direct relationships. Bands 4 and 5 and 1 and 5 were found to possess inverse relationships. Those bands having direct relationships were; 1 and 4, 1 and 2, and 1 and 3. Bands 3 and 4 showed ssigns of being both inverse and directly related. It was found that the albumin fraction generally followed the patterns of the globulins except for band 5. Similar results were also found by Miller and Bale (1949) when they showed that the plasma and globulin fractions of dogs possessed similar trends when being metabolized.

D. GLUCOSE:

The perfusate glucose levels of the five perfusions were found to range from 65 to 170 mg %. Hutton (1958) found the glucose levels in Natrix to range from 15.7 to 96.0 gm %. The values of the present study were a little higher than Hutton's. However, Hutton found that glucose levels increased as much as 30 to 40% if the animal had been excited prior to these tests. Perhaps this is the reason for the higher levels, since the preparation for anesthetization of these animals did cause definite outward signs of excitement. In comparison, these glucose levels were considerably lower than those found by Sokal and Miller (1958) in rats (90 to 550 mg %).

As has been previously mentioned, the levels of blood glucose fluctuated in a cyclic or staircase manner throughout the five perfusions (Fig. 12). Similar trends have been observed in studies by Sokal and Miller (1958) and by Haft and Miller (1958) while working with isolated perfused rat livers.

Also, as in the total proteins, the present studies revealed inverse relationships between blood glucose turnover and liver weight (Table 7 & Fig. 15B). This can possibly be explained by the fact that the larger the organ the greater the non-productive tissue per gm of liver weight. Again, as in the urea production of rats, correlations between glucose production and liver weights as found by Sokal and Miller (1958) and Haft and Miller (1958) were very poor. This was perhaps due to the fact of uneven circulation within the multilobed rat liver.

Although the exact metabolic pathways involved with the fluctuating glucose levels were not determined in this study, tentative explanations are proposed. The decrease in the level of perfusate

glucose could be attributed to: (1) the use of glucose as an energy source perhaps in protein production as was pointed out in Figure 21; (2) glycogenesis; (3) lipogenesis as found by Haft and Miller (1958) in rats; or (4) the production of amino acids (proteins). The increase in the level of perfusate glucose may be due to: (1) glycogenolysis; (2) lipid oxidation; and (3) amino acid (protein) metabolism.

It was concluded from this study that the livers retained their ability to metabolize glucose up to five hours in this system.

E. BILE PRODUCTION:

Bile production was recorded in only two perfusions (1 and 2) due to the mechanical difficulties involved in cannulating the minute bile duct. The bile accumulation in perfusion 1 (3 hours) was 0.1 ml, and in perfusion 2 (5 hours) it was 0.15 ml. Miller (1951) found bile secretion totalling 0.7 to 1.5 ml in 6 hours of perfusion (rat livers).

The fact that the bile was not collected in the last three perfusions would lead to speculation that there might be a buildup within the liver and hence within the perfusate. From Table 9 it can be seen that the pH of the perfusate remained relatively constant throughout the five perfusions.

F. HISTOLOGY:

Microscopically there were some minor differences between the tissue of the perfused livers and those of the controls. The present studies revealed that 2 to 5% of the perfused tissue showed signs of necrosis according to the duration of the perfusion. These histological observations seem to closely follow those of Miller (1951) in which he found little necrosis in rat liver tissue perfused for 6 hours.

SUMMARY

A. URIC ACID:

1. It would appear that the perfusate levels of uric acid in the present study were physiological.
2. Endogenous uric acid production continued for 4 hours before showing signs of a nitrogen-sparing action taking place between hours 4 and 5.
3. A very significant positive correlation was found between uric acid production and liver weight.

B. PERFUSATE PROTEIN:

1. It would appear that the perfusate protein levels within the present study were physiological.
2. Plasma protein concentration changed considerably during the the perfusion. The amount of protein catabolized was closely equivalent to the amount resynthesized.
3. The perfusate proteins were found in three perfusions to level off after the fourth hour, possible showing signs of a nitrogen-sparing mechanism.
4. A significant inverse relationship existed between the perfusate protein and glucose levels.
5. A very significant negative correlation was found to exist between perfusate protein turnover and liver weight.
6. In all cases, the livers maintained their ability to metabolize the perfusate proteins for 5 hours.

C. PLASMA PROTEIN PATTERNS:

1. Electrophoresis revealed 5 distinct bands within the plasma protein fraction. The chemical nature and percentage composition of each was determined.
2. Most of the plasma protein activity took place within bands 4 and 5. Bands 2 and 3 were the next most active. Band 1 exhibited the least amount of change.
3. In this system globulin production was favored over albumin production.
4. The plasma globulins (except for band 5) and albumin fractions followed similar trends when being metabolized.

D. GLUCOSE:

1. Glucose levels were slightly higher than similar studies had revealed. This was probably due to the trauma of the anesthesia and the operation.
2. The glucose levels fluctuated in a cyclic or staircase manner.
3. A very significant negative correlation was found to exist between glucose turnover and liver weight.
4. An inverse relationship existed between the glucose and perfusate proteins levels.
5. The livers maintained their ability to metabolize glucose for 5 hours.

E. BILE PRODUCTION:

1. Bile production was recorded in the first two perfusions.

F. HISTOLOGY:

1. There was some necrosis (2 to 5%) found within the perfused tissues due to experimental procedures.

G. It was concluded that the livers within this system remained viable for 5 hours. This system would therefore be an excellent one for further study of hepatic function and particularly if applied to endocrine problems.

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TABLE 1

Perfusate Uric Acid Levels

A. Perfusate uric acid levels for each perfusion (mg %).

B. Total amount of perfusate uric acid produced at the end of each time interval (mg %). Cumulative values.

C. Total amount of perfusate uric acid produced/gm of liver tissue at the end of each time interval (mg %). Cumulative values.

A.

Per. No.	Wet Wt gm	Perfusion Time (Hours)								
	Liver	0	.5	1	2	2.5	3	4	4.5	5
1	20.0	1.5	2.7	2.9	2.8	---	3.3	---	---	---
2	10.0	2.8	3.4	3.5	3.6	3.6	3.7	3.9	3.9	3.9
3	15.5	2.5	3.3	3.3	3.6	4.0	3.9	3.8	3.8	3.8
4	8.0	2.5	2.9	2.9	3.0	---	---	---	---	---
5	14.3	2.9	3.8	3.9	4.0	4.4	4.5	4.9	4.9	4.9

B.

Per. No.	Wet Wt gm	Perfusion Time (Hours)								
	Liver	0	.5	1	2	2.5	3	4	4.5	5
1	20.0	---	1.2	1.3	1.3	---	1.8	---	---	---
2	10.0	---	0.6	0.7	0.8	0.8	1.0	1.1	1.1	1.1
3	15.5	---	0.8	0.8	1.0	1.5	1.4	1.4	1.4	1.4
4	8.0	---	0.4	0.4	0.5	---	---	---	---	---
5	14.3	---	0.9	1.0	1.1	1.5	1.6	2.0	2.0	2.0

C.

Per. No.	Wet Wt gm	Perfusion Time (Hours)								
	Liver	0	.5	1	2	2.5	3	4	4.5	5
1	20.0	---	0.06	0.06	0.07	---	0.09	---	---	---
2	10.0	---	0.06	0.07	0.08	0.08	0.10	0.11	0.11	0.11
3	15.5	---	0.05	0.05	0.07	0.10	0.09	0.09	0.09	0.09
4	8.0	---	0.05	0.05	0.07	---	---	---	---	---
5	14.3	---	0.06	0.07	0.08	0.10	0.11	0.14	0.14	0.14

TABLE 2

A Statistical Evaluation of Perfusate Uric Acid Production

A. Evaluation of perfusate uric acid production at the end of each time interval.

B. Evaluation of perfusate uric acid production/gm of liver tissue at the end of each time interval. Cumulative values.

C. Pearson r correlation coefficient for total perfusate uric acid produced at the end of each time interval. Cumulative values.

A.

Perf. Time	.5	1	2	2.5	3	4	4.5	5
X	1.20	1.30	1.30	---	1.80	---	---	---
X	0.60	0.70	0.80	0.80	1.00	1.10	1.10	1.10
X	0.80	0.80	1.00	1.50	1.40	1.40	1.40	1.40
X	0.40	0.40	0.50	---	---	---	---	---
X	3.90	4.20	4.70	3.80	5.80	4.50	4.50	4.50
X	0.75	0.84	0.94	1.26	1.45	1.50	1.50	1.50
X^2	3.41	3.98	4.79	5.14	8.76	7.17	7.17	7.17
s^2	0.0924	0.1125	0.0925	0.1650	0.1167	0.2100	0.2100	0.21
SS	0.37	0.45	0.37	0.33	0.35	0.42	0.42	0.42
s	0.30	0.34	0.30	0.41	0.34	0.46	0.46	0.46

TABLE 2B

B.

Perf. Time	.5	1	2	2.5	3	4	4.5	5
X	0.060	0.060	0.070	---	0.090	---	---	---
X	0.060	0.070	0.080	0.080	0.100	0.110	0.110	0.110
X	0.050	0.050	0.070	0.100	0.090	0.090	0.090	0.090
X	0.050	0.050	0.070	---	---	---	---	---
X	0.060	0.070	0.080	0.100	0.100	0.110	0.110	0.110
X	0.280	0.300	0.370	0.280	0.380	0.310	0.310	0.310
X	0.1580	0.0184	0.0275	0.0264	0.0362	0.0323	0.0323	0.032
$X10^{-3}$ SS	0.1	0.4	0.1	0.3	0.2	0.3	0.3	0.3
$X10^{-4}$ s^2	0.25	1.00	0.25	1.50	0.66	1.50	1.50	1.50
s	0.005	0.010	0.005	0.012	0.008	0.012	0.012	0.012

TABLE 2C

c.

x= Liver Wt gm	y= .5	1	2	2.5	3	4	4.5	5
20.0	1.20	1.30	1.30	---	1.80	---	---	---
10.0	0.60	0.70	0.80	0.80	1.00	1.10	1.10	1.10
15.5	0.80	0.80	1.00	1.50	1.40	1.40	1.40	1.40
8.0	0.40	0.40	0.50	---	---	---	---	---
14.3	0.90	1.00	1.10	1.50	1.60	2.00	2.00	2.00
x= 67.8	y= 3.90	4.20	4.70	3.80	5.80	4.50	4.50	4.50
x^2= 1008.7	y^2= 3.41	3.98	4.79	5.14	8.76	7.17	7.17	7.17
xy	58.47	62.50	69,23	52.70	90.58	61.30	61.30	61.30
r^2	0.9806	0.7620	0.9092	0.9570	0.8823	0.3644	0.3644	0.364
r	0.91**	0.87*	0.95**	0.99**	0.94**	0.60	0.60	0.6 0

* significant at 0.05 level
** significant at 0.01 level

TABLE 3

Perfusate Protein Levels

A. Perfusate protein levels for each run (gm %).

B. Total amount of perfusate protein produced or utilized (+or -) at the end of each time interval (gm %).

C. Total amount of perfusate protein produced or utilized/gm of liver tissue (gm %).

D. Total amount of perfusate protein produced or utilized/gm of liver tissue (gm %). Cumulative values.

A.

Per. No.	Wet Wt Liver	Perfusion Time (Hours) 0	.5	1	2	2.5	3	4	4.5	5
1	20.0	6.4	6.2	6.9	7.5	---	7.8	---	---	---
2	10.0	3.9	2.4	2.1	2.0	3.4	4.1	2.9	2.2	2.0
3	15.5	3.9	5.0	4.0	6.2	4.8	5.4	6.4	6.5	6.4
4	8.0	4.1	4.9	5.6	5.5	---	---	---	---	---
5	14.3	3.2	2.8	3.3	3.6	3.8	3.9	4.3	4.2	2.9

B.

Per. No.	Wet wt Liver	Perfusion Time (Hours) 0	.5	1	2	2.5	3	4	4.5	5
1	20.0	---	-0.2	+0.7	+0.6	---	+0.3	---	---	---
2	10.0	---	-1.5	-0.3	-0.1	+1.4	+0.7	-1.2	-0.7	-0.2
3	15.5	---	+1.1	-1.0	+2.2	-1.4	+0.6	+1.0	+0.1	+0.1
4	8.0	---	+0.8	+0.7	-0.1	---	---	---	---	---
5	14.3	---	-0.4	+0.5	+0.3	+0.3	+1.0	+0.4	-1.0	-1.4

C.

Per. No.	Wet Wt gm Liver	Perfusion Time (Hours) 0	.5	1	2	2.5	3	4	4.5	5
1	20.0	---	+0.01	+0.04	+0.03	---	+0.02	---	---	---
2	10.0	---	+0.15	-0.03	+0.01	+0.14	+0.07	-0.12	-0.07	0.02
3	15.5	---	+0.07	-0.07	+0.14	-0.09	+0.04	+0.07	+0.01	0.01
4	8.0	---	+0.01	+0.09	-0.01	---	---	---	---	---
5	14.3	---	+0.03	+0.04	+0.02	+0.02	+0.07	+0.03	+0.07	0.09

TABLE 3D

D.

Per. No.	Wet Wt. Liver	Perfusion Time (Hours)								
		0	.5	1	2	2.5	3	4	4.5	5
1	20.0	---	+0.01	---	---	---	+0.09	---	---	---
2	10.0	---	---	---	-0.19	---	+0.21	---	---	-0.21
3	15.5	---	+0.07	-0.07	+0.14	-0.09	---	---	+0.12	-0.01
4	8.0	---	---	+0.19	-0.01	---	---	---	---	---
5	14.3	---	-0.03	---	---	---	---	+0.18	---	+0.16

TABLE 4

Pearson r Correlation Coefficient for Average Perfusate Protein Levels during each Perfusion

	x= Liver wt (wet)gm	y= Av.Protein Levles
x;y	20.0	0.90
	10.0	0.20
	15.5	0.10
	8.0	0.19
	14.3	0.17
x y	67.8	0.75
x^2 y^2	1008.74	0.1230
xy	---	9.30
r^2	---	0.8014
r	---	-0.90*

* significant at 0.05 level

TABLE 5

Percent Composition and Chemical Nature of Electrophoretic Bands

Band No.	% Composition	Gm %/Band	Chemical Nature of Bands
1	16.2	0.62	Albumin
2	10.7	0.42	Glyco- & Lipo- Proteins
3	9.6	0.35	" " "
4	26.7	0.90	" " "
5	36.7	1.45	Glycoprotein - Gamma Glob.

TABLE 6

Perfusate Glucose Levels

A. Perfusate glucose levels for each run (mg %).

B. Total amount of perfusate glucose fluctuation at the end of each time interval (+ or -).

C. Total amount of perfusate glucose fluctuation/gm of liver tissue at the end of each time interval.

A.

Per. No.	Wet Wt gm Liver	Perfusion Time (Hours)								
		0	.5	1	2	2.5	3	4	4.5	5
1	20.0	72.0	65.0	66.0	---	---	---	---	---	---
2	10.0	84.0	114.0	18.0	80.0	72.0	72.0	72.0	72.0	72.0
3	15.5	87.0	84.0	110.0	117.0	141.0	128.0	165.0	156.0	170.0
4	8.0	113.0	83.0	126.0	100.0	---	---	---	---	---
5	14.3	114.0	157.0	41.0	110.0	116.	151.0	---	102.0	147.0

B.

Per. No.	Wet Wt gm Liver	Perfusion Time (Hours)								
		0	.5	1	2	2.5	3	4	4.5	5
1	20.0	---	-7.0	+12.0	-9.0	---	---	---	---	---
2	10.0	---	+29.0	+4.0	-38.0	-10.0	0	0	0	0
3	15.5	---	-3.0	+26.0	+7.0	+24.0	-13.0	+37.0	-9.0	+14.0
4	8.0	---	-30.0	+44.0	-9.0	---	---	---	---	---
5	14.3	---	+43.0	-16.0	-31.0	+6.0	+35.0	---	-49.0	-46.0

C.

Per. No.	Wet Wt gm Liver	Perfusion Time (Hours)								
		0	.5	1	2	2.5	3	4	4.5	5
1	20.0	---	+0.4	+0.6	-0.5	---	---	---	---	---
2	10.0	---	+2.9	+0.4	-3.9	-1.0	0	0	0	0
3	15.5	---	+0.2	+1.7	-0.5	+1.6	-0.8	+2.4	-0.6	-0.9
4	8.0	---	-3.7	+5.4	-1.1	---	---	---	---	---
5	14.3	---	+2.9	-1.1	-2.1	+0.4	+2.4	---	-3.4	+3.2

TABLE 7

Pearson r Correlation Coefficient for Perfusate Glucose Turnover After Two Hours of Perfusion

	x= Liver wt (wet) gm	y= Glucose Turnover
x;y	20.0	1.5
	10.0	7.3
	15.5	2.4
	8.0	10.2
	14.3	6.2
x y	67.8	27.6
x^2 y^2	1008.74	201.78
xy	---	310.46
r^2	---	0.9244
r	---	-0.96**

** significant at 0.01 level

TABLE 8

Body Weight, Length, Surface Area and Liver Weight of Liver Donors

Perfusion	Liver wt (wet) gm	Liver Donor wt	Liver Donor Length (cm)	Liver Donor Surf.Area cm^3
1	20.0	672.0	185.0	71.0
2	10.0	474.0	173.0	41.9
3	15.5	496.5	169.0	55.8
4	8.0	232.0	118.0	36.0
5	14.3	480.6	175.0	53.0

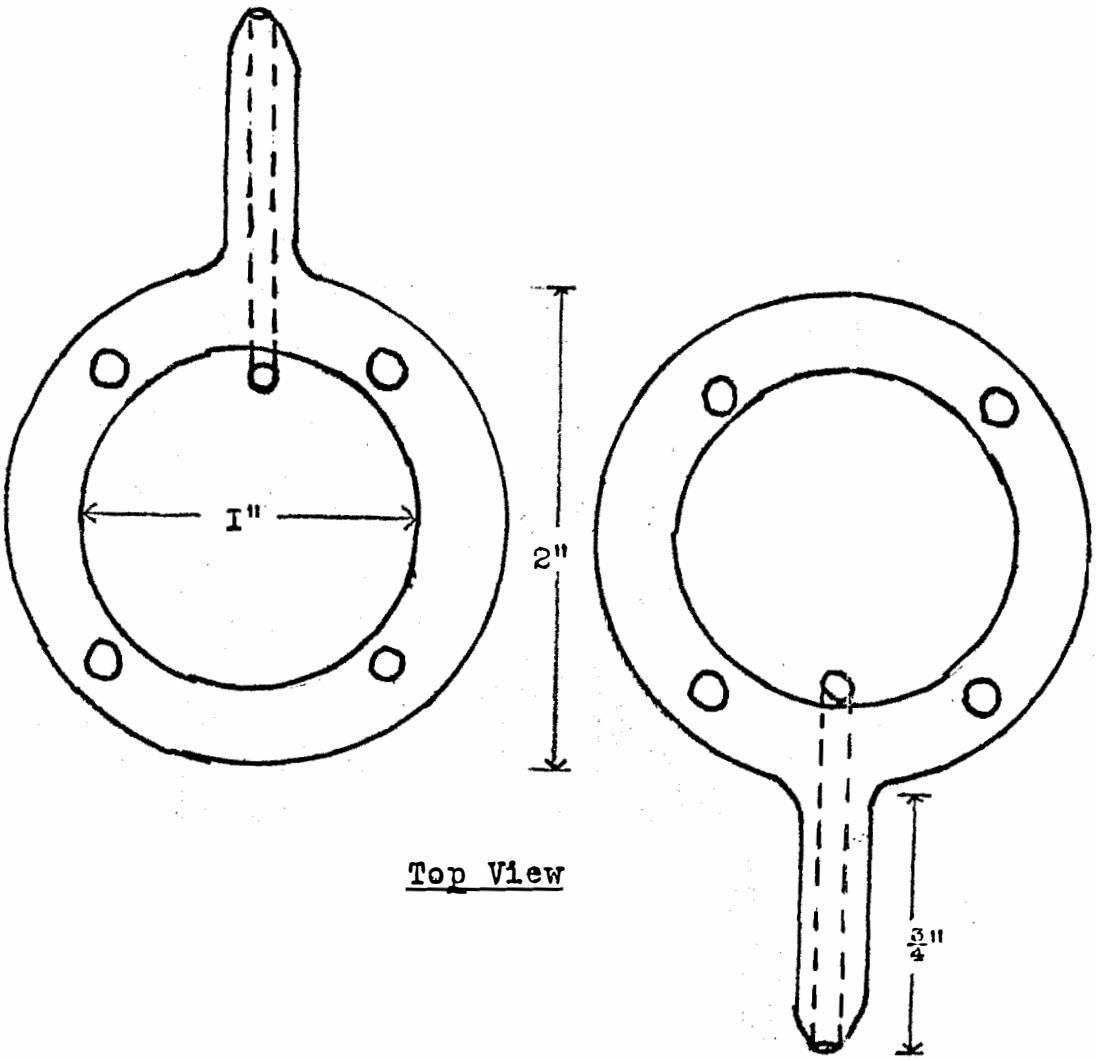
TABLE 9

Perfusate Flow Rates and pH Values

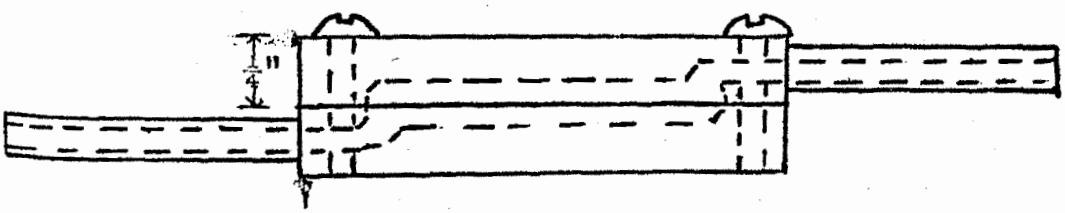
Perfusion	Average Flow Rate (ml/hr)	pH Range
1	192.0	7.30-7.40
2	72.0	7.30-7.40
3	204.0	7.40-7.50
4	180.0	7.40-7.50
5	195.0	7.35-7.45

FIGURE 1

Clot Filter



Top View

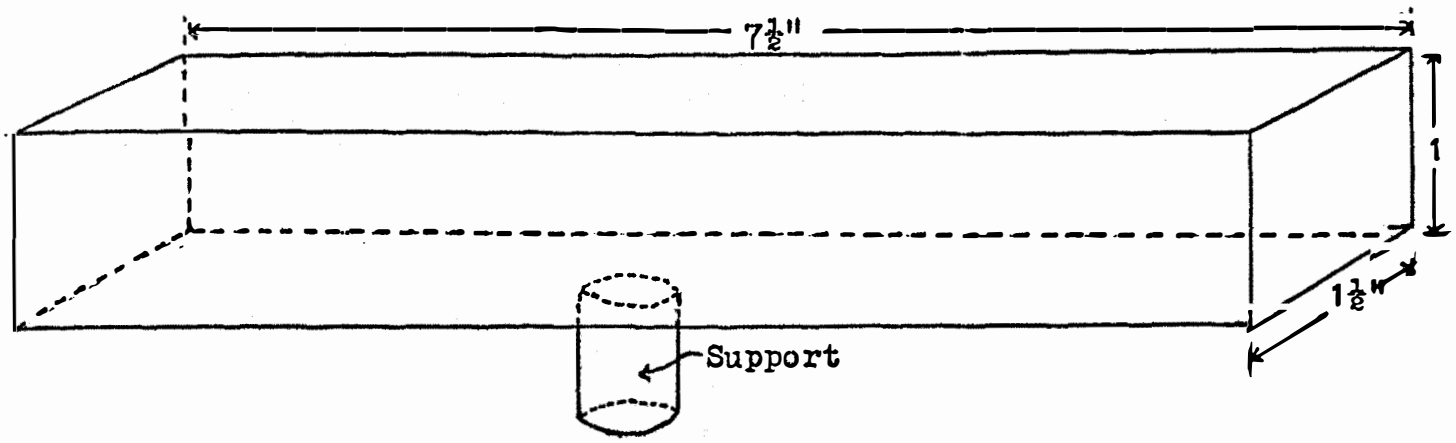


Side View

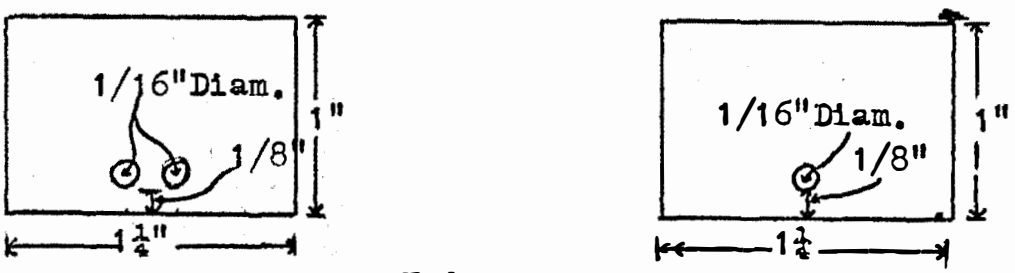
FIGURE 2

Diagram of Liver Chamber

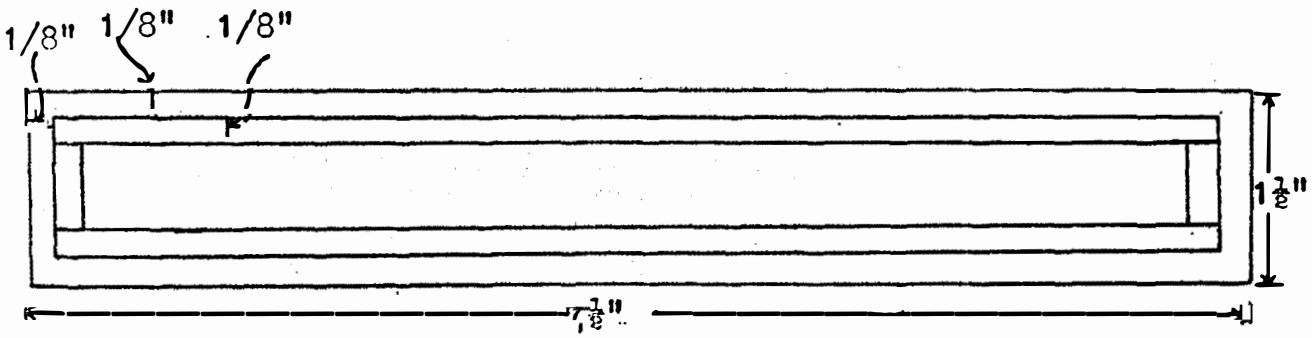
(1/8" Leucite)



Side



Ends



Inside Top

FIGURE 3

Diagram of Perfusion Apparatus Housing

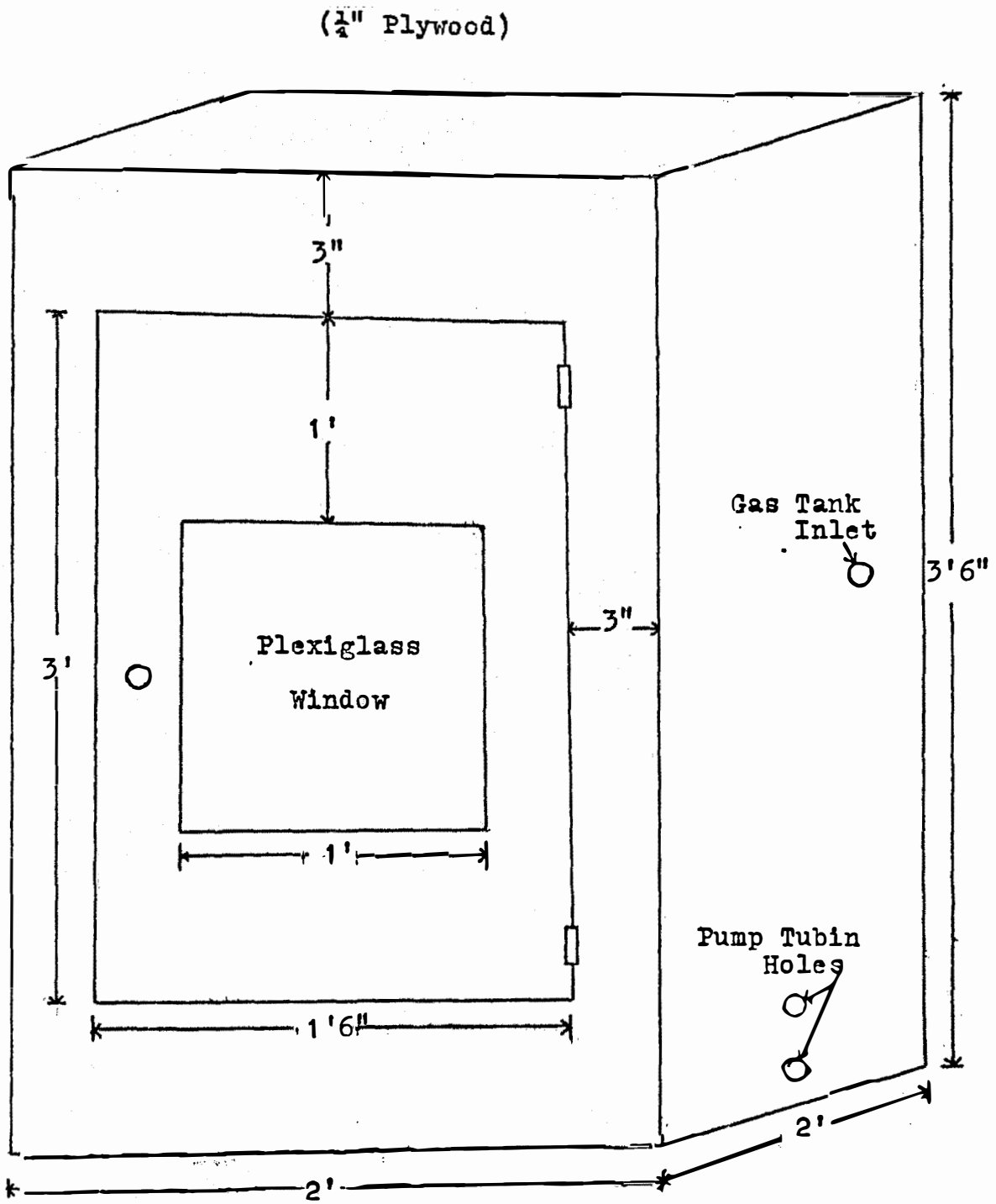

(¼" Plywood)
3"
1'
3'
Plexiglass
Window
1'
3"
Gas Tank
Inlet
3'6"
Pump Tubin
Holes
1'6"
2'
2'

FIGURE 4

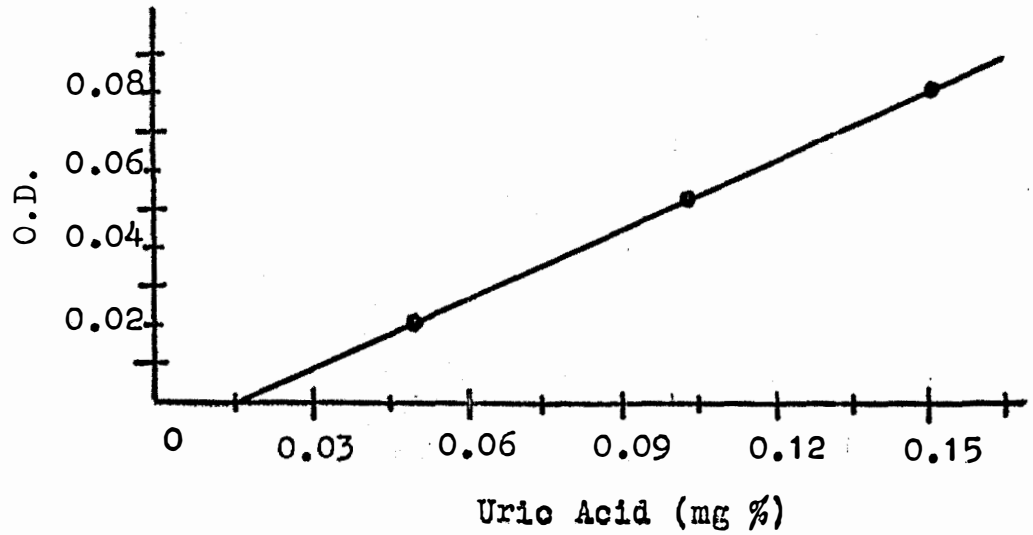
Standard Uric Acid Curve

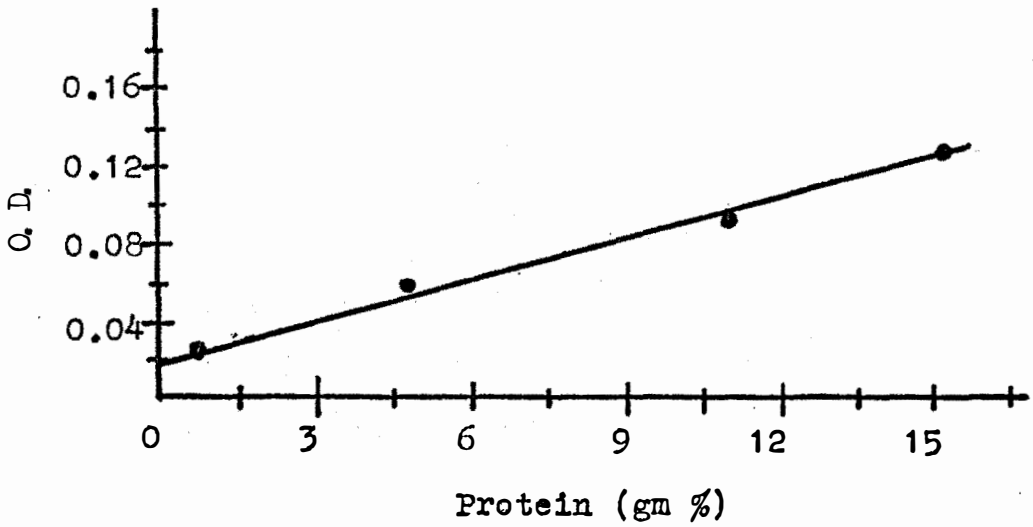
FIGURE 5

Standard Protein Curve

FIGURE 6

Standard Glucose Curve


O.D.
0.08
0.06
0.04
0.02
0
0.03
0.06
0.09
0.12
0.15
Uric Acid (mg %)


O.D.
0.16
0.12
0.08
0.04
0
3
6
9
12
15
Protein (gm %)

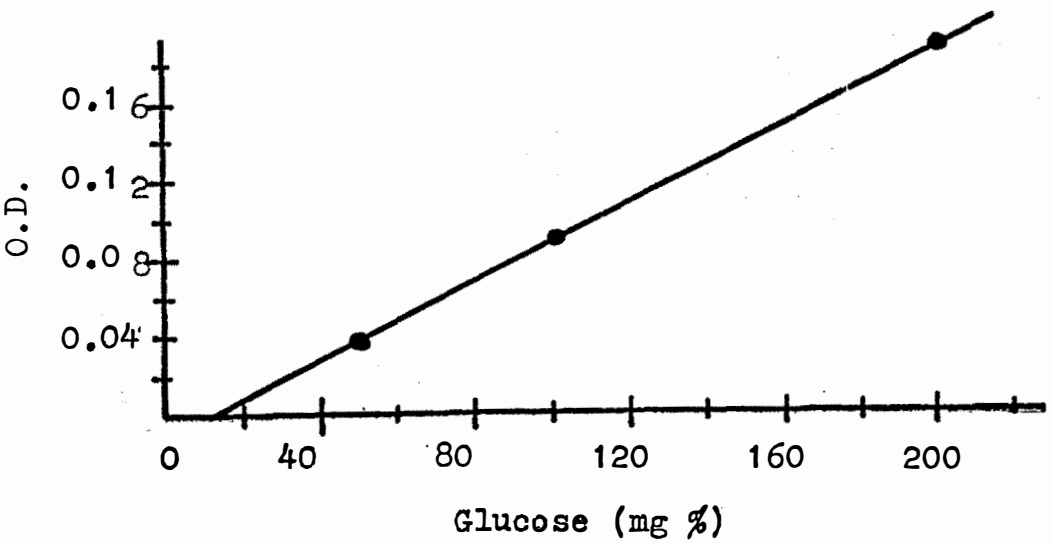

O.D.
0.16
0.12
0.08
0.04
0
40
80
120
160
200
Glucose (mg %)

FIGURE 7

Perfusate Uric Acid Levels

A. Perfusion 1

B. " 2

C. " 3

D. " 4

E. " 5

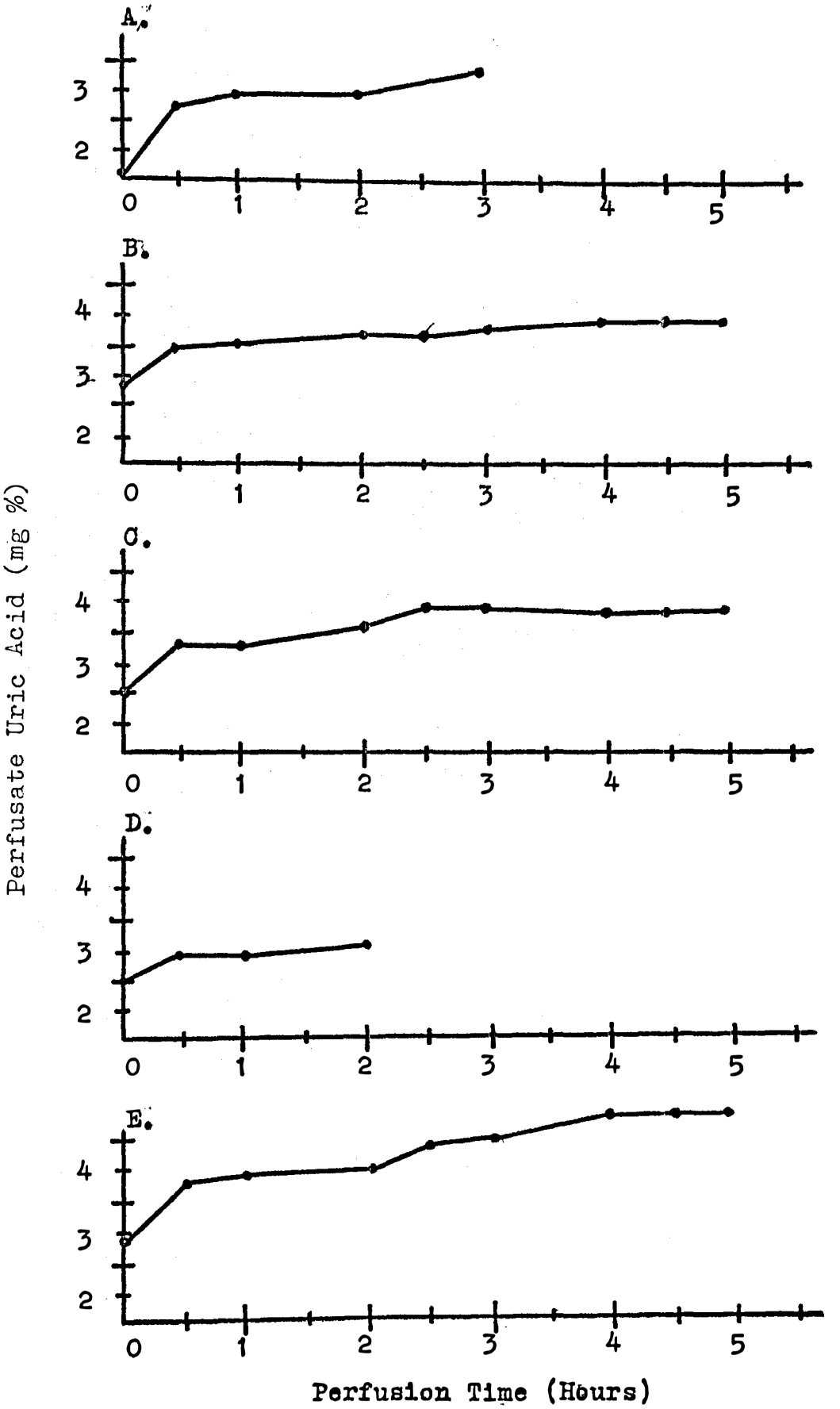

A.
B.
C.
D.
E.
Perfusate Uric Acid (mg %)
Perfusion Time (Hours)

FIGURE 8

Relationship between Perfusate Uric Acid Production and Liver Weight

A. Perfusion Hour 0.5

B. " " 1.0

C. " " 2.0

D. " " 2.5

E. " " 3.0

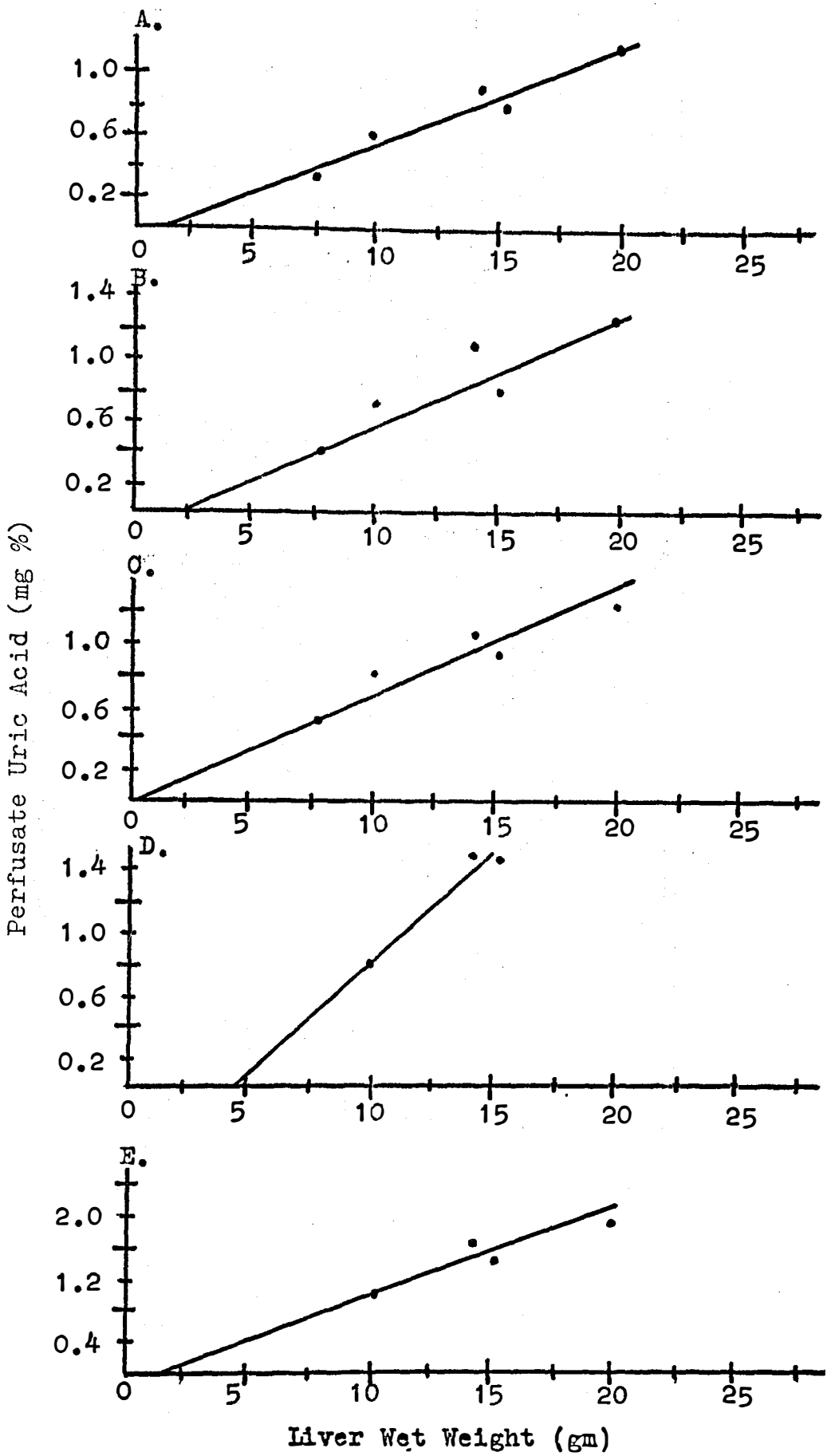

A.
B.
C.
D.
E.
Perfusate Uric Acid (mg %)
Liver Wet Weight (gm)

FIGURE 9

Perfusate Protein Levels

A. Perfusion 1

B. " 2

C. " 3

D. " 4

E. " 5

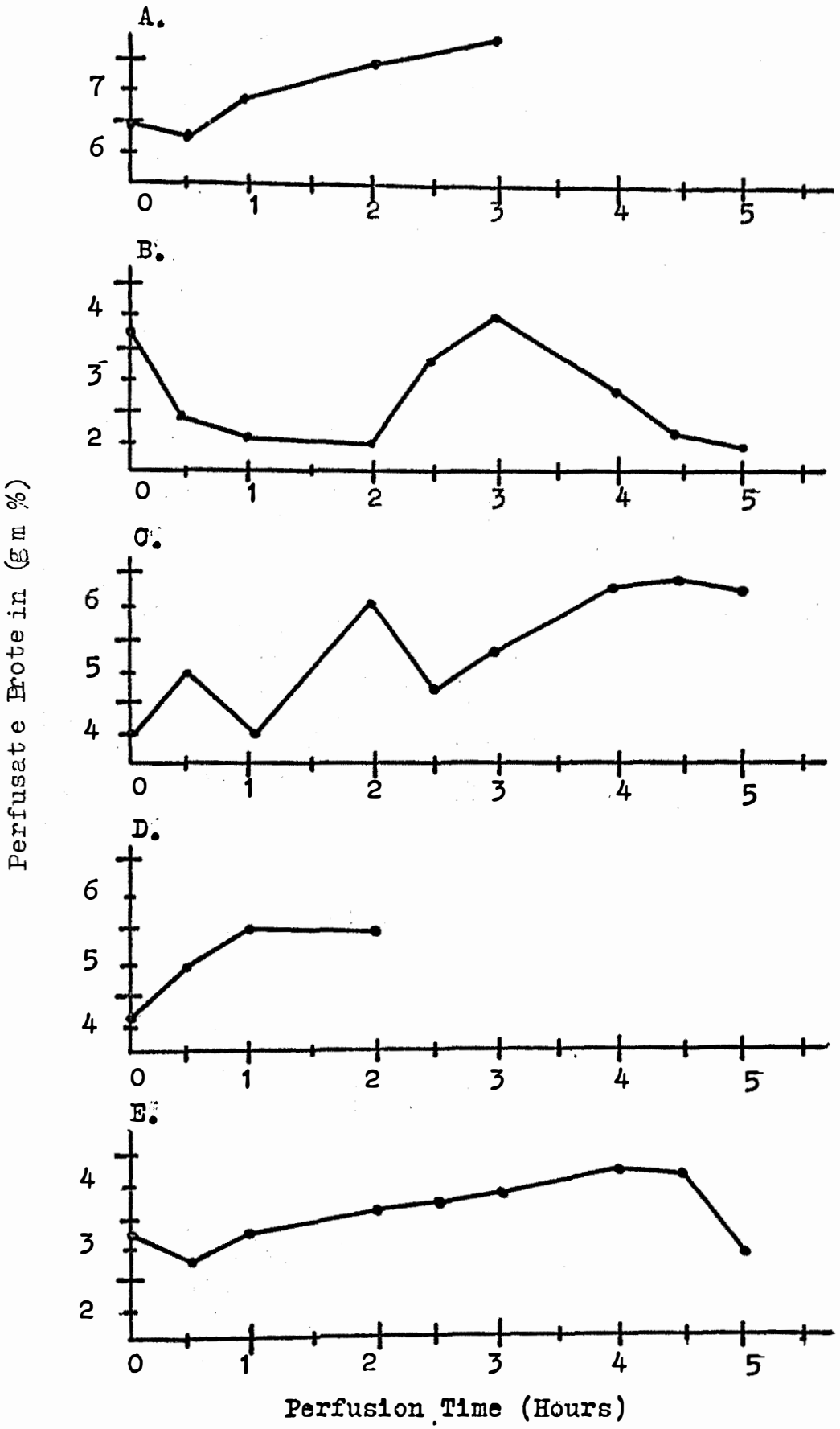
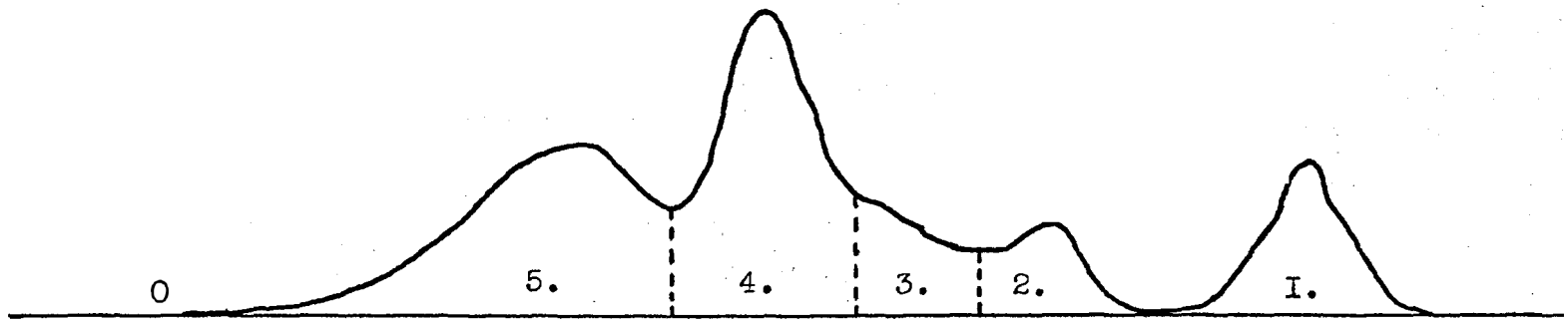

A.
7
6
0
1
2
3
4
5
B.
4
3
2
0
1
2
3
4
5
C.
6
5
4
0
1
2
3
4
5
D.
6
5
4
0
1
2
3
4
5
E.
4
3
2
0
1
2
3
4
5
Perfusate Protein (gm %)
Perfusion Time (Hours)

FIGURE 10

Electrophoretogram of the Plasma Proteins of Natrix fasciata fasciata

0. Origin
I. Albumin
2. Glyco- & Lipo- Proteins
3. " " "
4. " " "
5. Gamma Globulin (glycoprotein)


0
5.
4.
3.
2.
I.

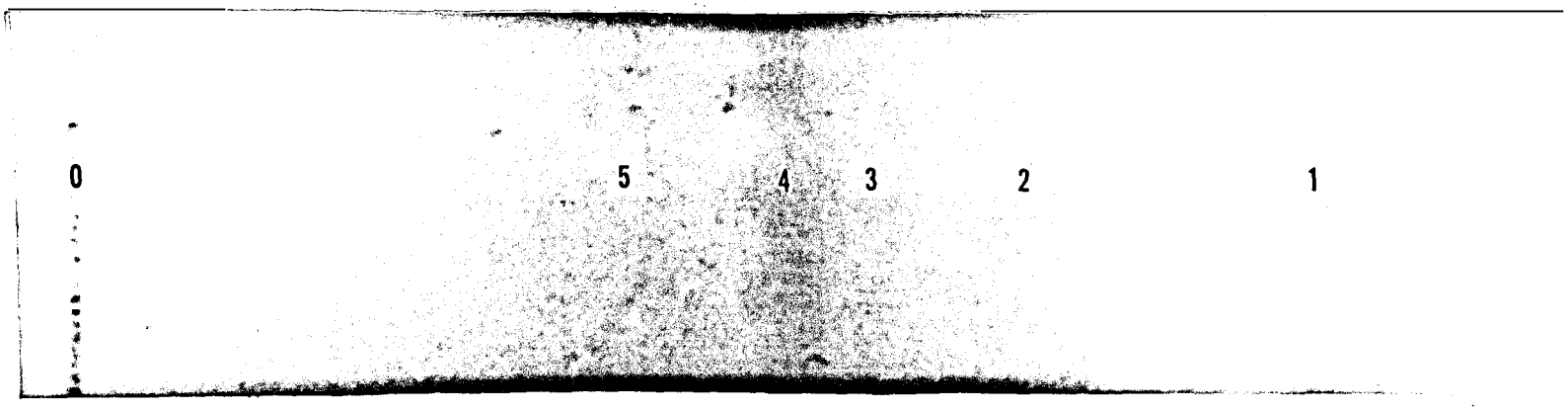

0
5
4
3
2
1

FIGURE 11

Relationship between the Percent Composition of each Electrophoretic Band and Perfusion Time

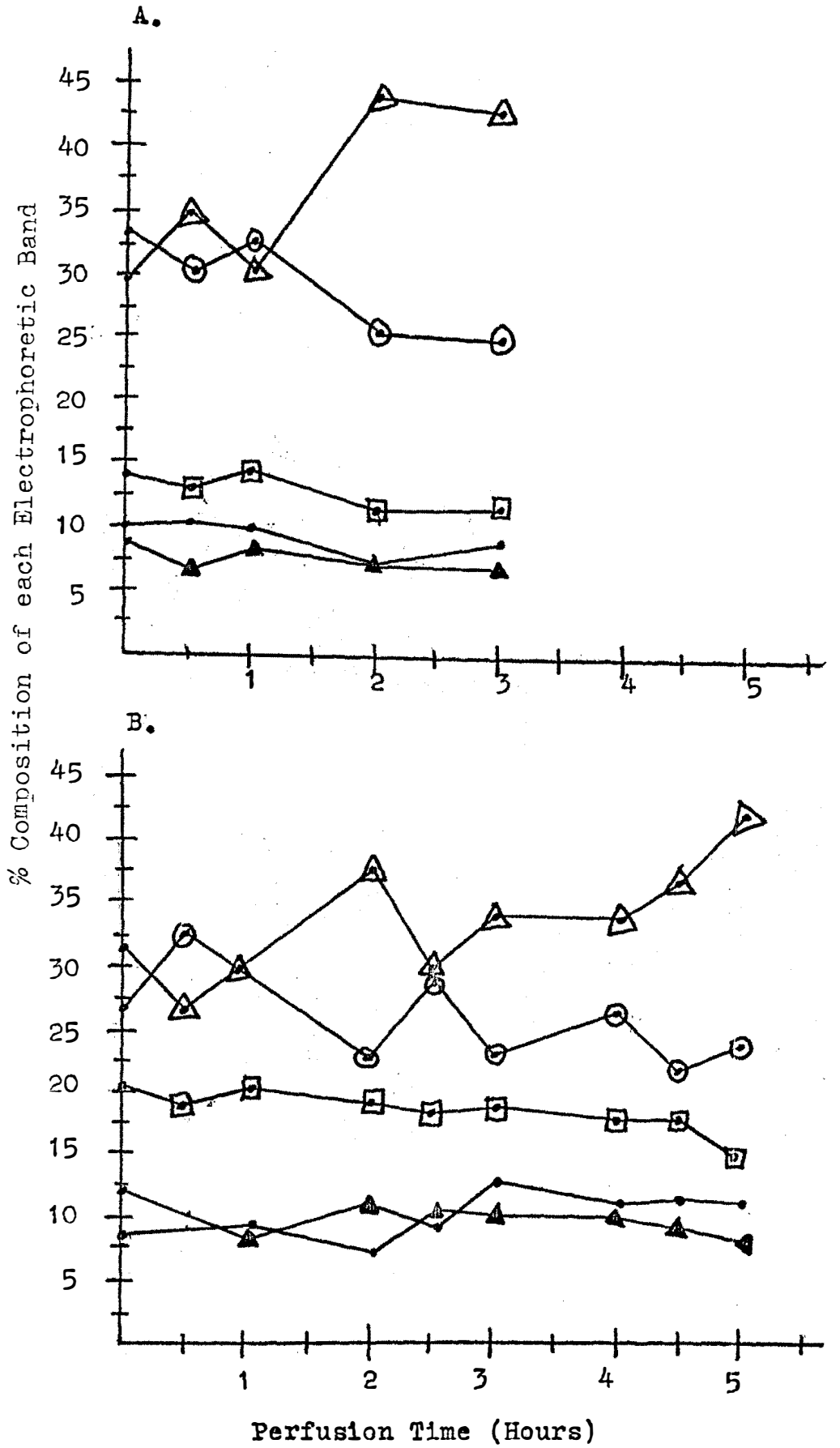
A. Perfusion 1

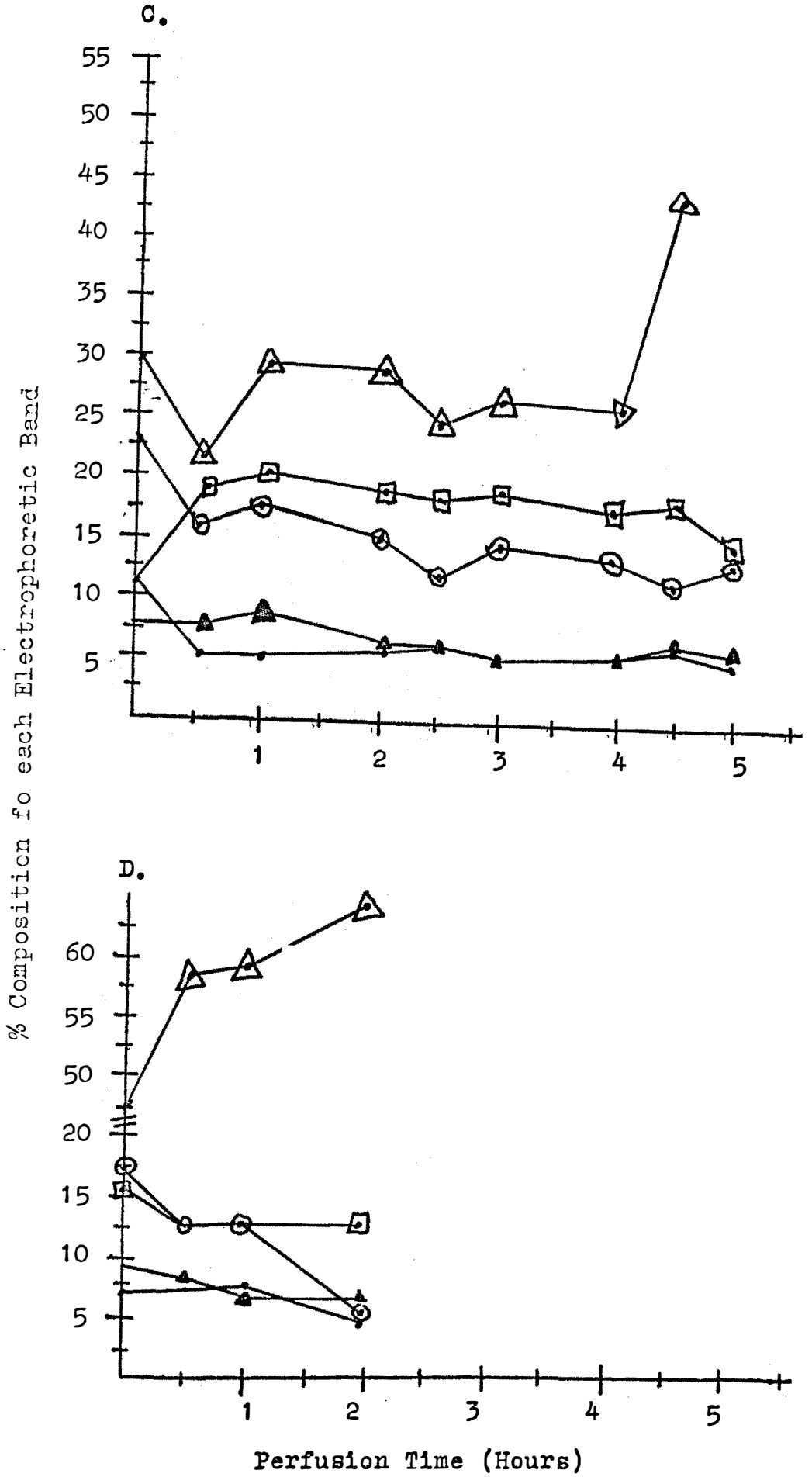
B. " 2

C. " 3

D. " 4

E. " 5


A.
B.
45
40
35
30
25
20
15
10
5
1
2
3
4
5
% Composition of each Electrophoretic Band
Perfusion Time (Hours)


C.
55
50
45
40
35
30
25
20
15
10
5
1
2
3
4
5
D.
60
55
50
20
15
10
5
1
2
3
4
5
% Composition fo each Electrophoretic Band
Perfusion Time (Hours)

E.

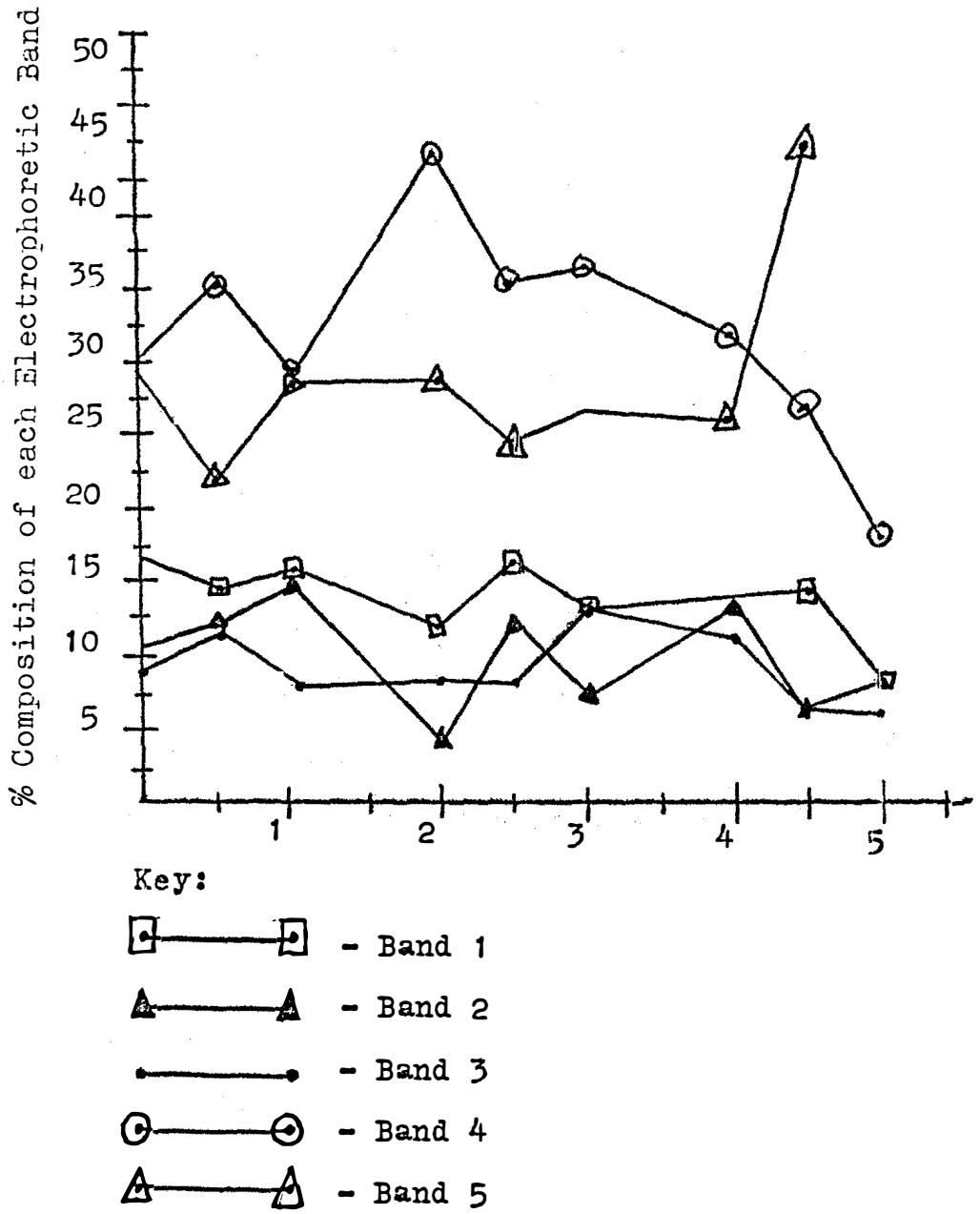

% Composition of each Electrophoretic Band
50
45
40
35
30
25
20
15
10
5
1
2
3
4
5
Key:
- Band 1
- Band 2
- Band 3
- Band 4
- Band 5

FIGURE 12

Perfusate Glucose Levels

A. Perfusion 1

B. " 2

C. " 3

D. " 4

E. " 5

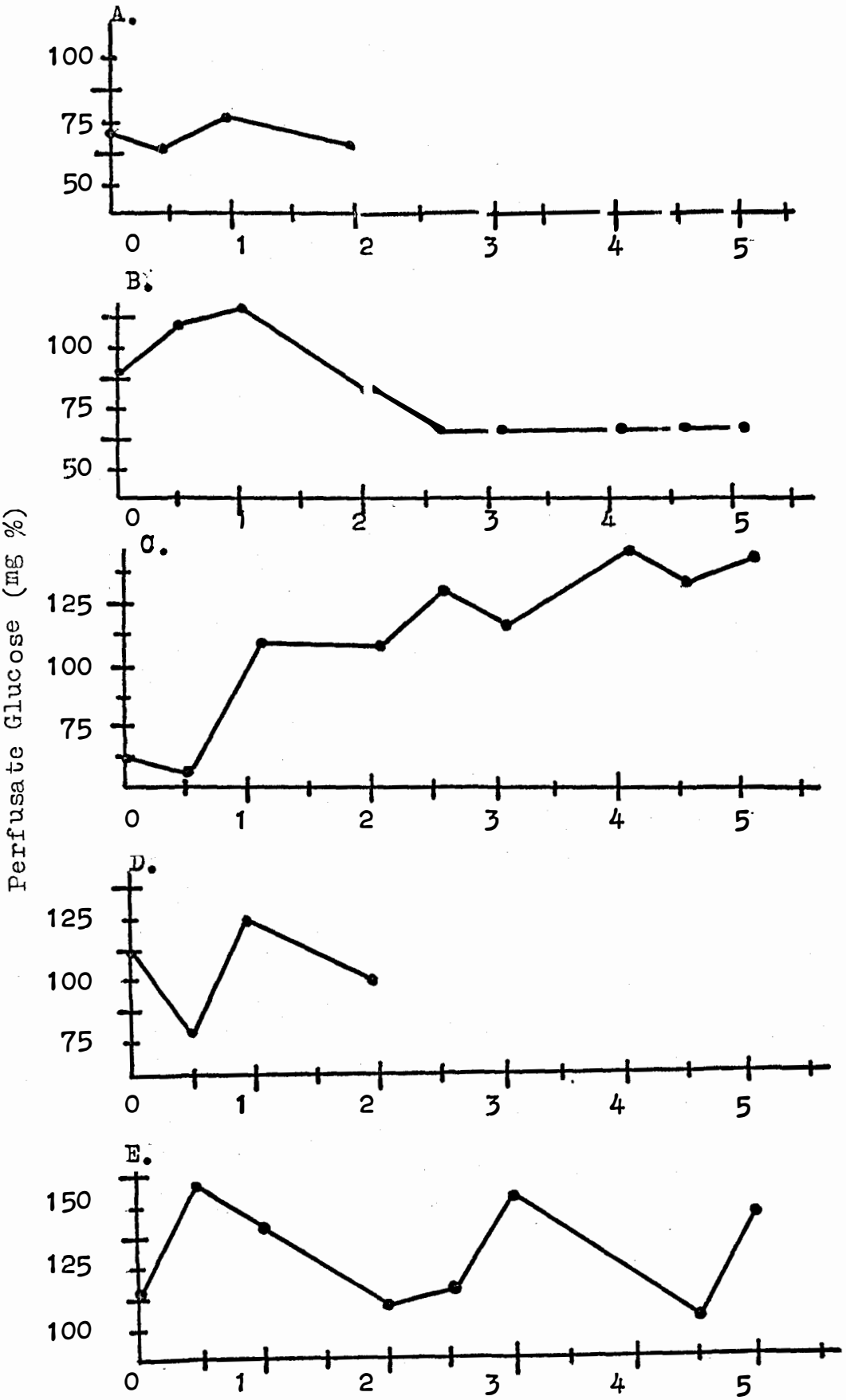

A.
100
75
50
0
1
2
3
4
5
B.
100
75
50
0
1
2
3
4
5
C.
125
100
75
0
1
2
3
4
5
D.
125
100
75
0
1
2
3
4
5
E.
150
125
100
0
1
2
3
4
5
Perfusate Glucose (mg %)
Perfusion Time (Hours)

FIGURE 13

Relationship between Perfusate Protein and Perfusate Glucose Levels

A. Perfusion 1

B. " 2

C. " 3

D. " 4

E. " 5

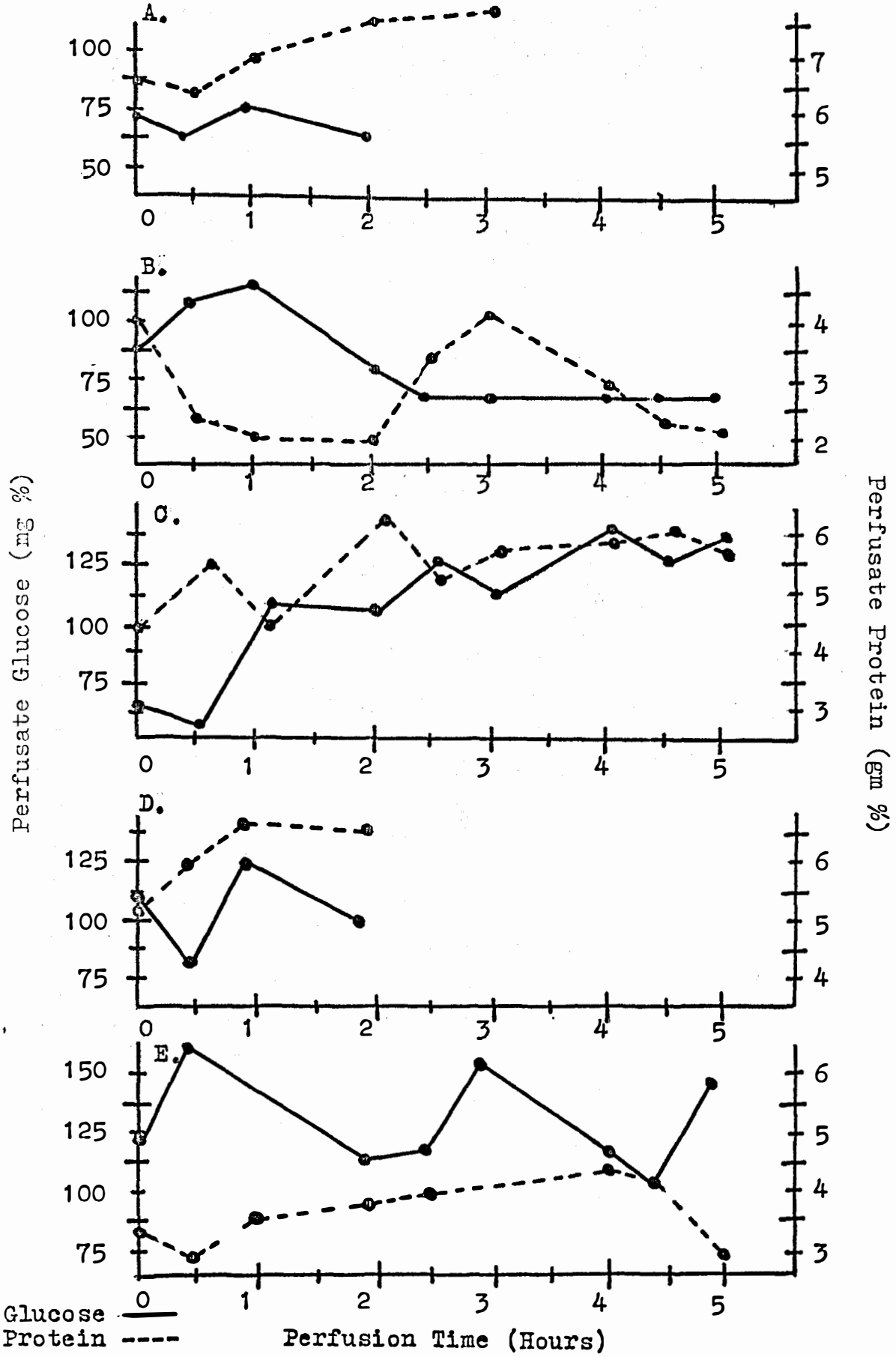
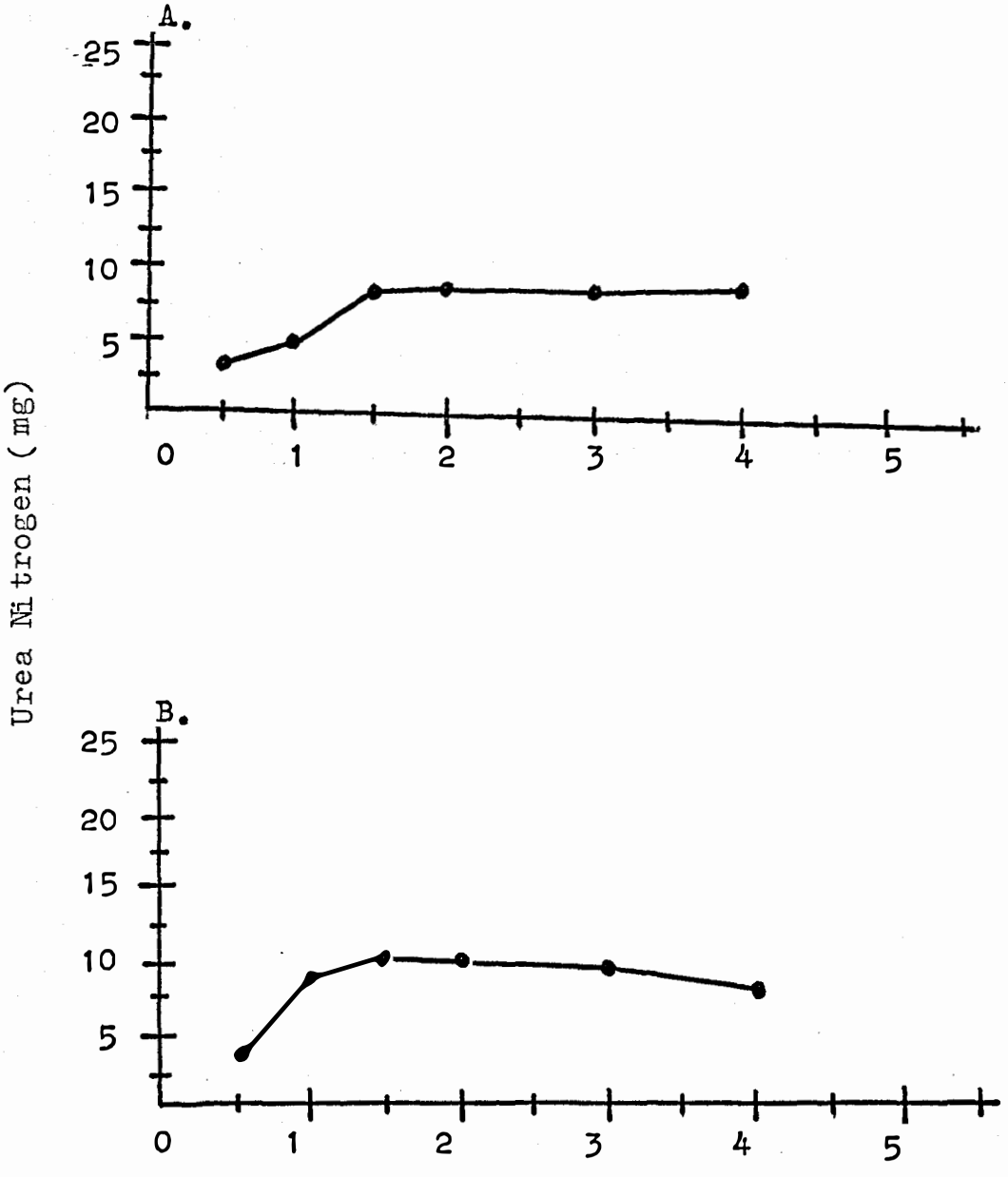

A.
B.
C.
D.
E.
Perfusate Glucose (mg %)
Perfusate Protein (gm %)
Glucose
Protein
Perfusion Time (Hours)

FIGURE 14

The Effects on Urea Production Incident to the Addition of the Amino Acid Mixture with Graded Amounts of Glucose

A. Amino acid supplement added plus 0.5 gm of glucose.

B. Amino acid supplement added plus 1.2 gm of glucose.



Taken from:
Miller, L. L., W. T. Burke, D. E. Haft. 1955. Fed. Proc. 14:710.

FIGURE 15

A. Relationship between average perfusate protein turnover and liver weight.

B. Relationship between perfusate glucose turnover and liver weight (after 2 hours of perfusion).

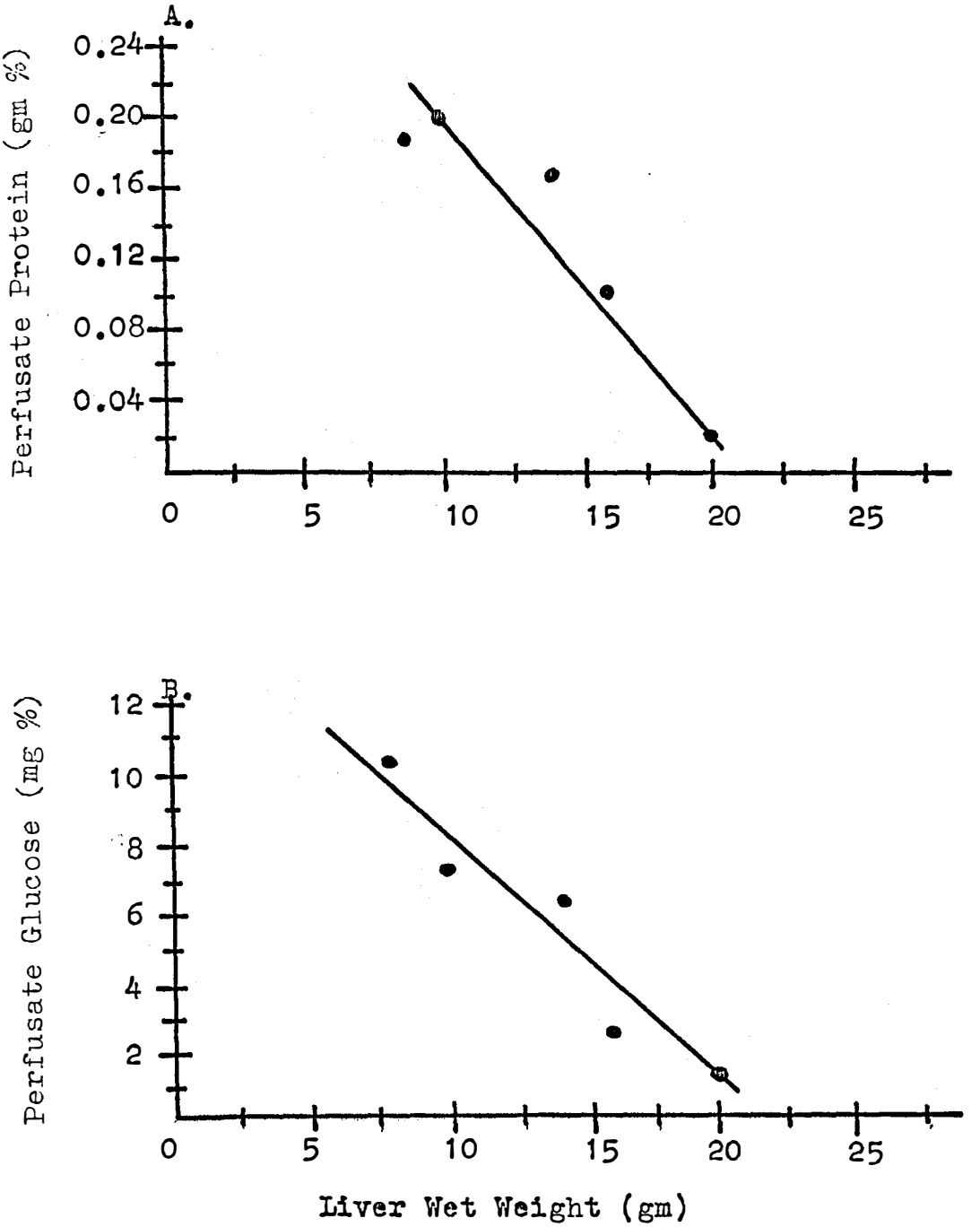

A.
Perfusate Protein (gm %)
0.24
0.20
0.16
0.12
0.08
0.04
0
5
10
15
20
25
B.
Perfusate Glucose (mg %)
12
10
8
6
4
2
0
5
10
15
20
25
Liver Wet Weight (gm)

FIGURE 16

Exposed Snake Liver

FIGURE 17

Perfusion Apparatus

A. Perfusate Reservoir.

B. Clot Filters.

C. "Lung".

D. Gas Humidifier.

E. Liver Chamber.

C
B
D
B
E
A

FIGURE 18

Liver Chamber

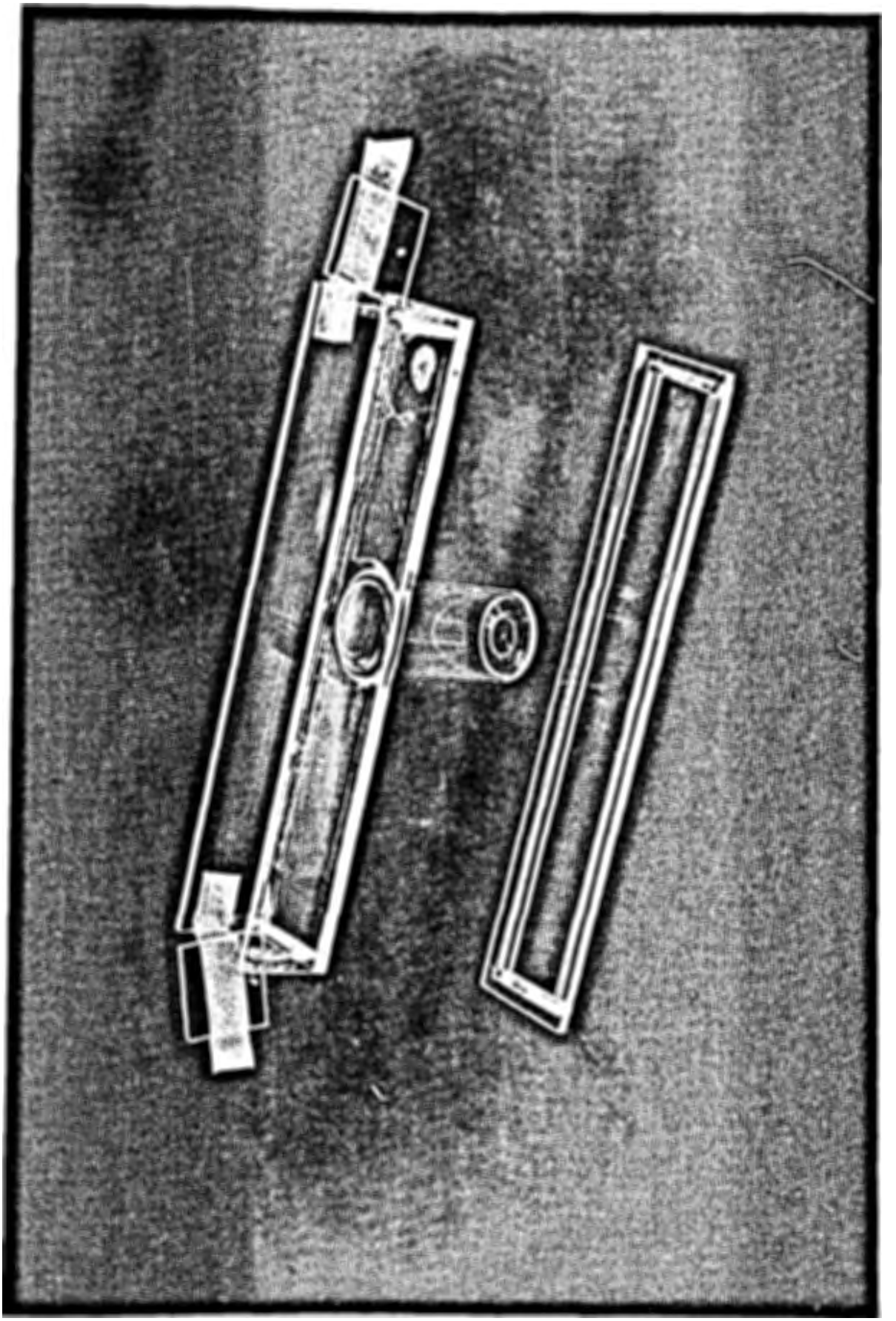


FIGURE 19

Perfusion Apparatus Housing

LIVER PERFUSION

FIGURE 21

Operating Platform

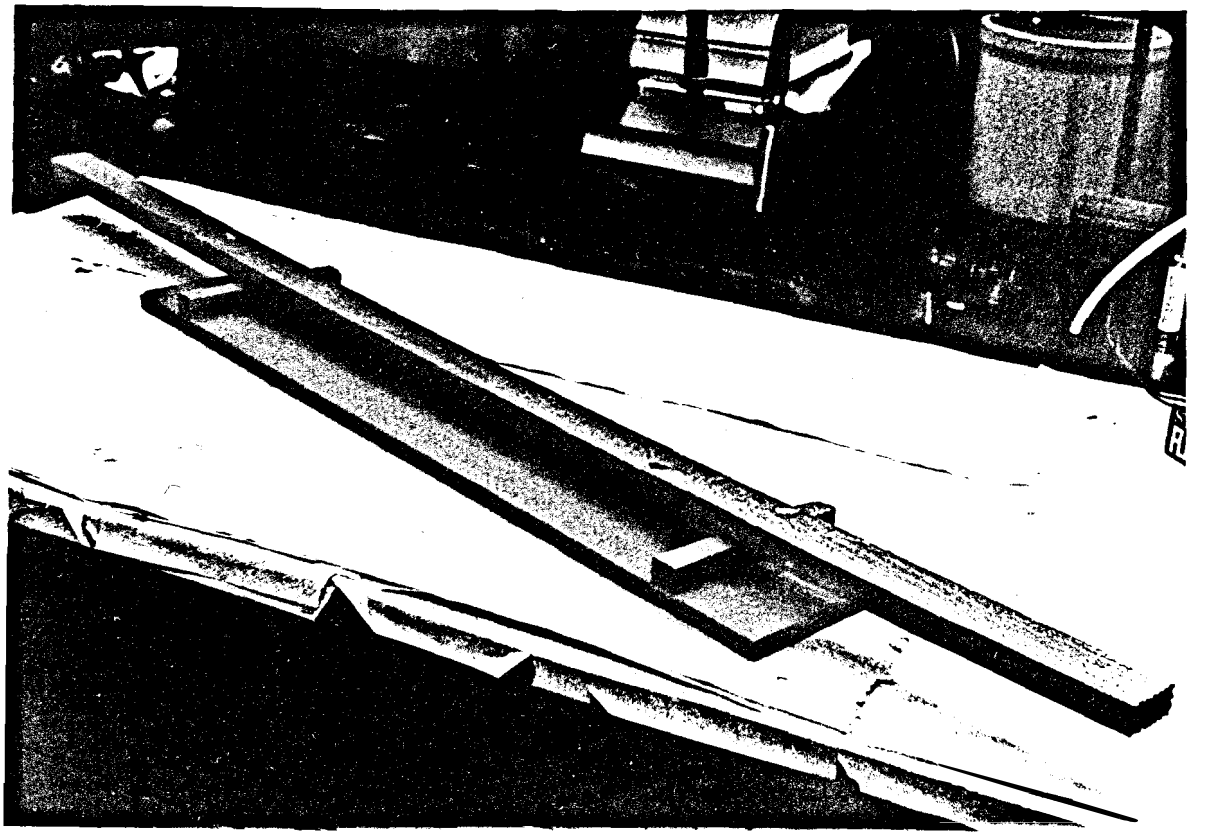
FIGURE 20

Cannulae

A. Postcaval Vein

B. Portal Vein

C. Bile Duct



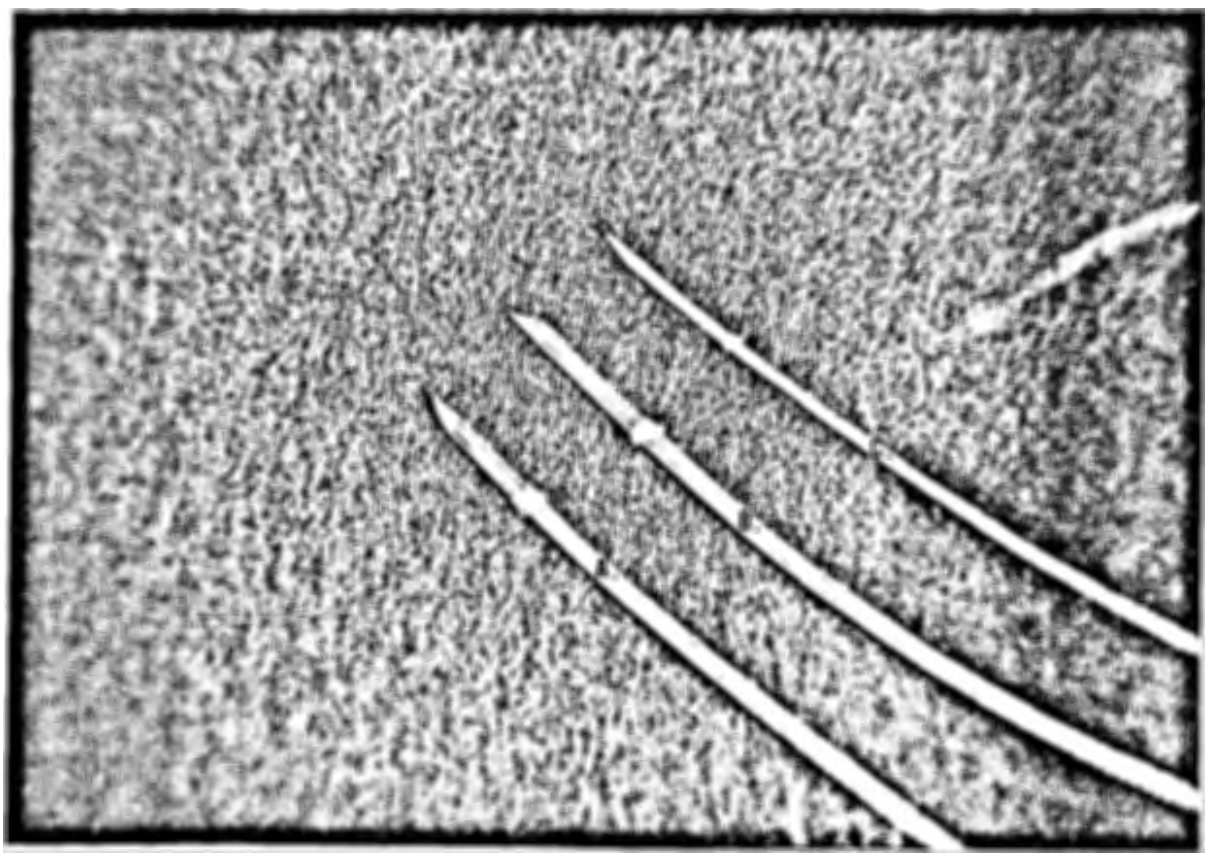


FIGURE 22

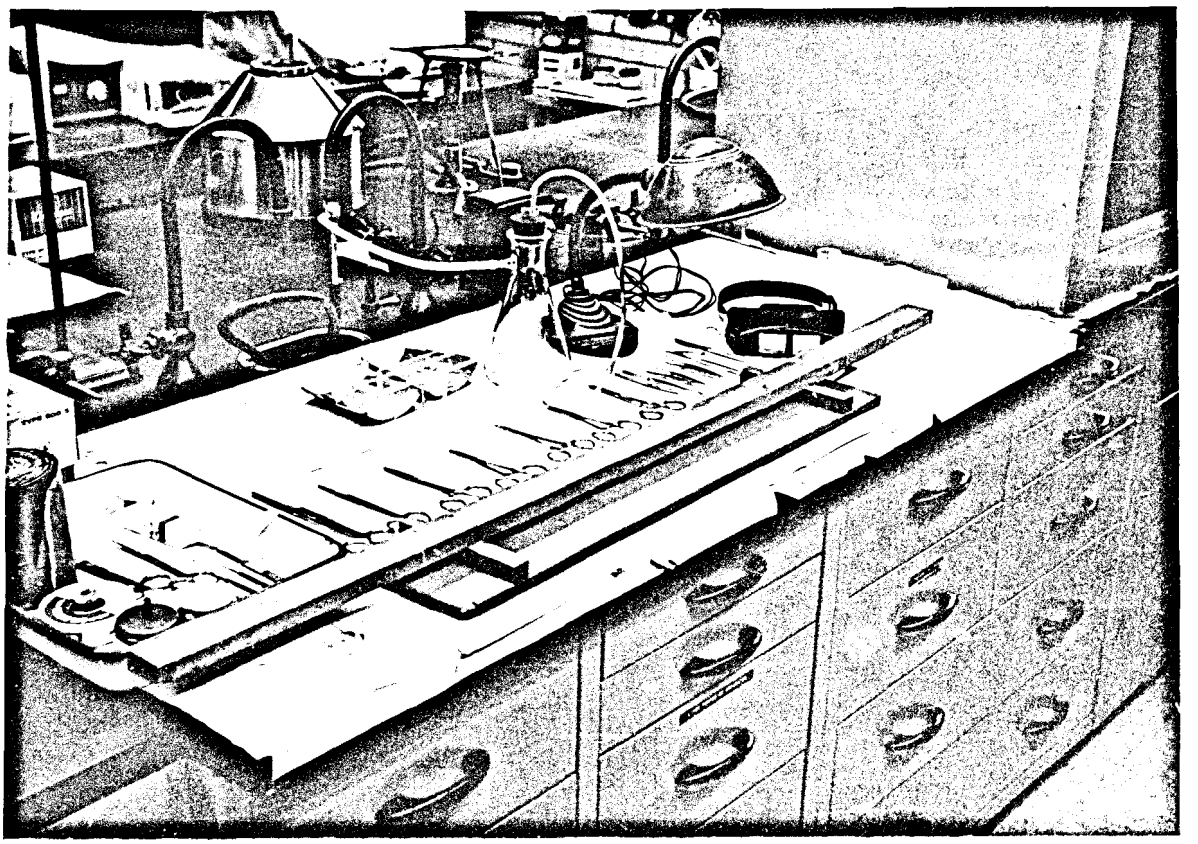
Surgical Area

FIGURE 23

Exposed Portal Vein and Bile Duct

A. Portal Vein

B. Bile Duct



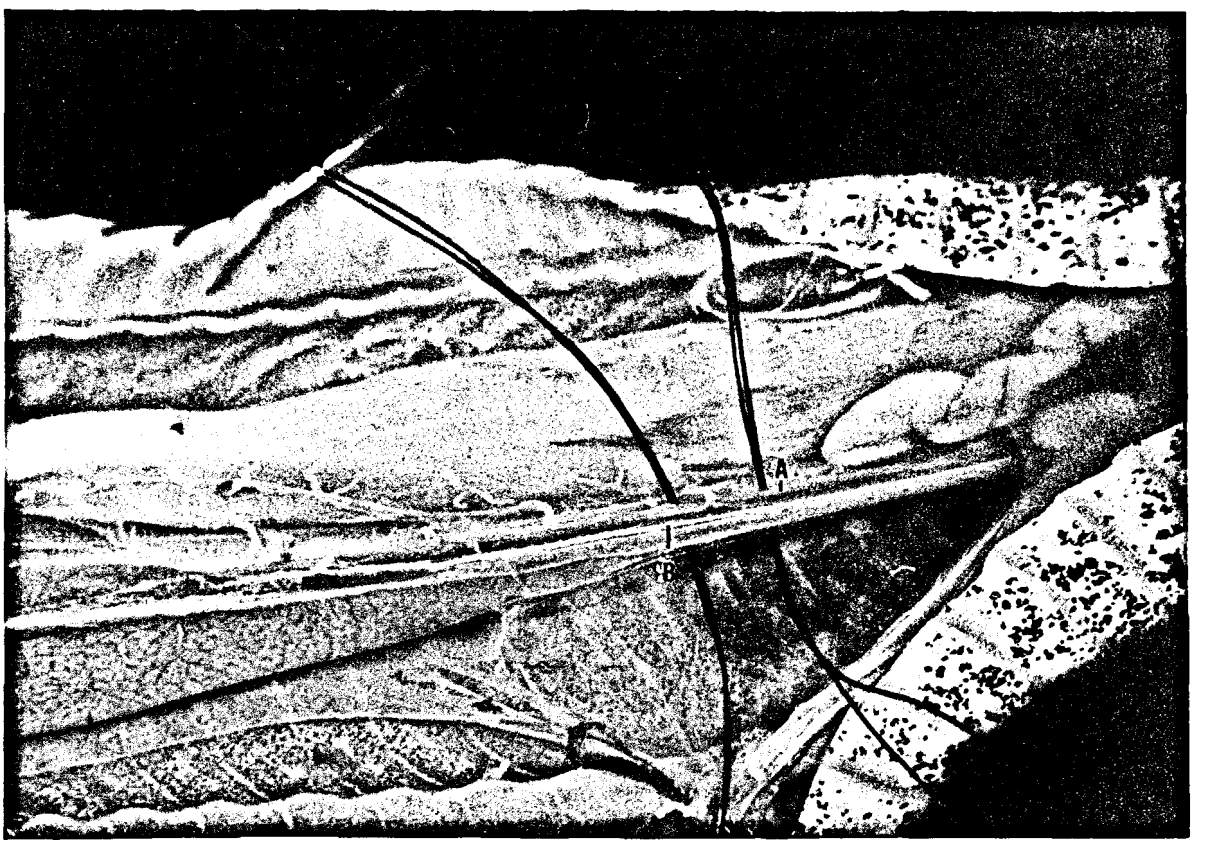
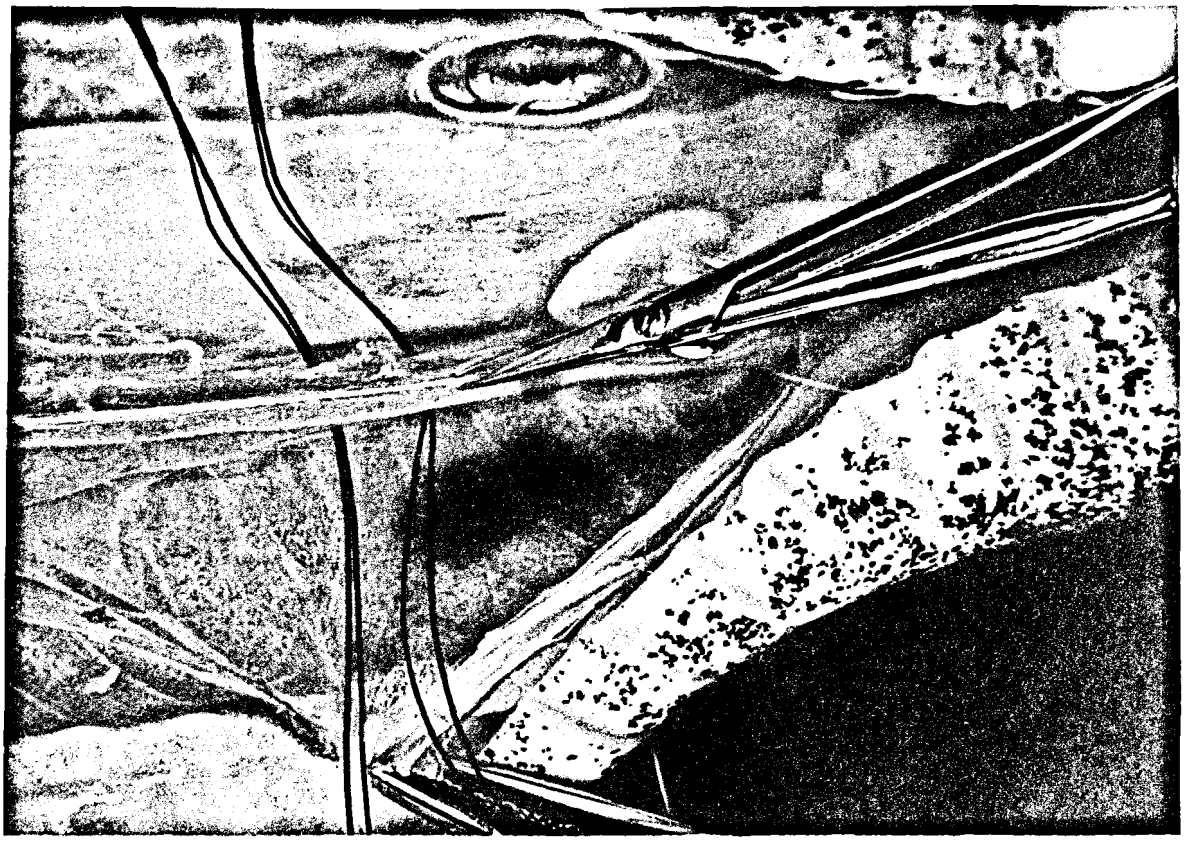

A
B

FIGURE 24

A. Bile Duct Cannulation (top).

B. Cannulated Portal Vein and Bile Duct (bottom).



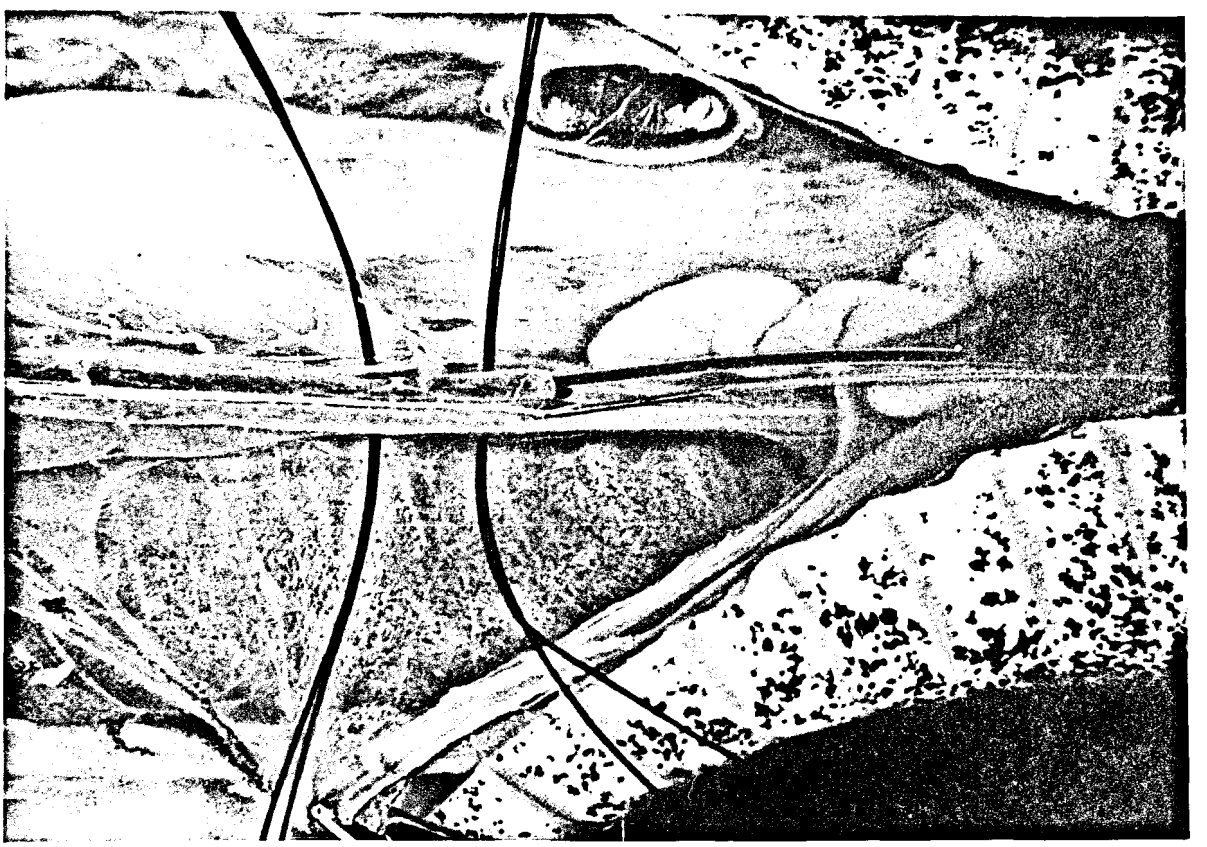
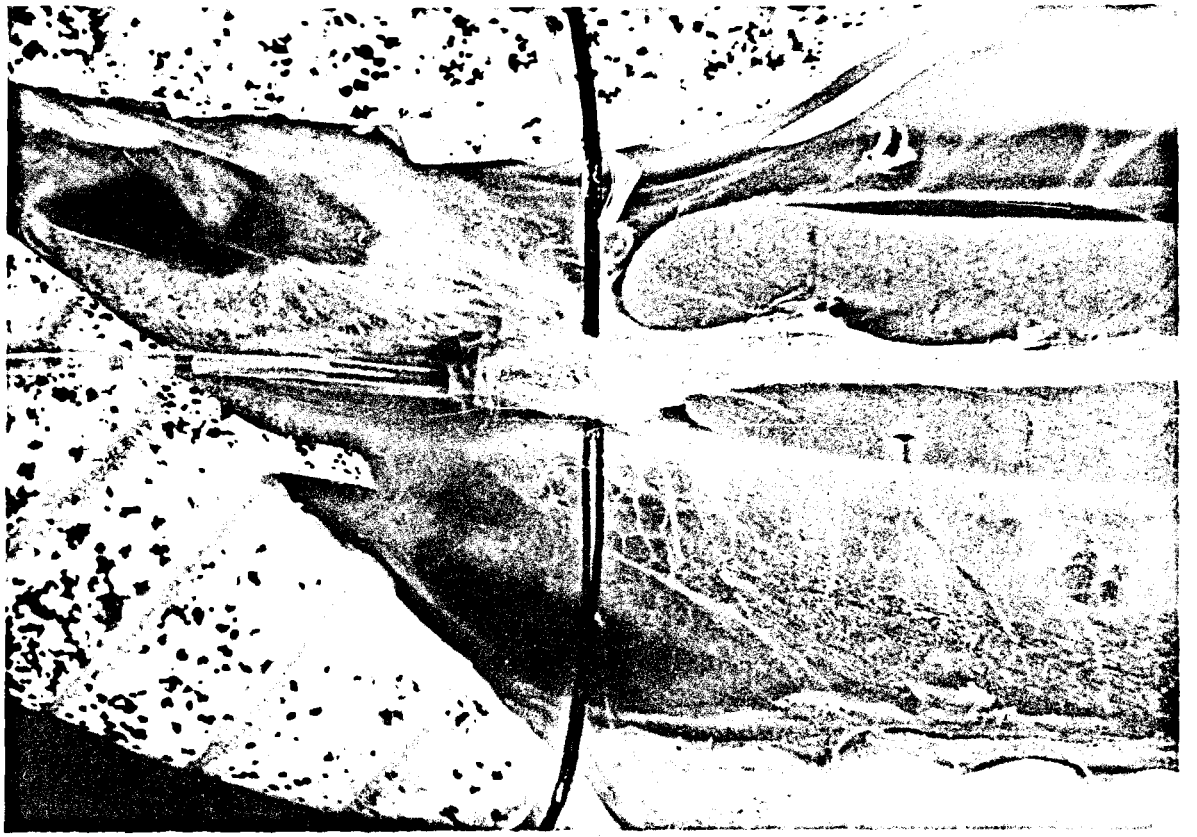


FIGURE 24

D . Cannulated Postcaval Vein (top).

C . Postcaval Vein Cannulation (Bottom).



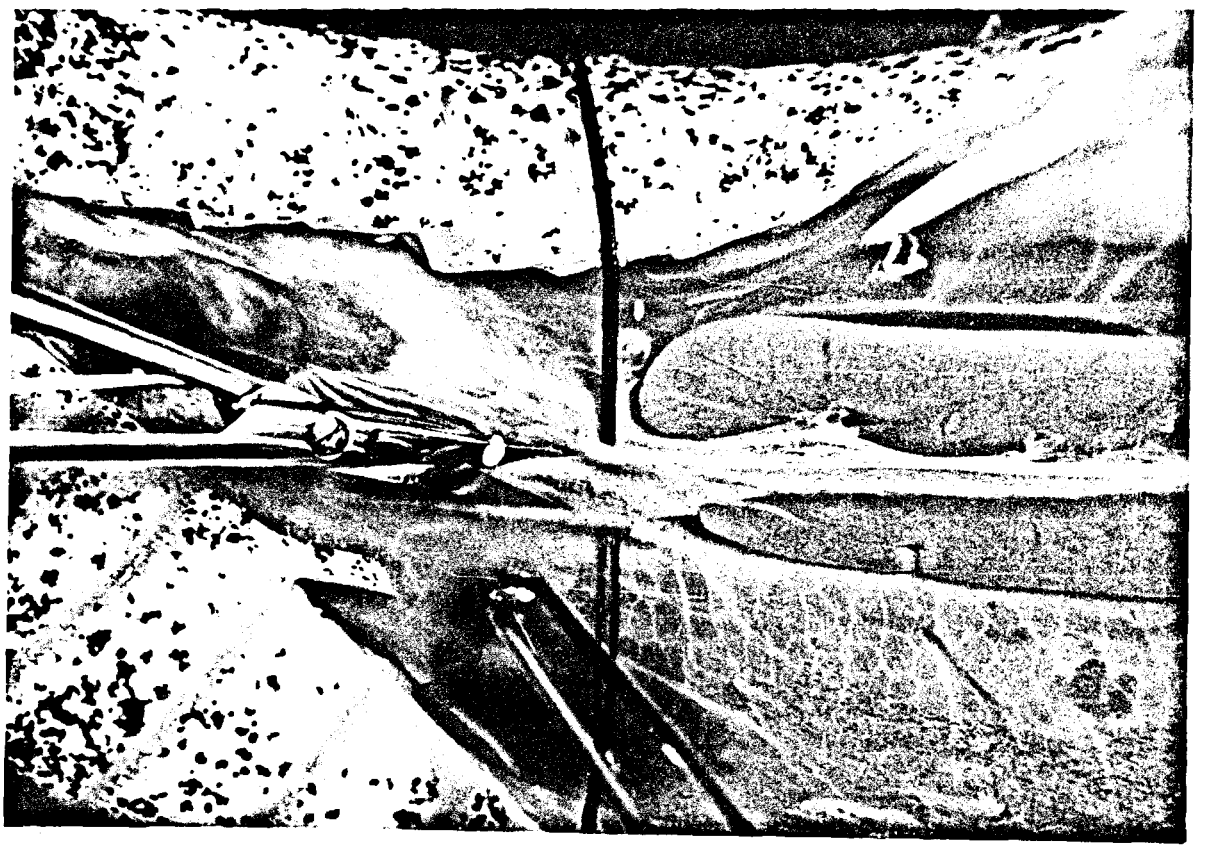
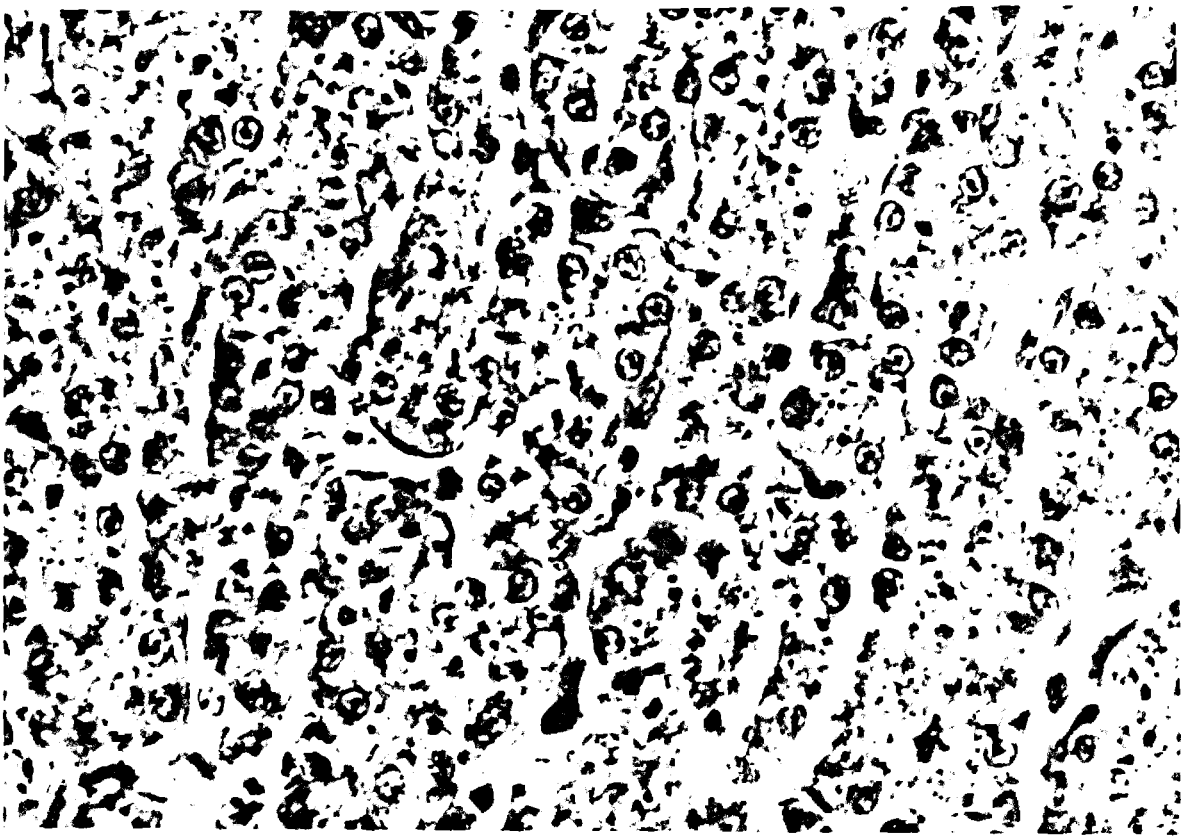


FIGURE 25

Photomicrographs of Control and Perfused Liver Tissue

A. Control (top).

B. Perfusion 1 (bottom).



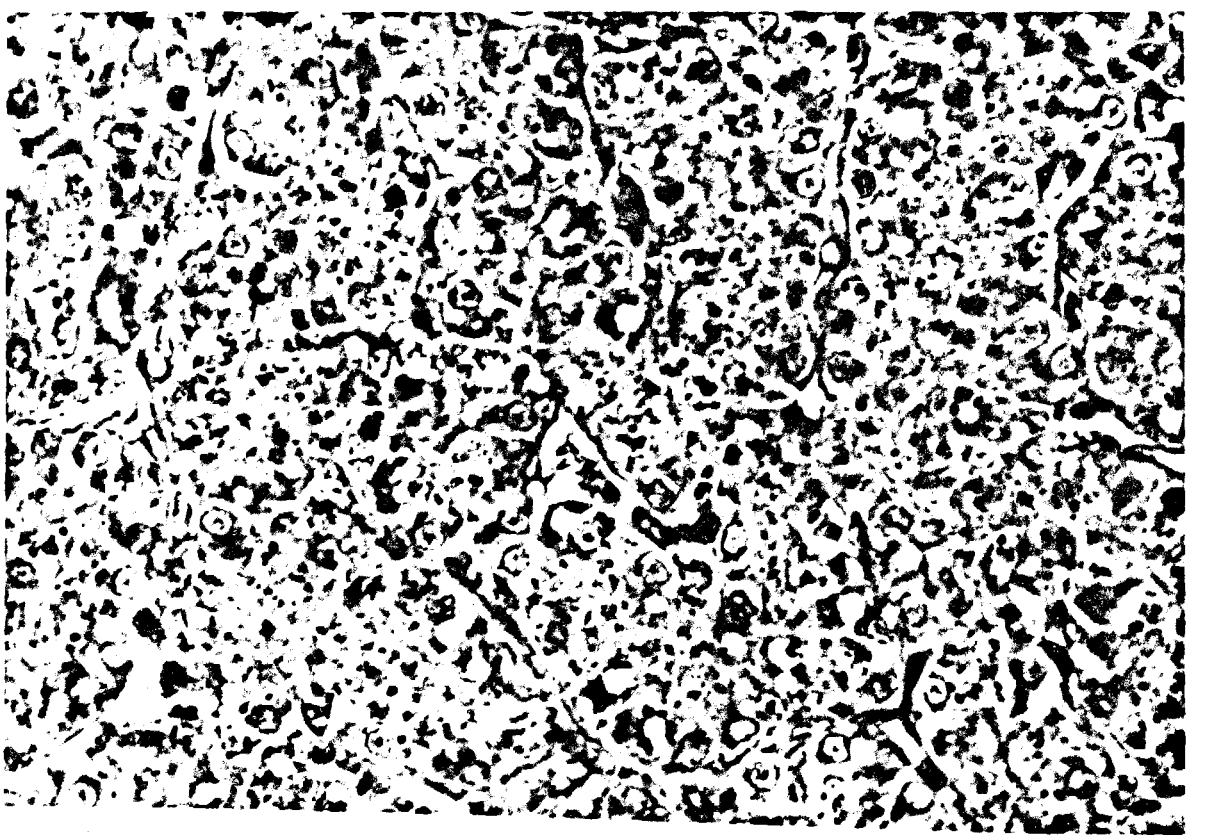
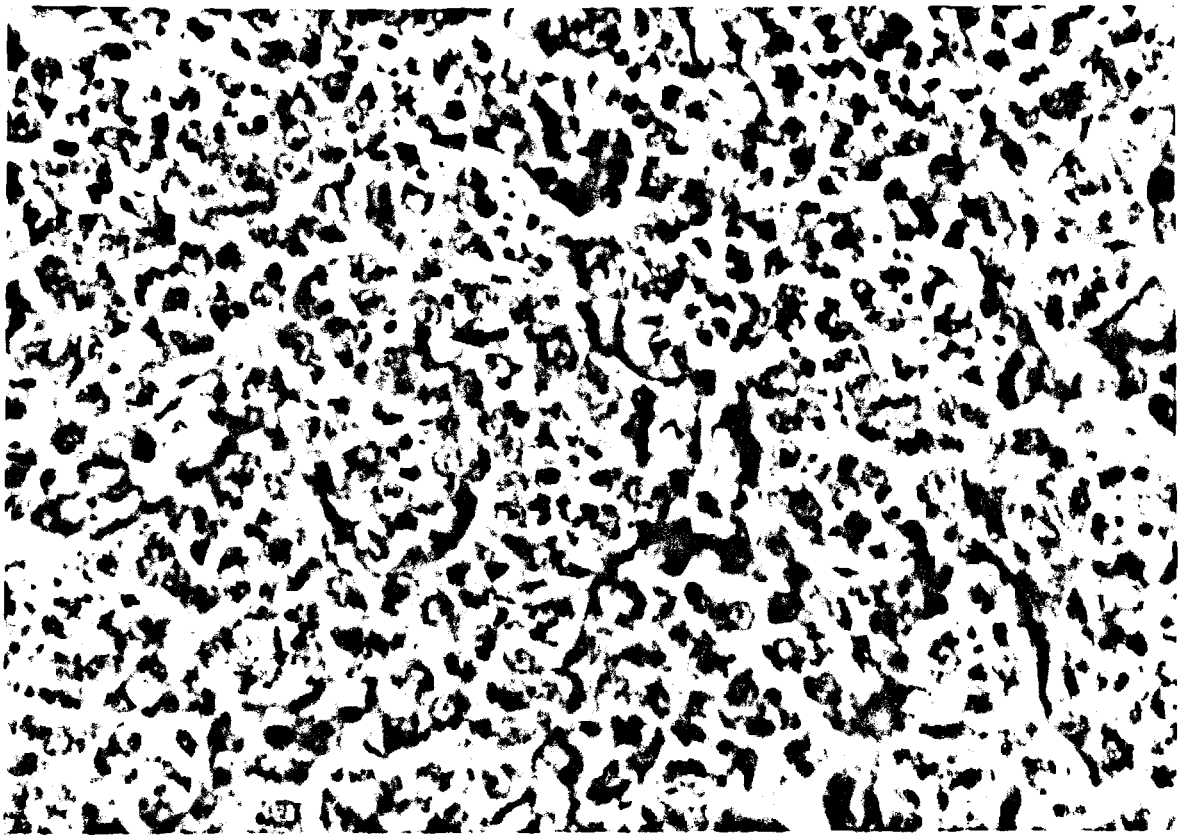


FIGURE 25

C. Perfusion 2 (top).

D. Perfusion 3 (bottom).



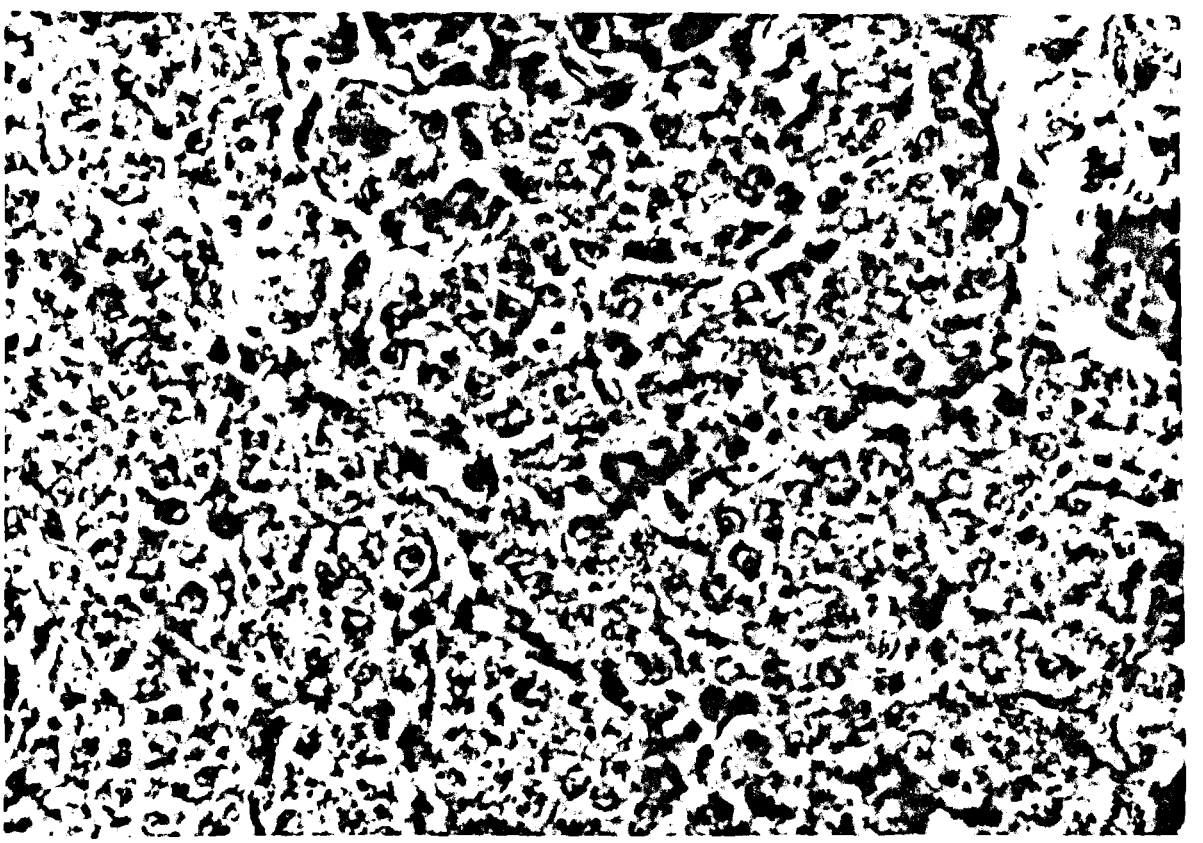
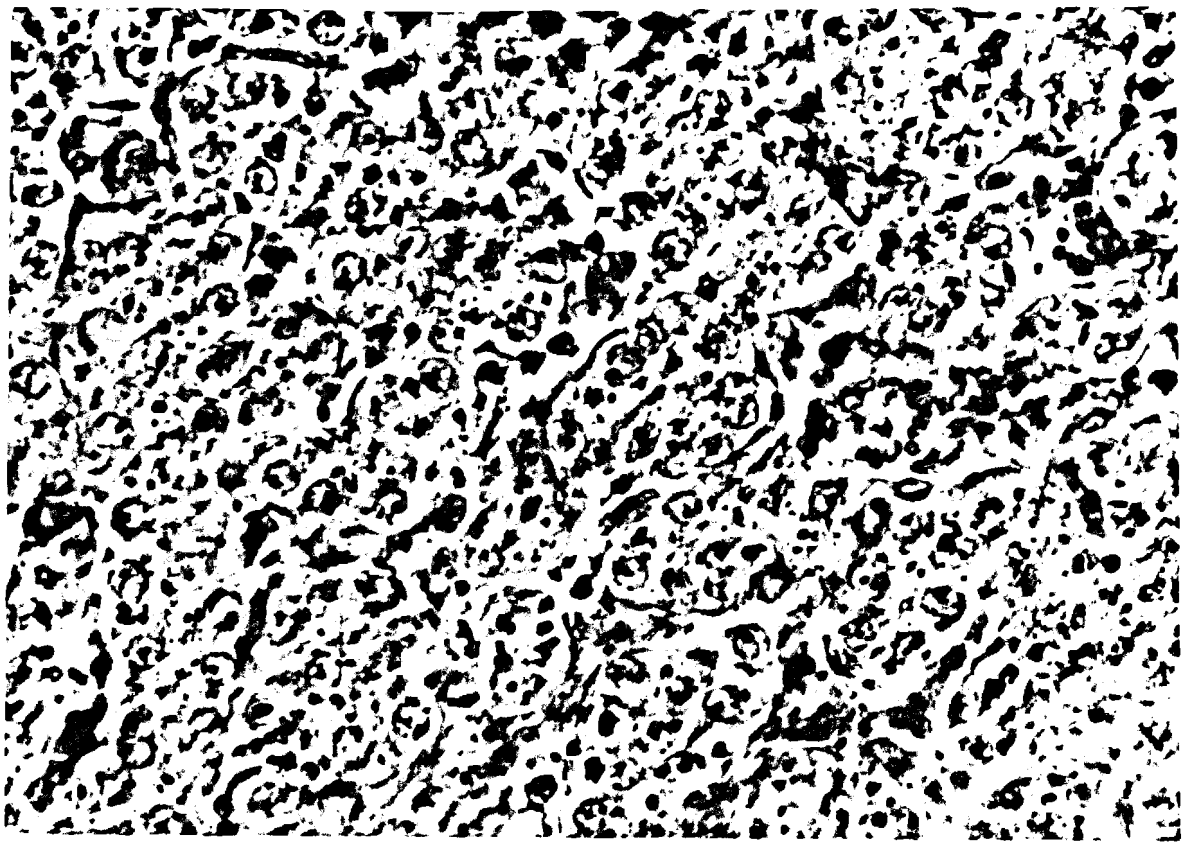
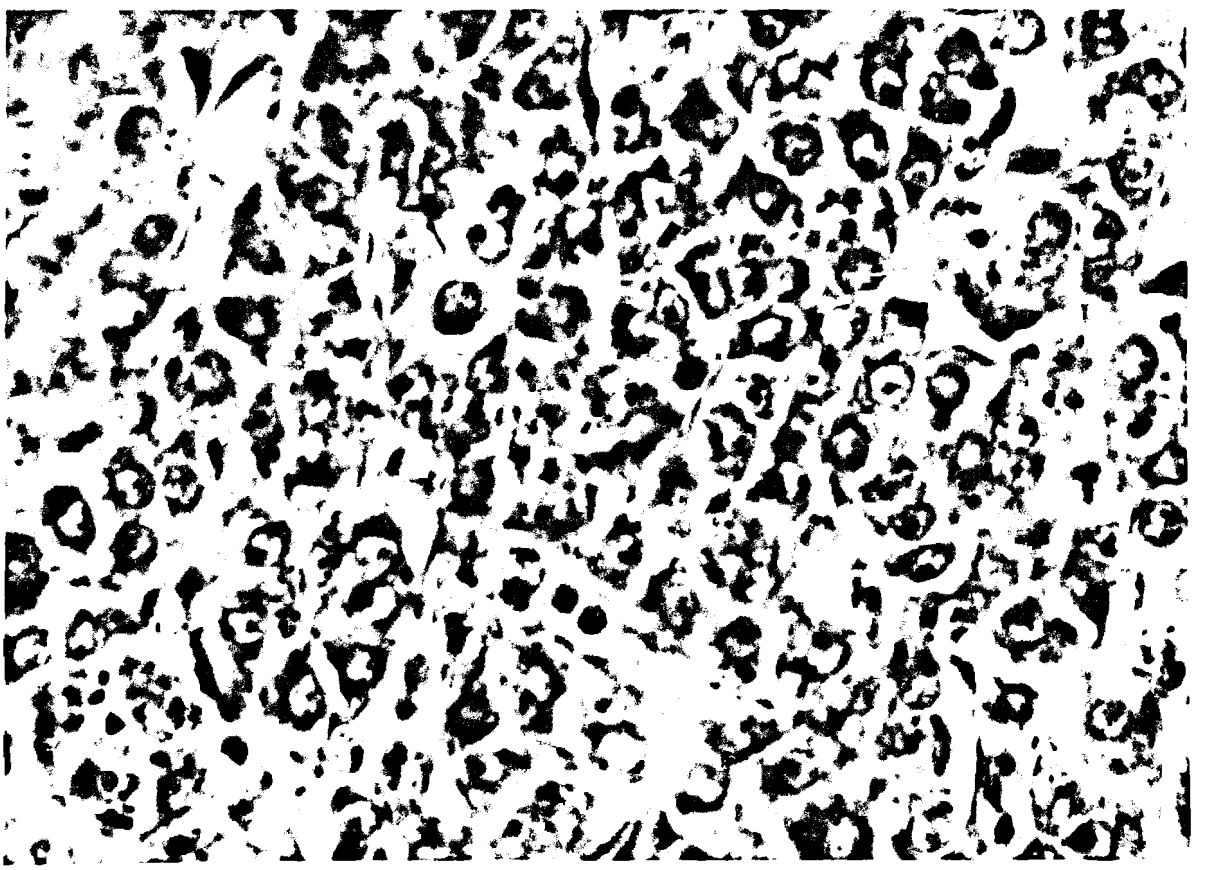


FIGURE 25

E. Perfusion 4 (top).

F. Perfusion 5 (bottom).





VITA

James Eldridge Turner was born October 1, 1942, in Richmond, Virginia. He received his primary education in the Richmond City schools and graduated from Thomas Jefferson High School in June, 1961. He attended the Virginia Military Institute in Lexington, Virginia, where he majored in Biology. While at V. M. I. he was a Dean's List student, received the Army ROTC Medal for being the most outstanding Army ROTC cadet in his junior class, was battalion commander of the Corps of Cadets, was a distinguished military student, and was named to Who's Who Among Students in American Universities and Colleges (1964-1965). He received the B. A. Degree from V. M. I. in June, 1965, and was commissioned a Second Lieutenant in the U. S. Army at this time. He was accepted for graduate studies at the University of Richmond in February, 1966. While at the University he was elected to membership in Beta Beta Beta Honorary Biological Soxiety. He became interested in the field of physiology at the University through the influence of Dr. F. B. Leftwich who later became his advisor. He received his Master of Science Degree in Biology from the University of Richmond in June, 1967. Mr. Turner was awarded a Teaching Assistantship at the University of Tennessee, where he will continue his graduate studies toward the Doctor of Philosophy Degree in September, 1967.