THE VALIDITY OF AN INDIRECT BLOOD PRESSURE METHOD ON RATS AND THE EFFECT OF DIETARY CHOLINE CHLORIDE ON BLOOD PRESSURE

by

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INTRODUCTION

In the early 1900's, many workers found that choline had some effect on blood pressure, but the main interest was focused on acetylcholine, which had activity many times greater than that of choline.

When further attention was centered on choline rather than its derivatives, many authors could not agree on the action of the compound. The variability in the action of choline was later attributed to purification techniques. The general consensus was that choline acted as a parasympathomimetic. In the 1940's and 1950's it was found that choline could raise blood pressure when fed to unilateral nephrectomized rats on either low protein or low sodium diets.,

The metabolism of choline in the body was a further point of interest. Intravenous injections of choline lowered blood pressure, but this action was very transient because choline was rapidly metabolized into inactive compounds. The effects of high levels of dietary choline on blood pressure have not been elucidated. There is some question as to whether this route of administration of choline chloride will lower blood pressure or have no effect. Both of these hypotheses were considered in an experiment described in this thesis which was concerned with the effects of high dietary choline chloride on the blood pressure of rats.

Since blood pressures were studied, a technique had to be used which was accurate, could be done rapidly, and could be repeated many times without losing validity. Many indirect blood pressure methods have been used on rats for experimental studies with very few being comparable to the direct arterial pressures. A technique evaluation will be made comparing

the indirect blood pressure technique used for recording blood pressures of rats in the choline experiment with a direct technique in unanesthetized rats with chronically implanted cannulas in the abdominal aorta.

REVIEW OF LITERATURE

Direct and Indirect Blood Pressure in Rats

Hamilton et al. (1934) used a sensitive and responsive manometer for determining pressure pulses in skin or vessels of lightly morphinized rats. Blood pressures recorded were a little higher than those found by other authors using direct arterial cannulation.

Griffith (1935) described an indirect method for determining the blood pressure of rats. He removed the dorsum from the back foot while the rat was under pentobarbital sodium. By microscopically observing blood flow in the foot pad of a rat, blood pressure determinations were made during intermittent occlusions of larger leg vessels. The point where blood started flowing was read on the mercury manometer as the systolic pressure end point. These readings were compared with direct cannulation of the femoral artery, abdominal aorta, and left carotid artery. The readings were within six mm Hg of the direct readings. Griffith et al. (1935) repeated this procedure in studying hypertension in rats following intracisternal kaolin injections. Although the animals were anesthetized, the method was very comparable to the direct blood pressure readings.

McMaster (1941) used the blood flow procedure on the mouse. The hind legs were made transparent in the focused and cooled beam of a carbon arc light. Blood pressure readings were comparatively lower than those of carotid artery cannulation. Continued cuff inflation caused edema and gave readings 10 to 15 mm Hg lower. Duncan et al. (1943) used the flow procedure of blood pressure determination in the rat and found that pressure

readings were repeatable within four mm Hg. Their values agreed within five mm Hg of pressures taken from aortic cannulation. Anesthesia lowered the blood pressure reading from a 90 to 144 mm Hg range to 70 to 120 mm Hg.

Woodbury and Hamilton (1937) worked on a method to find the normal systolic and diastolic pressures without using anesthesia or anticoagulant. Cannulas were inserted in the femoral and carotid arteries by using procaine and a binocular loupe. Blood pressure readings were taken during and after anesthesia on birds, turtles, rats, and mice. The pressure measurements decreased 23 percent with general anesthesia.

Byrom and Wilson (1938) introduced the plethysmographic method for measuring systolic blood pressure in the anesthetized rat. When the pressure in the tail occluding cuff was reduced, blood re-entered the tail and increased tail volume. The initial increase in tail volume, which indicated systolic blood pressure, was detected by a water plethysmograph. Then systolic pressure was recorded by a mercury manometer attached to the cuff. In 88 rats studied, the average blood pressure recorded by the plethysmographic method was ten mm Hg lower than direct carotid recordings. The difference was attributed to cuff size.

Diaz and Levy (1939) found indirect blood pressure recordings by compressing the tail with a cuff regulated by a pressure bulb and a mercury manometer. In their method, the cuff pressure was raised above the systolic pressure and the end of the rat's tail was cut off. The end of the tail was placed in sodium citrate to avoid clotting. As the pressure in the cuff was lowered, blood dripped and then flowed freely into the sodium citrate solution. When this method was compared to direct aortic cannulation, the direct pressures averaged ten mm Hg higher than the indirect

method. The point at which blood flowed freely was more comparable to direct aortic pressures. In 1,207 rats studied, the pressures ranged from 90 to 140 mm Hg.

Williams et al. (1939) introduced a water plethysmographic method for recording indirect blood pressure in unanesthetized rats. The recordings agreed consistently with authors doing direct aortic pressures in anesthetized rats. End points were determined from the increase in tail volume which caused a rise in the fluid in the oncometer tube attached to the tail occluding cuff.

Shuler et al. (1944) compared the Williams et al. (1939) indirect blood pressure method with a direct carotid artery cannulation. A five mm diameter occluding cuff was found to be the most accurate in comparing the indirect method with the direct method. Direct systolic blood pressures recorded by an optical manometer were consistently within two or three mm Hg on 168 comparisons. It was found that this precision was not attained by the indirect method in the rat unless the width of the cuff was correlated to the circumference of the tail. A three mm occluding cuff did not stop flow until a pressure of 270 mm Hg was applied. Using the five mm occluding cuff, pressures recorded by the indirect method were higher than those of the direct method at pressures greater than 120 mm Hg and lower than the direct recordings below 120 mm Hg. The seven mm cuff always gave lower blood pressure values.

Wu and Visscher (1948) used the plethysmographic procedure in the mouse. The comparison with direct carotid artery cannulation of anesthetized mice showed that the indirect method was five mm Hg higher with pressures above 80 mm Hg, and slightly lower with blood pressures between 40 and 60 mm Hg.

Proskauer et al. (1945), using rats, studied the effects of temperature on direct and indirect blood pressure recordings. Elevation of cutaneous and rectal temperature of unanesthetized rats caused a rapid rise in blood pressure. Depressing the body temperature caused a blood pressure decrease. The comparison of the two methods were within 15 mm Hg. Olmsted et al. (1951b) found a similar effect of heat on indirect blood pressure recordings of rats. The rise in blood pressure at 45°C could be caused by either splanchnic vasoconstriction, increased cardiac output, or both. Sobin (1946) found that warming the tissue to 44°C produced maximal vasodilatation of the caudal artery. Local warming of the tail was found to prevent technique errors. The time for effective vasodilatation was 15 to 20 minutes. The size of tail cuff made a great difference in accuracy. By direct recording with a microinjection technique at the base of the tail, the indirect pressure correlated with the direct, but fell at a rate of four percent per cm from the base of the tail. Using a ten mm length cuff, the plethysmographic recording compared to the femoral artery recording regardless of age or weight.

Rosett and Handler (1957) found that the width of the occluding cuff must vary from rat to rat. By varying the inside diameter of the cuff, they were able to keep indirect blood pressure readings within 6 mm Hg of the direct carotid recordings in 204 tests.

Medoff and Bongiovanni (1945), using Albino Westmar and Gray Norway rats, found no variation of blood pressures in sex or species. However, increasing hypertension occurred in rats over 200 days old.

Ablondi et al. (1947) compared blood pressure measurements in the rat from the tail, foot, and femoral artery. The increase in the tail volume

was measured by a photocell attached to the upper surface of the tail cuff. The tail always had to be heated to give a volume change, but the foot did not. Heating the ventral surface of the rats gave higher blood pressures and greater tail volumes than were normally found. Indirect pressures were taken from the foot and tail simultaneously, and then rats were anesthetized and blood pressures were taken from the femoral artery and tail simultaneously. There was agreement between the indirect heated tail and the direct femoral arterial pressure, but not between the unheated tail and the femoral arterial pressures. The indirect pressures from the heated tail and normal foot were comparable on some rats. Kersten et al. (1947) used the photoelectric system for indirect blood pressure recordings in rats. Heating was necessary in their method, and the rats were found to respond differently to heat on various days. The average pressure found was 117 mm Hg.

Friedman and Freed (1949) used a method for indirect blood pressure determinations in the rat that was similar to that used in humans. A ten mm cuff was inflated to exceed the pressure of the caudal artery. As the cuff pressure was lowered, the systolic pressure was picked up by a microphonic manometer and displayed on an oscilloscope. The greatest pulsatile sounds occurred at diastole. Repeated pressure determinations on the rats did not vary over four to ten mm Hg.

Caster et al. (1956) demonstrated the microphonic technique for indirectly measuring the systolic and diastolic blood pressures in the rat. As compared to the direct carotid cannulation, the method was relatively accurate. Vasoconstriction of the tail, amount of heat necessary for dilatation of the caudal artery, and cuff size were still sources of inaccurate measurements. Systolic and diastolic pressures averaged 122 and

65 mm Hg, respectively.

Olmsted et al. (1951a) studied methods of pulse detection in the rat by means of mechanical, audiometric, and electrical impedance. Because of less movement artifact recorded, the mechanical method was selected over the other two methods to be used. Pulse waves were detected by a strain gauge bridge (Statham) and amplified by a direct writing oscillograph. The correlation between carotid artery pressure and pulsatile detection was low at pressures of 56 to 100 mm Hg, and high at pressures of 130 to 180 mm Hg. The advantage of this technique was that permanent records were made and the subjective errors of watching the cuff pressure gauge and trying to observe end points were eliminated.

Del Greco et al. (1953) modified the microphonic technique to eliminate animal heating, anesthesia, and movement artifact. The arterial pulse was recorded graphically. This measurement was compared to direct blood pressure from carotid cannulation. The carotid pressure was about six mm Hg higher than the microphonic technique.

Korol and McShane (1963) measured blood pressure in unanesthetized rats with a model XE60A infant's earpiece oximeter. A cuff around the ankle occluded blood flow. When arterial pulse occurred, there was a rise in the oxyhemoglobin curve. When compared to the direct blood pressure method done by Still et al. (1956), the oximeter determinations were eight to ten mm Hg lower than the direct blood pressure recordings.

Still (1952) first implemented the chronic aortic cannulation for drug injection to study the effects on the kidney. The polyethylene tubing (No. 10) was inserted into the abdominal aorta with the tip of the cannula slightly anterior to the renal arteries. The cannula was exteriorized on

the dorsal surface of the neck and heat sealed after each injection. Still and Whitcomb (1956) found this method to be excellent in recording direct blood pressure. The polyethylene tube had a diameter of 25 percent of the cross sectional area of the aorta. Blood pressures were recorded directly from the cannulated unanesthetized rats, and blood samples were easily collected. Still et al. (1956) recorded the blood pressure from the aortic cannulas after intra-arterial injections of histamine and vasopressin.

Pradham et al. (1956) cannulated the carotid artery in mice and recorded blood pressure using a model P23D Statham pressure transducer with a Brush recorder.

Weeks and Jones (1960) modified the Still et al. (1956) technique by using the polyethylene tubing (No. 10) only in the aorta, and then bringing the cannula out subcutaneously on the dorsal surface of the neck with a larger tubing. The cannula in the aorta ended caudal to the renal artery. The cannula was connected to a Statham P23G pressure transducer. A comparison was made between the direct method and the indirect photoelectric tensometer method. Tensometer blood pressure varied as much as 25 mm Hg, but by removing and replacing the cuff, the pressures varied up to 70 mm Hg. The variation in tensometer measurements existed regardless of the location of arterial cannulation. Weeks (1966) modified the earlier designed cannulas. The polyethylene tubing (No. 10) portion was looped to prevent slippage out of the aorta and aneurisms caused by cannula movement. The cannulas were functional for 19 weeks. Larger rats were used so they would not outgrow the cannula.

Popovic and Popovic (1961) inserted polyethylene cannulas (No. 10, No. 20, and No. 50) from the left carotid artery into the aorta, and other

cannulas from the right external jugular vein into the right superior vena cava of rats and squirrels.

Weeks and Davis (1964) passed a cannula from the right jugular vein into the right ventricle of rats. Blood samples were more easily collected from this type of cannulation than from aortic cannulas.

Slusher and Browning (1961) inserted polyethylene tubing (No. 10) from the brachial vein into the jugular vein of rats. The cannulas came out the back of the neck and were used for injections directly into the heart. Rappaport et al. (1961) used polyethylene tubing (No. 10) to cannulate mesenteric veins. This operation was similar to that of Still et al. (1956) and Weeks and Jones (1960) except that cannulas were exteriorized in the abdominal area.

Role of Choline in the Circulatory System

Calder (1942) demonstrated in rats that a deficiency of the entire vitamin B complex decreased the blood pressure. If the heat-stable portion (choline, pyridoxine, and riboflavin) was extracted from the diet, a rise in blood pressure occurred.

DeKleine (1944) found that dried brewer's yeast lowered the blood pressure of patients treated for albuminuria. The yeast contained 1620 mg of choline per pound. He then treated hypertensive patients between 45 and 76 years of age with yeast mixed in a glass of water. Nine out of 12 patients showed dramatic reductions in blood pressure in two to four weeks.

Honorato and Ivanovic (1944) found that choline deficiency increased prothrombin time in the rat. After ten days of a choline deficient regime,

the increased prothrombin times returned nearly to normal. The males showed a greater increase in prothrombin time than the females.

Honorato and Vadillo (1944), using the Williams et al. (1939) technique for indirect blood pressure measurements, found that the blood pressure of rats was not altered by choline deficient diets. However, there was an incidence of hepatic and renal damage in the rats.

Grollman and Harrison (1945) found that of the vitamins tested for their effects on blood pressure, choline appeared to exert a slight but definite pressor action in animals with a mild degree of hypertension. When choline was given in high doses (three percent of the dried weight of the diet) with a low sodium diet, the usual drop in blood pressure was negated. The authors stated that the pharmacodynamic action of choline may be to interfere with the excretion of sodium by hypertensive animals.

Sobin and Landis (1947), using male weanling rats of the Sherman and Sprague-Dawley strain, found that neither an acute nor a chronic choline deficiency permanently altered the blood pressure if the rats were maintained on choline deficient diets. Initially, the blood pressure rose, then returned to normal. Renal lesions which occurred were not characteristic of the renal abnormalities associated with hypertension.

Engel (1948) found that prolonged feeding of choline deficient diets to rats produced anemia. The hemoglobin levels in the deficient animals ranged from 6.25 to 11.25 gms/100 ml of blood. The anemia was prevented by dietary supplements of either choline chloride or DL-methionine. The choline chloride also prevented the nutritional edema. Alexander and Engel (1952) observed similar results in rats using a low protein and low choline diet; the concentration of blood and tissue lipid remained constant

while the blood protein values decreased.

Fischer and Garrity (1953) observed in choline deficient rats that plasma albumin concentrations did not change prior to renal lesions. Serum α and β-globulin increased in rats with degenerative kidneys. When choline was administered orally to speed up recovery, the serum globulin fraction increased.

Firstbrook (1950) became interested in choline treatment for atherosclerosis following the demonstration of the many lipotropic activities of choline. He found that choline chloride lowered the blood cholesterol in a rabbit when given orally in a capsule mixed with cholesterol.

Hartroft et al. (1952) observed atheromas in the major arterial trunks of rats that were maintained on a low choline diet up to 216 days. The initial lesions consisted of stainable lipid in the endothelial cells of the intima. In the later stages, the proliferation of the intimal cells caused formation of small plaques. The adjacent media underwent necrosis and calcification resembling that in man.

Buckley and Hartroft (1954) observed that nine of 25 rats fed a basal choline deficient diet developed aortic sclerosis. High doses of vitamin D gave the same results. Choline prevented the sclerotic lesions in both the choline deficient and excessive vitamin D groups. Hartroft and Buckley (1954) observed that rats fed 35 percent ethyl laurate frequently developed lipoidosis of coronary arteries and sclerosis of the aortas. In the control group fed 0.85 percent choline chloride, neither male nor female rats developed sclerotic lesions.

Wilgram et al. (1954b) observed that choline chloride prevented fat deposition in the myocardial muscle in 25 male rats. Choline was necessary

as a lipotropic agent for a healthy cardiovascular system. Wilgram and Hartroft (1955) observed aortic sclerosis and myocardial necrosis in rats of all ages. Choline supplements were used for treatment and prevention. Wilgram et al. (1954a) reported that 0.85 percent choline chloride prevented the development of lipomatous and atheromatous plaques in the coronary arteries of rats receiving a diet containing high fat and cholesterol.

Morrison and Rossi (1948) found that choline supplements caused re-absorption of aortic atherosclerotic plaques in the majority of cholesterol fed rabbits. Morrison (1949) fed a group of three month old rabbits an atherosclerotic diet containing 0.5 mg choline chloride and atherosclerosis was prevented in 55 percent of the cases. A diet containing one percent choline chloride prevented the lesions in 78 percent of the cases.

Morrison and Gonzales (1950) treated humans with acute coronary thrombosis and myocardial infarction with choline. Choline appeared to prevent arterial atheromatous deposition and to reduce the deposition of cholesterol in the vascular wall atheromatous deposits. Over a three year period choline carbonate at a daily dosage of 6.32 gms was effective in reducing mortality due to recurrent coronary thrombosis.

Handler and Bernheim (1950a) reported that weanling rats on a choline deficient diet for one week and stock diets for the remaining experimental period developed systemic hypertension. When the rats were fed diets low in either sodium or protein, the hypertensive pressure returned to normal in three weeks. Moses et al. (1950) demonstrated with a choline deficient regimen for one week and stock diet for six months that 20 of 28 weanling rats had an elevated arterial blood pressure over 150 mm Hg. Similarly treated animals receiving either 0.4 mg percent or two mg percent choline,

or older animals surviving ten days on similar deficient diets, did not have hypertension six months later.

Knudson and Harris (1955) fed weanling rats a low choline diet for five days and then a normal diet for seven months. Eighteen percent of the control group had a mean arterial pressure above 124 mm Hg. Of the choline deficient rats, 34.7 percent showed a mean pressure above 124 mm Hg. There were no significant changes in total cholesterol and free cholesterol in plasma, aorta, heart, kidney, or liver of animals with elevated blood pressure.

Best and Hartroft (1949) observed that blood pressures remained normal in young rats maintained on diets deficient in choline for as long as seven months. If the rats were put on normal stock diets after being on choline deficient diets, almost all developed hypertension. Pressures taken directly from the femoral artery averaged 195 mm Hg. Arterial lesions were seen and the kidney became fibrotic with necrosis beneath the capsule. Rats which survived and were maintained on choline deficient diets did not become hypertensive. The author thought this may have been caused by slowing the rate of growth, lowering demands on the cardiovascular and renal systems. Hartroft and Best (1949), in further studies on hypertension of choline deficient rats, noticed the hearts of the animals were doubled in size. The arterioles of the rats showed advanced hypertension in less than one week on a choline deficient diet.

Schaefer et al. (1951) found that weanling pups on 0.05 percent choline chloride diet for 13 to 55 weeks had cirrhotic livers with some hepatic tissue having hyperplastic regenerative nodules. Hemoglobin values ranged from 2.6 to 3.4 gms/100 ml of blood. Hematocrits ranged around 10 percent,

and the total red blood cell count ranged from 2.1 to 2.6 million/mm^3. Both choline chloride and vitamin B_{12} were found to be helpful in the prevention and treatment of liver cirrhosis.

Pharmacological Actions of Choline

Hunt (1900) demonstrated a compound in a suprarenal extract which lowered blood pressure before atropine injection, and caused either a rise or had no effect after atropine injection. Hunt and Taveau (1906) identified the compound as choline. They obtained another compound from the suprarenal gland which was 100,000 times more potent than choline and was blocked by atropine. This compound was acetylcholine.

Hunt and Taveau (1911) summarized the work of earlier authors concerning the effects of choline on blood pressure. Low dosages of choline (two mg/kg) injected into the dog lowered blood pressure while higher dosages (five mg/kg) caused a rise. The drop in blood pressure was attributed to slowing of the heart and dilatation of the intestinal circulatory system. The above authors found in their work that injected choline caused a fall in blood pressure in the rabbit before atropine, and a rise or no effect after atropine injections. The aromatic derivatives of choline always gave a rise in blood pressure. Hunt (1915) found that over 90 percent of the injected choline left the blood stream in less than one minute. It was hypothesized by the author that choline had no physiological or pathological importance in the blood stream, but probably was converted to a more active form. ^{124}I choline readily yielded choline derivatives which had activity 50 times that of choline.

Ewins (1914) described acetylcholine as an oxidation product of choline having muscarinic actions. Simonart (1932) found that choline and its derivatives induced physiological effects characteristic of parasympathetic stimulation. Since choline possesses both nicotinic and muscarinic effects, one action of choline may sometimes overshadow the other. It was pointed out that choline probably forms more powerful derivatives which have a longer duration in the blood stream, and less nicotinic action than choline itself. Bernheim and Bernheim (1933) reported that acetylcholine was oxidized in the animal body to choline. This was attributed to esterases splitting off the acetyl group following hydrolysis of the ester linkage.

Luecke and Pearson (1945) found that no increase occurred in free or total choline of the liver, kidney, or plasma when sheep ingested 40 gms of choline daily for six days. The urine contained 0.7 to 2.5 percent of the ingested choline, but the urinary nitrogen equaled that of the choline nitrogen fed. It was suggested that the feeding of large quantities of choline stimulates the activity of choline oxidase, thus preventing any increase in the choline content of tissue or blood. According to the author there may have been some mechanism present which very rapidly converted ingested choline to another metabolite.

Jacobi et al. (1941) reported that the rat can synthesize and catabolize choline very rapidly. The rat liver, kidney, and brain tissues were examined, and it was found that the choline content of tissues of rats on a choline free diet increased with animal size. Choline synthesis was reduced by a high fat diet, but other dietary supplements, such as protein or cystine, did not influence the synthesis of choline. Synthesis

occurred at a limited rate, but this may not be great enough at all periods of the life span. Engel (1943) reported that two strains of rats differed markedly in their requirements for dietary choline.

de la Huerga and Popper (1952) found that the bulk of the choline administered orally was transformed into trimethylamine by intestinal bacteria and excreted in the urine as the oxide. Choline was not transformed into any other nitrogenous products by intestinal bacteria. Since choline was not found in the stools, the choline that was not converted to trimethylamine was absorbed. Chlortetracycline increased choline absorption by decreasing the bacteria that catabolized choline. Feeding of a high level of dietary choline after cessation of chlortetracycline renewed the production of choline using bacteria. Popper et al. (1952) found that the excretion rate of dietary choline for humans and rats was 60 and 40 percent, respectively. The authors stated that the trimethylamine, formed from bacterial conversion of choline, had no lipotropic action.

Bligh (1952) assayed choline by converting it to an active acetic ester, then compared it with a known amount of acetylcholine activity. The author found that the blood level of choline was between one to two μg/ml in humans. This level remained constant over a six month period and was unaffected by food or exercise. The blood choline levels in the cat and dog were similar to that of the human. Appleton et al. (1951) confirmed the previous work by constant intravenous infusion of choline in dogs at a rate of three to four mg/min. The choline plasma level of the dogs rose to about twice normal, but returned to normal within an hour. About 20 percent of the injected choline was accounted for in the urine, and the fate of the remaining amount was not known.

Nachmanshon and Machado (1943) isolated choline acetylase from brain and nervous tissue and found that it converted choline to acetylcholine in the presence of adenosine triphosphate. Ledda and Baldi (1965) found in the cat that the addition of choline to a perfusion fluid prevented the decrease of acetylcholine output from the superior cervical ganglion after repeated stimulation. Choline increased the initial output of acetylcholine from the ganglion.

Hodge and Goldstein (1942) examined the acute toxicity of choline hydrochloride in mice and rats. It was extremely hard to kill an animal with injections of choline or choline hydrochloride. The injectable dose that killed the mice was 700 mg/kg given subcutaneously, with death usually occurring in two to four minutes. Salivation, trembling, jerking, cyanosis, and respiratory paralysis were symptoms of lethal dosage. Injecting six mg of choline hydrochloride intraperitoneally into mice killed 47 percent. A solution of choline hydrochloride equivalent to 0.67 gm, given by stomach tube to rats, killed 40 percent, while a one gm dose killed 80 percent. The blood vessels of the diaphragm and stomach were engorged and the heart stopped in diastole. The LD_{50} for choline hydrochloride given intraperitoneally was about 320 mg/kg or 6.7 g/kg when given by stomach tube.

Hodge (1944) reported on the intraperitoneal toxicity of choline hydrochloride. Lethal doses were 40 to 60 mg/kg for intravenous administration, and 200 to 1000 mg/kg for subcutaneous administration. Rats of various ages and weights were given choline hydrochloride at concentrations of 200, 100, 40, and 20 mg/ml, and the LD_{50} for these concentrations was 29 to 34, 37 to 38, 41 to 49, and 59 to 75 mg/100 gms of body weight,

respectively. Hemorrhage found around the eyes was attributed to the intense parasympathomimetic action of choline.

Hodge (1945) reported on the chronic oral toxicity of choline chloride in rats. Choline chloride was added at various levels to a solid diet, then in a second experiment to the drinking water. Levels added to the food were 0.01, one, two, five, seven, and ten percent. An inverse relationship resulted between the increasing dietary choline and daily growth. Because the high choline diets were refused, the rats exhibited a severe loss of weight. Due either to refusal of food or lipotropic action of choline, no fat was found in the subrenal, mesenteric, or subcutaneous tissues. No histopathological changes were attributed to excessive choline. Choline chloride was added to the drinking water at 0.01, one, 2.7, four, and five percent by weight. Growth was inhibited as the percentage of choline chloride increased in the drinking water. At levels of 6.7 and ten percent of choline chloride in the drinking water, death resulted from self water deprivation. There were no toxic effects attributed to choline in either experiment, but rather, animals died from either starvation or water deprivation. Neuman and Hodge (1945) reported other experiments on the route of administration of choline chloride. Intraperitoneal injections were much more toxic than doses given by stomach tube. No correlation existed between toxicity and sex or weight. The LD_{50} for choline chloride given by stomach tube was 3.4 gm/kg and 6.1 gm/kg for solution concentrations of 500 to 670 and 200 to 400 mg/ml, respectively. The toxicity symptoms were jerking, convulsions, and chromodacryorrhea followed by depression, and finally, complete respiratory paralysis.

Roth and Allison (1950) reported that a level of 1.35 percent choline chloride in a diet containing 12 percent casein was well tolerated by rats. Choline chloride prevented the usual adverse physiological conditions, such as enlarged kidneys and tissue protein breakdown, usually observed when high methionine diets were fed. The excess methyl groups fed as choline appeared to be more easily metabolized than those fed as methionine.

Cornatzer (1954) reported that the toxicity of choline in the rat was related to the choline oxidase activity of the liver and kidney. However, the absence of choline oxidase in the liver of the guinea pig did not increase choline toxicity.

Steigman et al. (1952) reported that doses of three to nine gms of choline caused few toxic symptoms in liver disease treatment. The authors stated that the parenteral use of choline has been considered dangerous because of its similarity to acetylcholine. EKG, blood counts, urine analyses, blood pressure, and pulse rate evaluations were conducted on patients receiving two gms of choline in 500 cc of five percent glucose. Hemoglobin values, red blood cell counts, and white blood cell counts remained constant, but blood pressures initially dropped and later returned to the original level. Ten percent of the choline was found in the urine sample during the first four hours with only traces detected thereafter.

Goodman and Gilman (1965) reported that choline had the same pharmacological action as acetylcholine, but was less active. It was reported that the acute toxicity of choline, especially by mouth, was relatively low (five gm/kg for rats) as compared to some of its esters.

Role of Choline in the Liver

Hershey (1930) first noticed the importance of choline in the liver. Lecithin maintained normal liver fat and glycogen of eight depancreatized dogs for the experimental period of one year. Welch and Welch (1938) found that minimal dosages of choline worked best as a lipotropic agent. High dosages would not transfer from the liver as much fat per molecule of choline chloride as the minimal dosages.

du Vigneaud et al. (1939) found that one purpose of choline was to enhance the homocystine utilization. Choline makes possible the in vivo methylation of homocystine to methionine. Perlman and Chaikoff (1939) observed that a second function of choline was to speed up phospholipid formation in the liver. The phospholipid movement from the liver was proportional to the amount of choline ingested up to 0.30 mg per rat.

Gyorgy and Goldblatt (1940) described choline as a member of the vitamin B complex. Pathological conditions of the kidney parenchyma caused by fatty livers could be improved by pyridoxine, riboflavin, or thiamine, but not completely prevented unless choline was present.

Griffith and Wade (1939b) observed that the livers of 24 day old male rats enlarged eight to 12 times on a choline deficient diet. A high fat diet was not required for hepatic lipid deposition. Cystine supplementation enhanced fatty livers while methionine had an opposite effect. Griffith and Wade (1939a) found that choline (two mg/gm diet) was required to prevent fatty livers. This was eight times the requirement to prevent hemorrhagic kidneys.

du Vigneaud et al. (1940) established that methionine furnished the methyl groups for choline and creatine synthesis. Griffith and Wade (1940) found that maximum effects of choline deficiency were observed if casein was fed at 15 or 25 percent of the diet, but 40 percent casein completely stopped nephritic degeneration. Supplementation of 0.05 percent cystine to a 25 percent casein diet increased toxicity of choline deficiency. The ratio of methionine to cystine in the protein was important. Mulford and Griffith (1942) confirmed the importance of the methionine to cystine ratio. In the absence of choline, 30 percent casein supplied sufficient methionine for the prevention of choline deficiency symptoms.

McKibbin et al. (1944) demonstrated the choline deficiency syndrome symptoms in puppies. There was a severe fatty infiltration of the liver, a rise in blood plasma phosphatase, and a fall in plasma cholesterol and cholesterol esters. The choline deficiency increased the prothrombin time and decreased the hemoglobin, packed cell volume, and plasma proteins. Although total liver lipids increased three to four times, total liver cholesterol remained constant.

Handler and Dubin (1946) suggested that hepatic necrosis and fibrosis of choline deficient rats resulted from chronic fatty infiltration. When the rats ingested an adequate quantity of good protein, the liver was protected from fatty infiltration.

Handler and Bernheim (1949) studied the effects of choline deficiency in the hamster. The choline deficient hamsters had some liver fat accumulation and choline oxidase activity. The hamster's kidney contained less choline oxidase activity than the rat's kidney. Neumann et al. (1949) observed that young pigs and chicks on choline deficient diets did not

utilize methionine to supply methyl groups for the aminoethanol synthesis step in choline formation. Fatty liver infiltration and characteristic glomerular occlusions with tubular epithelial necrosis were observed. Johnson et al. (1951) observed a similar choline deficiency syndrome in calves. Unless the deficiency proceeded too far, calves responded quickly to choline treatment. Reid (1955) observed that guinea pigs developed the circulatory effects, such as anemia and decreased packed cell volume, on choline deficient diets without fatty livers or hemorrhagic kidneys.

Hartroft (1950) fed rats choline deficient diets for seven months. After 24 hours, small fat droplets appeared in the centrolobular cells of the liver. In seven to ten days, fat globules were found in the parenchymal cells. The parencymal cell walls stretched and ruptured from the fat accumulation which caused large fat saturated areas in the liver. These large areas atrophied after three months and slowly disappeared. Fibrous tissue replaced the areas of fat accumulation and new parenchymal regenerative nodules were eventually seen replacing the necrotic parenchymal cells.

Plough et al. (1952) cured hepatic cirrhosis in rats with 30 percent dietary casein, while four percent casein supplemented with methionine and choline was not effective. Kock-Weser et al. (1953) found that choline mobilized the fat from liver and prevented fatty liver cirrhosis, but did not repair liver cells.

Hartroft and Ridout (1951) reported on the long-term effects of fatty livers in the rat caused by a choline deficient diet. The fat from the cysts in the liver escaped as minute emboli into the biliary tree and blood stream ending in the heart, lungs, and kidneys. The fat droplets in the heart capillaries resembled those found in ischemic infarcts.

Artom (1953) described the role of choline in the oxidation of fatty acids by the liver. In male rats, choline not only mobilized fatty acids as plasma phospholipids, but also helped to oxidize fatty acids.

Ackerman et al. (1953) found that choline synthesis was not detected in rat liver slices incubated with L-methionine and 2-dimethylamino-ethanol. This was believed to be due to the rapid oxidation of choline to betaine by choline oxidase. Bernheim and Webster (1937) reported that the alcohol group of choline is rapidly oxidized by the liver and the product formed depends on the pH. Sidransky et al. (1963) fed rats low levels of methionine with and without 0.25 percent choline. Choline oxidase remained normal in rats fed low methionine, but decreased in activity in the liver and kidney of choline deficient animals. Humoller and Zimmerman (1953) found with high fat diets that the choline oxidase activity of the liver decreased and the choline content rose. Excessive dietary fatty acids inhibited choline oxidase activity. Handler and Bernheim (1942) reported similar evidence showing the inability of choline to enter into transmethylation reactions when choline oxidase was inhibited by fatty acids. Oxidation of choline was a necessary step in mobilizing phospholipids.

Benton et al. (1957) found that rats fed butterfat grew faster than those fed corn oil, but more choline was required in the butterfat diet than the corn oil diet to maintain normal liver lipid concentration. Harper (1958) thought rats developed fatty livers on a low protein and normal choline diet because some of the enzymes necessary for complete mobilization of fat from the liver were not synthesized.

Ohta et al. (1963) found the severity of fatty infiltration and liver cirrhosis decreased by feeding rats a normal diet instead of a choline

deficient diet. Four to six weeks were required to mobilize the liver lipid and halt further cirrhosis. The fibrous trabeculae persisted, but were thin and compressed. Hoffbauer et al. (1963) observed that rats with fatty livers caused by a choline deficiency seemed to be resistant to fat accumulation during a second choline deficiency period. Regeneration nodules, a generation of liver cells formed during the choline deficient conditions, regressed when choline was added. When choline was deleted from the diet again, the nodules were more resistant to degeneration. Haines and Mookeya (1965) found that choline added to a choline deficient diet for rats restored the liver fat and plasma triglycerides to normal in 24 hours.

Bianchi and Azzone (1964) observed that choline oxidation proceeded through the site of the first respiratory chain phosphorylation. By blocking this phosphorylation step with rotenone, the oxidation of choline to betaine aldehyde and betaine was inhibited. Kagawa et al. (1965) concluded that the rate of choline oxidation was controlled by intramitochondrial concentrations of adenine nucleotide and the magnesium ion.

Role of Choline in the Kidneys

Griffith and Wade (1939a) found marked hemorrhagic degeneration of the kidneys within ten days in weanling rats fed choline deficient diets. This syndrome was prevented or corrected by amounts of choline supplementation (0.4 mg/gm of food) too small for correction of fatty livers. Choline requirements were directly related to cystine and methionine content of dietary protein. The kidney became enlarged and discolored, and

the capsule was grossly hemorrhagic. Engel and Salmon (1941) reported that sex did not make a difference in the effects of the deficiency syndrome.

Christensen (1942) described the renal changes in rats on choline deficient diets. The cortical capillaries were congested and capsular blood vessels were ruptured. The cortical tubules had undergone necrosis with occasional tubular calcification caused by the appearance of hyaline or granular droplets. Treatment and recovery were complete when choline was added to the diet, but sometimes the glomeruli remained small and the tubules retained some casts and were slightly dilated. Dessau and Oleson (1947) described the kidney syndrome of weanling rats on choline deficient diets. The first stage was a change in venous stasis which caused an extreme cyanosis of the outer renal cortex. During this stage, hemorrhage did not occur because red blood cells were not present in the lumen of the tubule. The venous stasis was followed by epithelial degeneration and necrosis of the tubules in the outer cortex. The lack of glomeruli involvement proved venous rather than arterial disturbance. Hartroft (1948) observed intracellular fat droplets deposited in the proximal convoluted tubules of the cortex causing renal lesions. Renal ischemia and necrosis were the results of tubular swelling and compressing of the cortical capillary plexus. Weanling rats were more susceptible to tubular deposits than older rats.

Griffith (1941) prevented hemorrhagic renal conditions of rats on low choline diets by the addition of 0.8 percent methionine. The choline requirements varied inversely with the dietary methionine and were increased by dietary cystine.

Wachstein (1944) demonstrated in weanling rats on choline deficient diets that atherosclerotic lesions in the aorta and coronary arteries were like the lesions present in the small vessels of the kidney.

Handler (1946) reported that weanling and adult rats housed in group cages failed to develop renal lesions on choline deficient diets which were effective for single caged rats. The weanling rats on a six percent casein diet did not develop hemorrhagic kidneys until 35 to 45 days, whereas, high dietary protein concentrations produced lesions in six to ten days. Adult rats rarely developed kidney lesions on choline deficient diets regardless of protein supplement.

LaLich et al. (1949) observed that during prolonged choline deficiency in weanling rats an acidic proteinaceous material collected in Bowman's capsule and compressed the glomerular tuft. This accounts for hypertension, anemia, and pulmonary infection and may be responsible for hyalinization and fibrosis of the capsule.

Olson and Deane (1949) observed changes in the adrenal gland and kidney of weanling rats which were fed a choline deficient diet. The physiological changes in the kidney were those described previously by other authors. It was found that the adrenal gland becomes very enlarged with most of the enlargement being contributed to the zona glomerulosa.

Handler and Bernheim (1950b) found that the ingestion of an adequate choline-low protein diet caused the disappearance of renal hypertension in partially nephrectomized rats. The addition of 1.6 percent choline to the diet demonstrated a definite hypertensive action. This pressor action of choline was noted before and was considered a pharmacological action of choline in potentially hypertensive rats.

Handler and Bernheim (1951) observed that renal decapsulation, after choline deficient rats became hypertensive, caused blood pressure to drop to normal for the remaining period. Decapsulation of the kidney before the rats became hypertensive from the choline deficient diet prevented hypertension. Decapsulation before the hemorrhagic syndrome appeared caused death because the rats could not survive the pathological changes.

MATERIALS AND METHODS

The two main objectives of this experiment were: 1) to compare blood pressure measured from chronically implanted aortic cannulas in rats to blood pressures measured indirectly from the tail and 2) to evaluate the effect of a high choline chloride diet on the blood pressure of rats.

Animals, Care and Management

Two groups of caesarean derived Charles River's[1] rats were used in the experiment concerned with the effects of high choline chloride diets on blood pressure. The first group (Group I) consisted of 13 females and seven males about six months of age and ranged in weight from 300 to 650 gms. The second group (Group II) consisted of 19 males and ten females about four months old, which ranged in weight from 200 to 350 gms.

A third group of rats (Group III) were used for the direct and indirect blood pressure comparisons. This group was obtained from Frank and Sons.[2] These rats were about four months old and weighed from 250 to 300 gms. Since blood pressure comparisons were made the day after surgery, it was not necessary to use the disease-free Charles River's rats for this part of the experiment.

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[1]Charles River's Breeding Laboratories, Inc., North Wilmington, Massachusetts.

[2]H. Frank and Son's, Lammon, Wisconsin.

A randomly selected fourth group of rats (Group IV) also from Frank and Sons, was used for a trial feeding experiment to determine how much choline chloride the experimental rats could tolerate. These rats were about four months of age and ranged in weight from 200 to 250 gms.

Housing

The rats in Groups I and II were housed individually in eight by ten inch cages containing a feeding jar and a water bottle attachment. Each cage was marked with a three by five inch card specifying the rat's number, original weight, and type of diet. The animal room was temperature and humidity controlled.

The rats in Groups III and IV were housed in similar cages as those in Groups I and II but in a different room to avoid contamination of the disease-free rats.

Diets

Rats in Groups I and II were fed specially prepared purified diets. Randomly selected rats from each group were fed a control diet containing 0.1 percent choline chloride. The remaining rats in both groups were fed the experimental diet with three percent choline chloride. The composition of these two diets was:

	Control diet percent	Experimental diet percent
Casein[1]	18.0	18.0
Dextrose[2]	63.8	60.9
Corn oil[3]	8.0	8.0
Cellulose[4]	5.0	5.0
Mineral mix[5]	5.0	5.0
Vitamin mix[6]	0.1	0.1
Choline chloride	0.1	3.0
Total	100.0	100.0

Both diets contained four Calories per gm and 15 percent protein. This provided the rats with the National Research Council's recommended 80 Calories per day when a daily food consumption of 20 gms per day was used.

All ingredients in the diets were weighed, then thoroughly mixed for ten to 15 minutes in a Patterson-Kelly Twin Shell Blender.[7] Four thousand gms of each of the above diets were prepared weekly and stored in plastic

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[1]General Biochemicals, Chagrin Falls, Ohio.

[2]Clinton Corn Processing Company, Clinton, Iowa.

[3]General Biochemicals, Chagrin Falls, Ohio.

[4]Jones and Foster (1942) NaCl, 13.9%; KH_2PO_4, 38.9%; $MgSO_4$, 5.73%; $CaCO_3$, 38.1%; $FeSO_4$-$7H_2O$, 2.7%; KI, 0.08%; $MnSO_4$-$2H_2O$, 0.44%; $ZnCl_2$, 0.26%; $CuSO_4$-$5H_2O$, 0.048%; and $CoCl_2$-$6H_2O$, 0.002%.

[5]Thiamine-HCl, 0.5%, Riboflavin, 0.8%; Niacin, 4.0%; Pyridoxine, 0.5%; Ca-Pantothenate, 4.0%; Biotin, 0.04%; Folic Acid, 0.2%; Menadione, 0.5%; Cyanocobalamin (B_{12}), 0.003%; Inositol, 10.0%; p-Amino Benzoic Acid, 10.0%; Corn starch, 65.36%; α-Tocopherol Succinate, 2.2%; Vitamin A Palmitate (250,000 IU/gm), 1.5%; and Vitamin D_2 (500,000 IU/gm), 0.4%.

[6]Nutritional Biochemicals Corporation, Cleveland, Ohio.

[7]Patterson-Kelly Co., Inc., East Stroudsburg, Pennsylvania.

containers. The vitamins and minerals for the diets were similarly mixed and stored between the weekly preparations of the feed. The rats were fed ad libitum, and water was available at all times.

Rats in Group III were fed commercially prepared pellets[1] ad libitum. Group IV rats were fed the same pellets but ground and supplemented with choline chloride. The nine rats of Group IV were divided equally into three groups. One group was fed ground commercial pellets, the second group was fed the ground pellets plus five percent choline chloride by weight, and the last group was fed ground pellets plus seven percent choline chloride. After two weeks, it was evident that the rats on the five and seven percent choline chloride would only eat enough to prevent starvation. For this reason, it was decided that the rats in Groups I and II would continue on the arbitrarily preset level of a three percent choline chloride diet.

Experimental Procedure of Blood Pressure Comparisons

Rats in Group III, which were used for blood pressure comparisons, were selected at random from available rats. The single female in the group, which later died following a cardiac puncture, came from Group I. Because the experiment was aimed solely at comparing the direct and indirect blood pressure techniques, neither sex nor age was considered important.

From June 1, 1967 to July 1, 1967, abdominal aortic cannulas were

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[1]Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois.

inserted into 19 rats selected for Group III. The direct and indirect blood pressures were taken simultaneously on the first day after surgery. After blood pressures were recorded, the rats were not used again. Statistical evaluations, comparing the two blood pressure methods, were made.

Cannula preparation

The cannulas which were implanted in the abdominal aorta of rats were made of three graduated sizes of polyethylene tubing[1] fused together with heat (see Figure 1). The construction of the cannulas and the surgical procedure for inserting the cannula in the aorta were similar to that done by Weeks (1960). The section of the cannula which was inserted into the aorta, was made of polyethylene tubing (PE 10) 11 cm long. This section was flared at one end by directing a stream of heated air into the end of the tubing. One end of the second section, a 15 cm length of PE 50 tubing, was inserted into the flared PE 10 tubing and sealed with finger pressure after heated air was applied to the joint. A number 30 HNC Nyclad copper wire[2] was pushed through the PE 50 into the PE 10 tubing to prevent occlusion when the joint was sealed. The copper wire was taken out after this step and replaced by a 27 HNC Nyclad copper wire in the PE 50 tubing. Then PE 160 tubing of eight cms in length was placed over the PE 50 tubing and heat welded. This section was used for support and protection of the portion of the cannula to which the blood pressure apparatus was

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[1]Clay-Adams, Inc., New York, New York.

[2]Consolidated Wire and Associated Companies, Chicago, Illinois.

Figure 1. Constructed polyethylene cannula

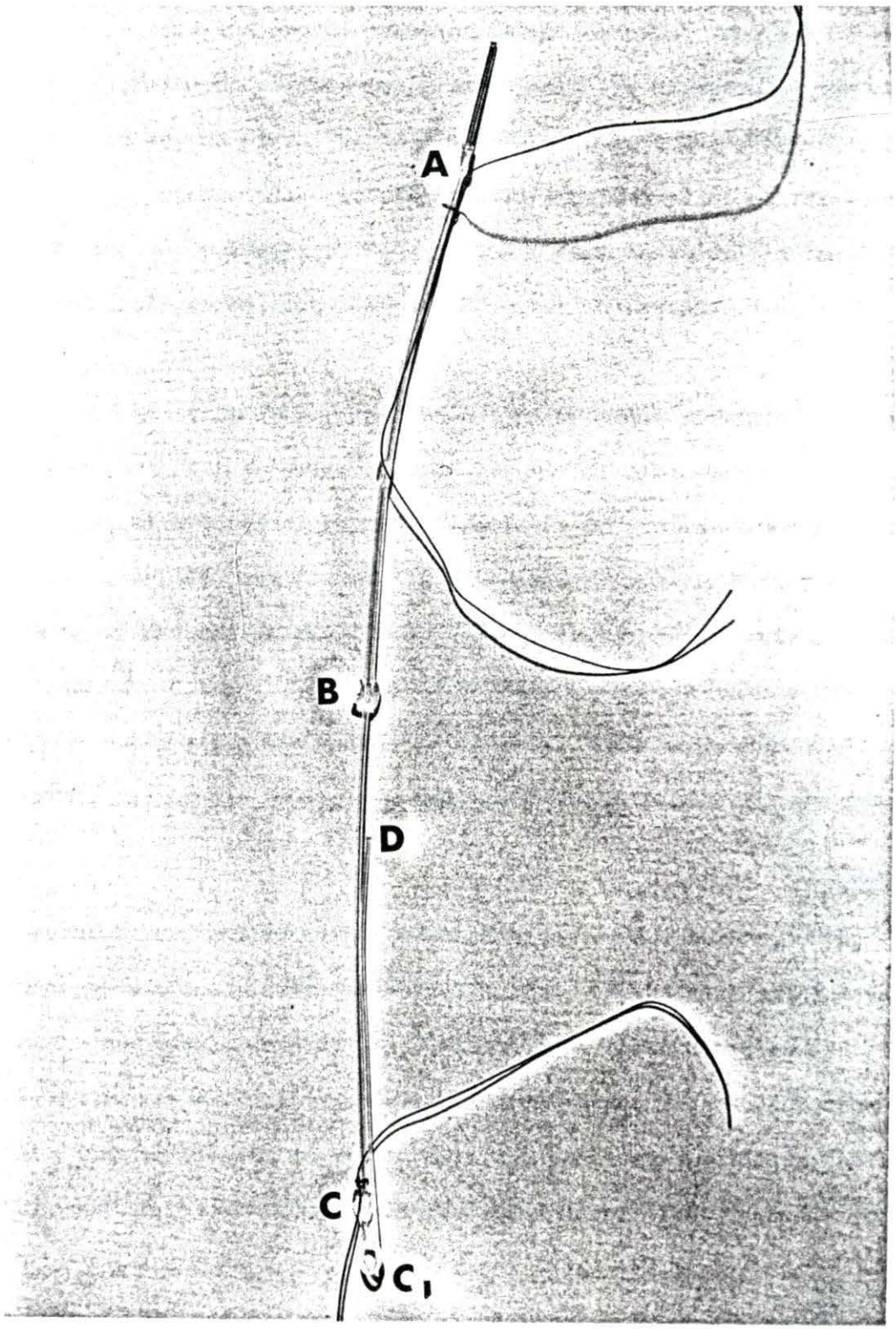
A-C - Polyethylene tubing (PE 50) with stainless steel pin inserted

A to B - Polyethylene tubing (PE 160) placed over the PE 50 section for support

C - PE 50 and PE 10 heat sealed joint

C_1 - Permanent curl placed in PE 10 tubing

C to D - Polyethylene tubing (PE 10)


A
B
D
C
C1

attached. A removable stainless steel pin was placed in the end of the PE 50 tubing to keep a constant pressure in the cannula. The end of the cannula was heated with the pin in place to make the diameter of the tubing conform to that of the pin. The section of PE 10 tubing posterior to the PE 10 and PE 50 junction was curled around a 1/8 inch glass stirring rod and dipped into boiling water. This placed a permanent curl in the PE 10 tubing. The curl reduced the chances of aortic pulsations pushing the cannula out.

The sections of cannula were heat welded by means of hot air which passed through a 1/4 inch copper tube. One end of the tube had a blunt end 22 gauge needle attached to give a more direct stream of hot air on the area to be welded (see Figure 2). The copper tube was heated by a Bunsen burner. The end of the PE 50 tube containing the stainless steel pin was heated by placing a section of 3/32 inch heat shrinkable tubing[1] over the end and heating with a soldering iron. This end was cooled with water and the shrinkable tubing removed.

Surgery

Rats were anesthetized with ether and prepared for surgery. The abdominal area and areas over the dorsal surface of the neck and lateral to the lumbar vertebrae were clipped. The clipped area was cleansed with alcohol and the rat was placed on a rat board. Ether saturated cotton was placed in front of the rat's nose to maintain surgical anesthesia. All surgical instruments were autoclaved for 30 minutes. The prepared

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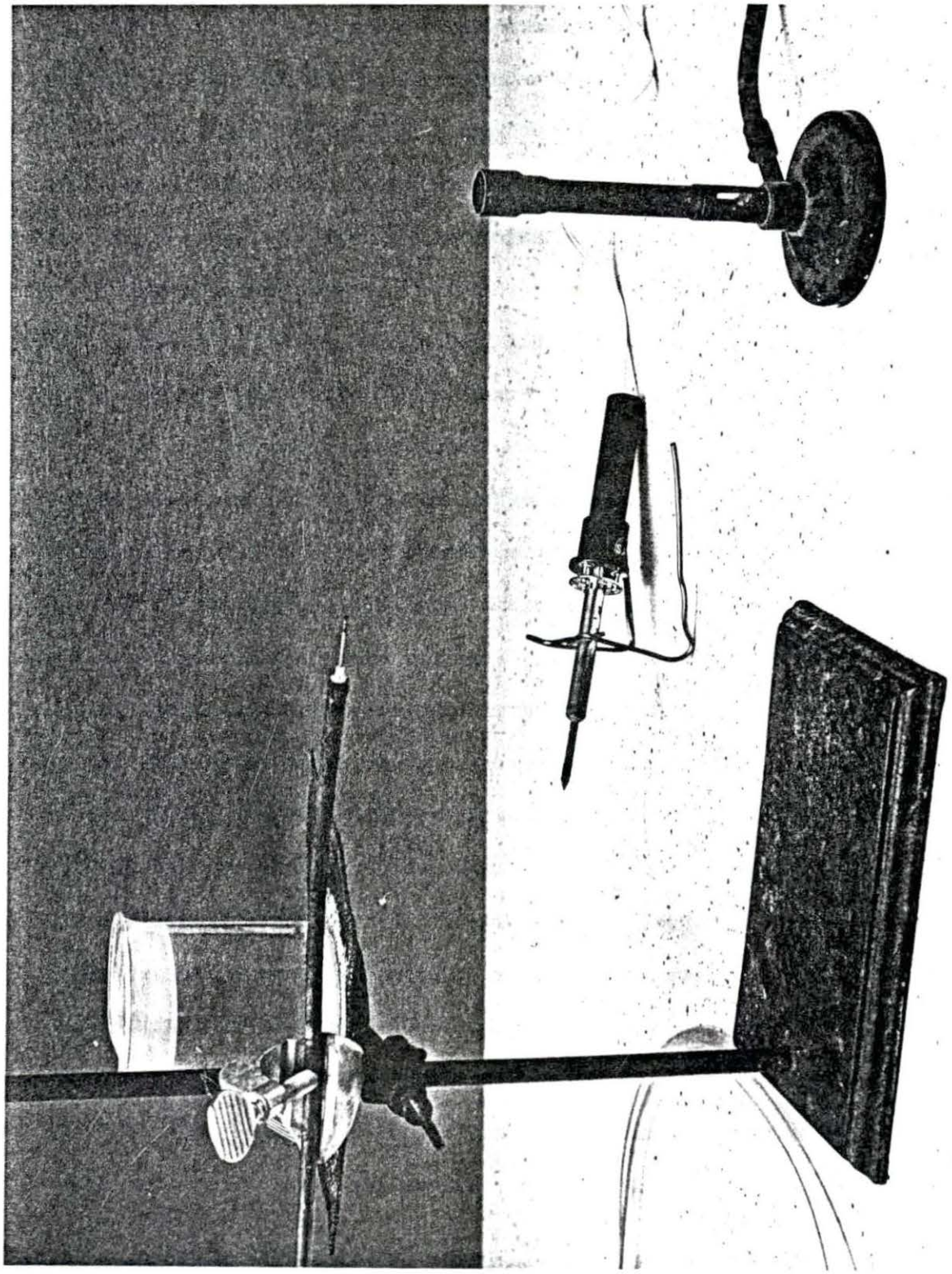
[1]Allied Electronics, Chicago, Illinois.

Figure 2. Apparatus used for constructing polyethylene cannulas



cannulas were filled with one percent neomycin sulfate[1] to help prevent infection and to prevent blood entering the cannula. Braided silk surgical suture size (00) was tied on the cannula at the anterior end and at the junction of the PE 10 and PE 50 tubings. An incision just posterior to the ears was made with a scissors. A similar incision was made in the skin anterior to the pelvic bone, posterior to the last rib, and lateral to the lumbar vertebrae. A 1/4 inch copper tube was inserted subcutaneously in the head incision and pushed out the posterior incision in the left flank. The cannula was inserted into the copper tube. The copper tube was pulled forward and removed, thus leaving the cannula in place subcutaneously. The rat was placed in dorsal recumbency on the rat board and the legs were attached to the board with rubber bands. About a two inch incision was made along the linea alba with a scalpel and the skin was separated from the underlying muscle tissue. Scissors were used to enter the abdominal cavity. The intestines were retracted laterally within the abdominal cavity. Sterile Q-tip applicators were used to clean the fascia and fat from the aorta just anterior to the iliac bifurcation. A needle nose hemostat was used to penetrate the psoas muscle and the muscles of the abdominal wall to the posterior incision. The posterior ligature on the cannula was gripped with the hemostat. The cannula was pulled through the muscle tissue to lay lateral to the aorta just between the genitofemoral nerve and the left ureter. The cannula was anchored to the psoas muscle by means of the posterior ligature on the cannula. The PE 10 tubing, which was to be inserted into the aorta, was cut off so that the cannula

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[1]Biosol, The Upjohn Company, Kalamazoo, Michigan.

end would lay posterior to the renal artery and anterior to the iliolumbar vein. While the aorta was occluded with the left hand, a 22 gauge needle was inserted in the aorta posterior to the iliolumbar vein and anterior to the iliac bifurcation. The PE 10 tubing was inserted into this puncture made by the needle and pushed anteriorly in the aorta (see Figure 3). Tetracyline hydrochloride[1] was sprinkled over the abdominal aorta near the cannulation site. The abdominal muscles were sutured with catgut, and the skin was closed with Michel clamps. The rat was placed in ventral recumbency so that the dorsal incisions could be sutured. The anterior incision was closed with the silk suture previously tied to the anterior section of PE 50 and 160 tubing. The rat was kept warm with a heat lamp after surgery in a one foot by one foot cardboard box. When normal movement was regained, the rat was moved to the original cage for use in the comparative blood pressure experiment (see Figure 4).

Blood pressure determinations

On the day following surgery, blood pressures were taken on the rat. Each rat was placed in a "rat holder"[2] with a temperature control unit attached. Sobin (1945) and Olmsted (1951) demonstrated that the temperature required to maintain maximum blood flow in the caudal artery of the tail was 40° to 42°C. A cuff with a length of 4.5 cms and a diameter of 1.5 cms was placed at the base of the rat's tail. A Pneumatic Pulse Pickup unit[3]

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[1]Polyotic, American Cyanamid Company, Princeton, New Jersey.

[2]E and M Instrument Company, Inc., Houston, Texas.

[3]E and M Instrument Company, Inc., Houston, Texas.

Figure 3. Surgical view

A - Polyethylene tubing (PE 10) shown after ligation to the iliopsoas muscle and permanently implanted into the abdominal aorta

B - Abdominal aorta with cannula inserted

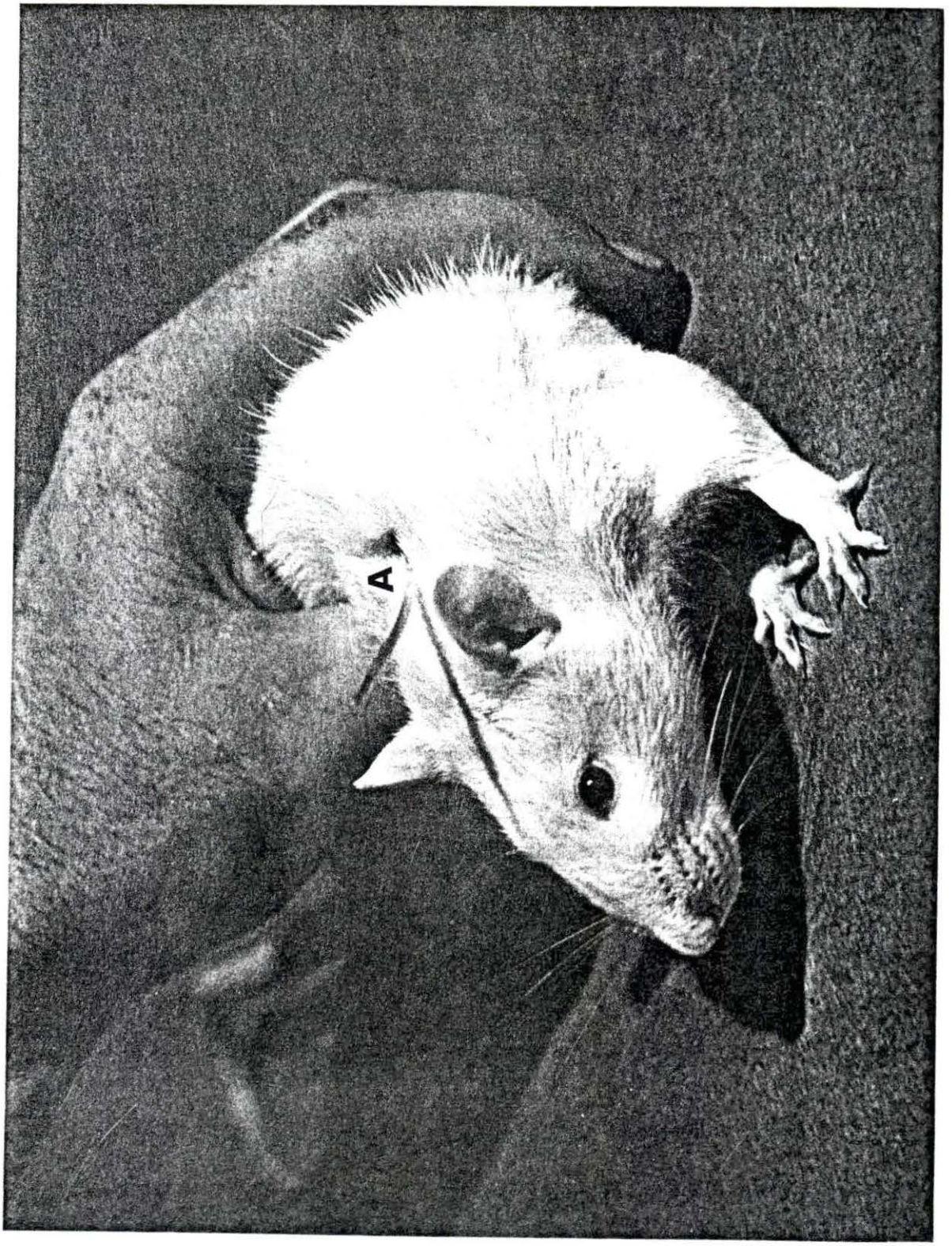
C - Iliopsoas muscle

D - Kidney


D
B
C
A

Figure 4. Cannulated rat

A - The exteriorized portion of cannula from which blood pressures were recorded


A

for recording indirect blood pressure was taped on the top of the tail about one half inch posterior to the cuff. The pulse pickup was connected to an Electrosphygmograph[1] which in turn was connected to a Transducer Monitor Coupler.[2] The Transducer Monitor Coupler permitted biological signals to be picked up by a physiograph transducer and recorded by the Grass model 7 Polygraph.[3] Indirect systolic blood pressure was recorded by raising the cuff pressure above that of the caudal artery and slowly lowering the cuff pressure until pulsations were observed on the polygraph recording. The equipment was calibrated after each rat (see Figure 5).

After clear indirect blood pressure pulses were recorded, the aortic cannula was connected to the transducer for the simultaneous recording of direct blood pressure. The cannula was connected to a Statham P23Dc[4] transducer which was connected directly to the Polygraph. A 0.04 ml fluid displacement in the cannula must occur for each 100 mm Hg of pressure recording.

A one percent heparin solution was flushed through the cannula by means of a reserve heparin bottle connected to the transducer. This insured better recordings by preventing blood clots forming in the end of the cannula. A direct comparison of the two recordings was obtained by drawing a vertical line from the point of reappearance of the indirect systolic blood pressure on channel one to the direct pressure point on

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[1] E and M Instrument Company, Inc., Houston, Texas.

[2] E and M Instrument Company, Inc., Houston, Texas.

[3] Grass Instrument Company, Quincy, Massachusetts.

[4] Statham Instrument, Inc., Los Angeles, California.

Figure 5. Equipment used for recording direct and indirect blood pressures

A - Grass Model 7 Polygraph

B - Transducer Monitor Coupler

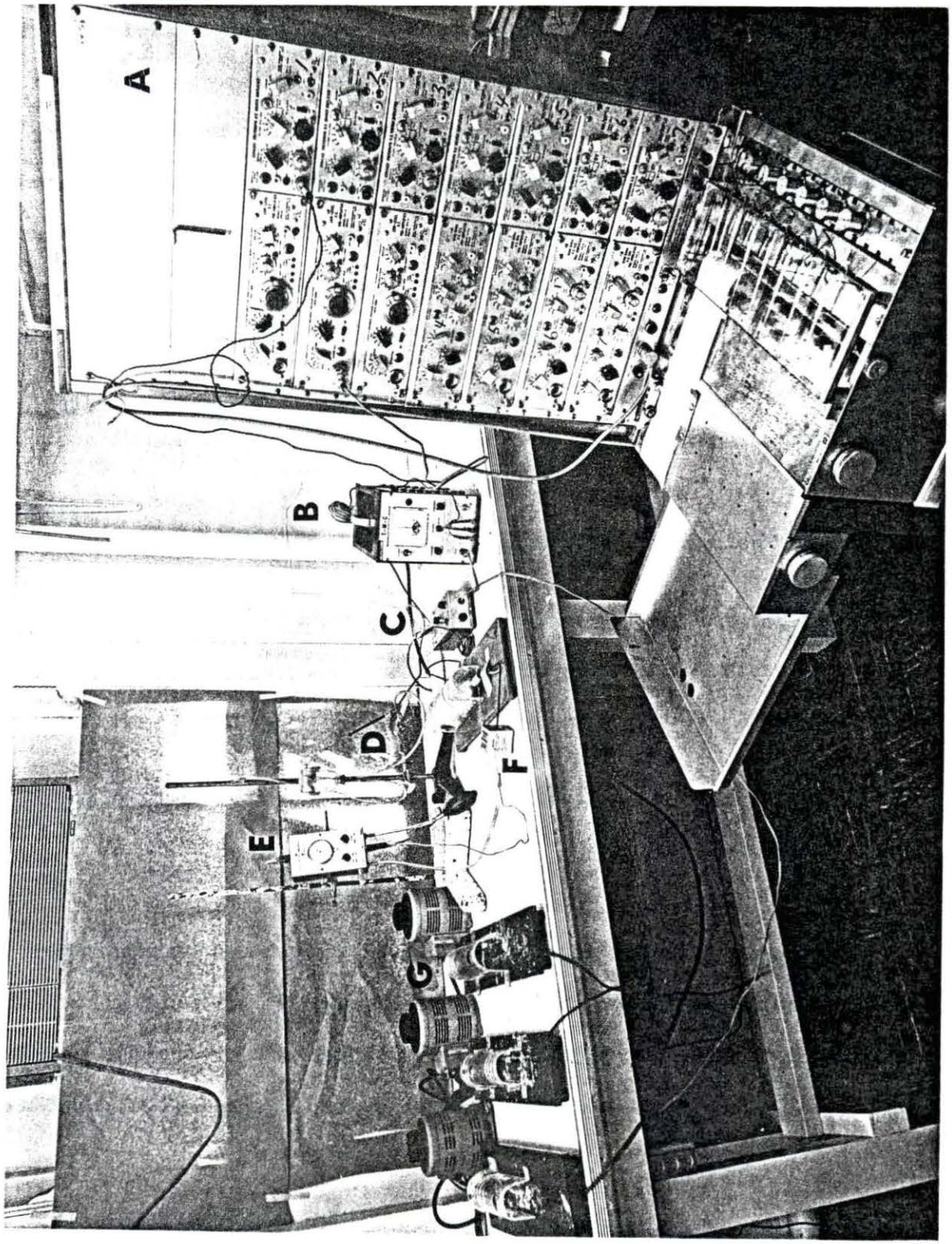
C - Rat holder with temperature control unit attached

D - Statham P23Dc pressure transducer with tuberculin syringe attached

E - Electrosphygmograph

F - Pneumatic Pulse Pickup unit

G - Homemade rat holders with rheostats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G

channel two (see Figure 6). In order to obtain a wider range of blood pressure values, each rat was recorded at a normal pressure and then at an elevated blood pressure. A vasopressin[1] dosage of 0.2 pressor unit was given with a tuberculin syringe via a three way stopcock valve connected between the cannula and the pressure transducer. Those rats which were not given vasopressin or whose responses were not recorded were compared solely on the normal recording. As stated previously, 19 rats were recorded only once and then returned to the cage. Several blood pressure values were determined from each recording to obtain better estimates of the average.

Experimental Procedure of Choline Supplementation

Group I and II rats were randomly distributed (one rat per cage) in the rack. A table showing the distribution of the two diets among the rats is shown below.

	Control diet	Experimental diet
Males	16	14
Females	12	14

Both groups of rats were divided so that 28 animals were placed on each diet. High mortality was encountered in Group I rats prior to beginning the study. Greater detail of this will be discussed in Results and Discussion. Since the Group II rats were replacement rats for Group I,

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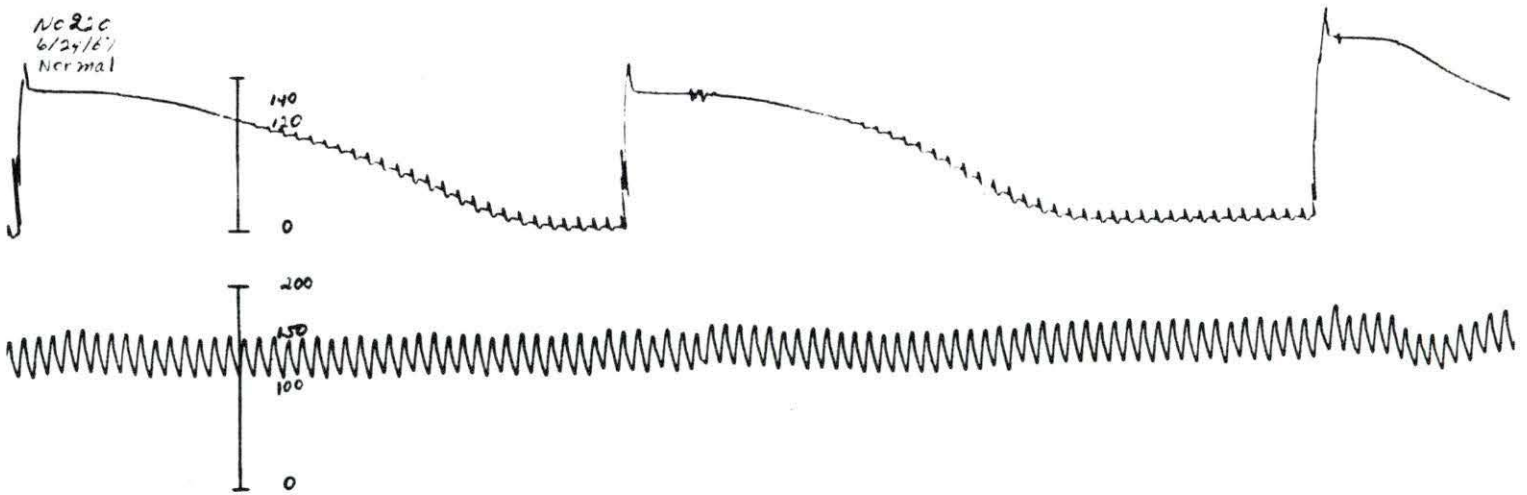
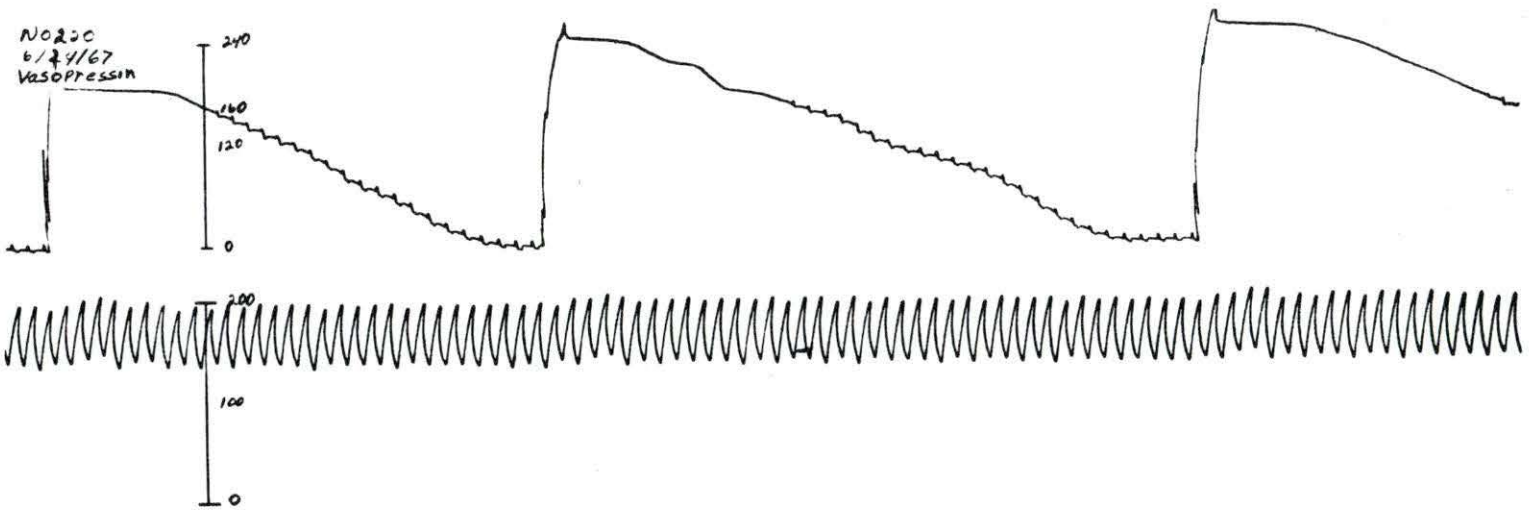
[1]Pitressin, Park, Davis and Company, Detroit, Michigan.

Figure 6. Indirect and direct blood pressure recordings during a normal response and following vasopressin injection


No 2 c
6/24/67
Normal
140
120
0
200
150
100
0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6/24/67
Vasopressin
240
160
120
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200
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the number of males and females fed the control diet was not equal.

On May 1, 1967, the study evaluating the effects of a high choline chloride diet on blood pressure began. Group I, which consisted of the older rats, began the experiment on May 1, 1967 and was killed the week of July 1, 1967. Because of order delay, Group II was placed on the experiment May 7, 1967 and was killed the week of July 7, 1967.

Blood pressure measurements

Indirect blood pressures were recorded once a week during the experimental period on the rats in Groups I and II. The recording technique for the indirect blood pressures was the same as that used in the comparative experiment. Three homemade and one commercial rat holders were used. Rheostats were used to control voltage to the heating filament in the three self-built rat holders. As soon as the blood pressure values were obtained, the rat was returned to his cage and replaced by another rat. An average of ten minutes was required for the caudal artery to dilate enough to record the tail pulse. Several recordings were determined on each rat so that an average systolic blood pressure value was established for that week.

Blood analyses

A total blood sample of five ml was taken from the abdominal aorta of each rat under ether anesthesia. A two ml portion of this sample was emptied into a test tube containing two drops of one percent heparin. This sample was used for all the blood analyses except the determination of prothrombin time and total cholesterol. The remaining three ml of blood in the syringe were emptied into a test tube containing 0.3 ml of 0.1 M sodium oxalate. The three ml portion of the blood sample was used

for the prothrombin time and total cholesterol determinations. Blood analyses were run in duplicate on each rat. After the blood samples were taken, the rats were killed with ether.

Hemoglobin The Cyanmethemoglobin method[1] was used for hemoglobin analysis. A Sahli pipette was used to pipette 0.02 ml of whole blood into five ml of a previously prepared standard reagent. The transmittance of each sample was read with a Beckman B Spectrophotometer at 540 mμ and compared to predetermined standards.

Packed cell volume Blood was drawn into heparinized capillary tubes and one end sealed with heat. The samples were centrifuged with a micro-hematocrit centrifuge and read with a micro-hematocrit reader.

Total white blood cell count Whole blood was drawn to the 0.5 mark of a leucocyte pipette and diluted with 0.1 N HCl. The pipettes were placed on a pipette shaker. After the diluent was removed from the capillary portion of the pipette, the sample to be counted was discharged on the counting chambers of the hemacytometers. Both chambers were counted on a light microscope under low power. The total count from each chamber was multiplied by 50 and the average of the two was recorded.

Prothrombin time A 0.1 ml sample of blood plasma and 0.2 ml of Symplastin[2] were placed in separate test tubes and warmed to 37°C

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[1]Hycel Cyanmethemoglobin Instructions. Hycel, Inc., Houston, Texas.

[2]Warner-Chilcott Laboratories Div., Morris Plaines, New Jersey.

in a water bath. After the two samples were poured together, the time for a clot to appear was determined by a stop watch.

Total cholesterol The AutoAnalyzer[1] was used for the determination of total cholesterol. The extracts were prepared by placing 0.5 ml of plasma in a centrifuge tube containing 9.5 ml of isopropanol. The samples were centrifuged and small portions placed in the sample cups. Duplicates were run on each sample and compared to previously prepared analyzed standards of 50, 100, 200, 300, and 400 mg percent.

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[1]Technicon Instruments Corporation, Ardsley, New York.

RESULTS AND DISCUSSION

Analyses

The collected data were organized and programmed for computer[1] evaluation. Correlation matrices were used to evaluate the indirect and direct blood pressure comparisons and analysis of variance was used for evaluation of the remaining data. The Student's t-test was applied to the results from the analyses of variance.

Indirect and Direct Blood Pressure Comparisons

The computed correlation matrices of the two methods were evaluated at the five and one percent levels of significance. The indirect systolic blood pressure was correlated with the direct systolic and direct mean blood pressures at the normal pressure and after vasopressin administration. All the variables compared had a significant positive correlation except the indirect systolic and direct systolic blood pressures evaluated after the injection of vasopressin. A table illustrating the correlation coefficients is shown below.

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[1]Computer Center. Iowa State University, Ames, Iowa.

Variables	Correlation Coefficients
NIS[1] and NDS[2]	0.7280[7]
NIS and NDM[3]	0.6104[8]
VIS[4] and VDS[5]	0.5285
VIS and VDM[6]	0.6448[9]

Further experimentation with larger samples could possibly improve the statistical evaluation of the correlation in which no significance at the five percent level was found. The correlation coefficient of this variable indicates a high positive correlation, but not enough to be significant with a sample size of seven. A least squares was computed and linear equations were found which could be used to directly convert the recorded indirect blood pressure to the direct blood pressure. It must be emphasized that these equations only apply to the rats used in this experiment. Further experimentation with larger samples could elucidate more

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[1]Normal indirect systolic blood pressure.

[2]Normal direct systolic blood pressure.

[3]Normal direct mean blood pressure.

[4]Vasopressin indirect systolic blood pressure.

[5]Vasopressin direct systolic blood pressure.

[6]Vasopressin direct mean blood pressure.

[7]Significant at the five percent level.

[8]Significant at the one percent level.

[9]Significant at the one percent level.

accurate equations which could be used in any experiment using the indirect blood pressure measurement on rats. Because of the lack of correlation, the equation for the prediction of direct systolic blood pressure from indirect systolic blood pressure after vasopressin was not computed. A table listing the equations is shown below.

Blood pressure	Equation
NIS and NDM	NDM = 66.76 + (.53)(NIS)
NIS AND NDS	NDS = 57.06 + (.74)(NIS)
VIS and VDM	VDM = 65.66 + (.64)(VIS)
VIS and VDS	Lack of correlation

An explanation for the failure of the VIS and the VDS to correlate significantly is not completely understood. It was very easy to obtain direct mean blood pressures in every animal, but the direct systolic blood pressures were more difficult to obtain, especially following vasopressin. Vasopressin caused a reflex slowing of the heart which sometimes hindered an accurate indirect systolic blood pressure recording. If the pressure in the occluding cuff was lowered at a rate such that the indirect systolic pressure was approached between two heart beats, the recorded value of the indirect systolic blood pressure would be low. After vasopressin, the rats became very restless which hindered the pickup of accurate indirect recordings. In this case, a slight movement caused a deflection to be recorded which was not a systolic pulse. The peripheral vasoconstriction caused by vasopressin may be another reason for this inaccuracy. The caudal artery may have been constricted too much for accurate indirect systolic pulse

pressures to be recorded. The indirect systolic pressure would still correlate with the direct mean blood pressure, but may not be high enough to correlate with the direct systolic blood pressure.

Only those rats which appeared to have accurate systolic blood pressures, without a damped pulse pressure, were used for the correlation of indirect systolic and direct systolic blood pressures. All the other rats were correlated at the two blood pressure values solely by their means. Dampening of the pulse pressure may have been due to: 1) a small leak at the junction of the PE 10-PE 50 tubing, 2) the aorta could have been partially occluding the open end of the PE 10 tubing, and 3) a small blood clot in the cannula which could not be completely flushed out. All of the rats which had dampened pulse pressure were still excellent experimental animals for correlation of direct mean blood pressures with the indirect systolic blood pressure.

The literature did not reveal any work showing the correlation coefficients of indirect and direct blood pressure measurements; however, many authors did make comparisons of various indirect and direct blood pressure techniques on a mm Hg basis. The results obtained in this experiment agreed somewhat with those obtained by Byrom and Wilson (1938). These authors found that the plethysmographic method for recording indirect blood pressure from the tail gave values ten mm Hg lower than the direct femoral blood pressure. Williams et al. (1939) found that the plethysmographic method was more comparable to the direct method in unanesthetized rats. The size of the occluding cuff may have been the decisive factor in making the indirect blood pressure more comparable to the direct blood pressure in the studies done by Williams et al. (1939) and Byrom and

Wilson (1938). A summary table illustrating the comparative blood pressures of the two methods described in this thesis is shown below.

	Blood pressure (mm Hg)	
	Normal response	Vasopressin response
Indirect systolic	107.7	145.9
Direct systolic	136.9	184.2
Direct mean	123.7	157.6

By comparing the indirect and direct blood pressure, the correlation coefficients were found to be highly significant, indicating that the indirect systolic blood pressure could be used to evaluate future data involving blood pressure studies on rats. The data point out that most evaluations will have to be done on a relative basis rather than exact blood pressure measurement. A further limitation of the indirect method appears to exist as the blood pressure is increased above normal. Further experimentation with drugs that do not cause peripheral vasoconstriction might demonstrate a high correlation with the direct blood pressure at all levels. If the limitations are taken into consideration, the indirect method may be used in place of the direct blood pressure method which has many more difficulties and involves more time.

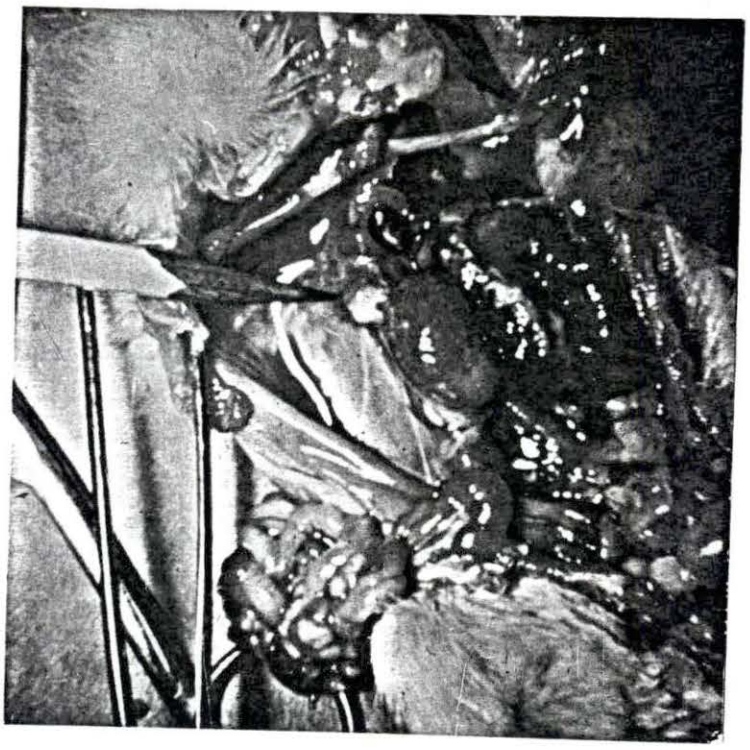
The experiment described in this thesis was originally planned to determine blood pressures every week for two months on 30 cannulated rats. In the first cannulated rats, the cannula entered the abdominal cavity on the animal's right side, and was ligated to the right iliopsoas muscle. The PE 10 tubing crossed the vena cava before entering the abdominal aorta.

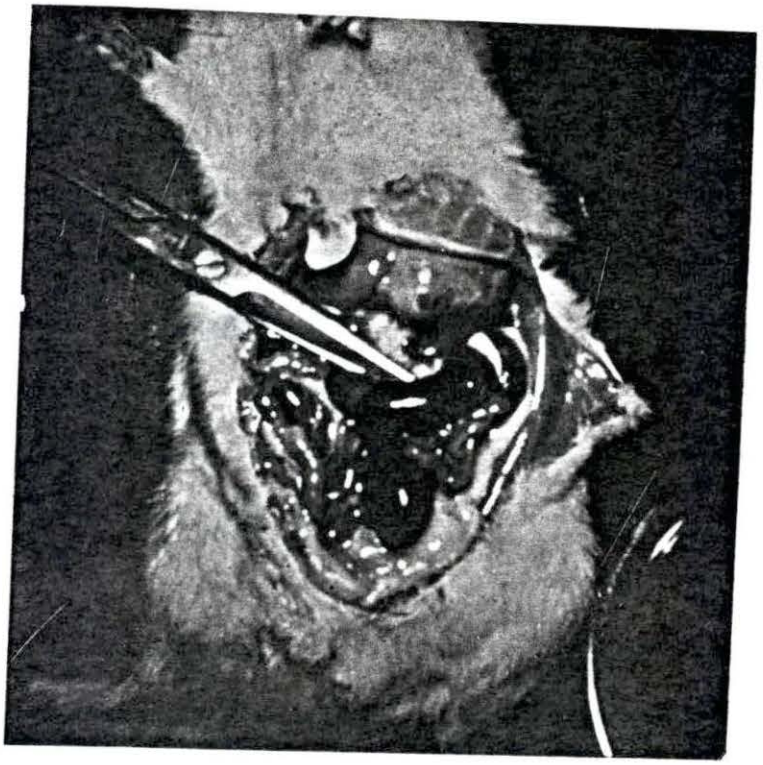
All rats cannulated with this procedure died about two hours after surgery from interrupted posterior circulation. After revaluating the technique, it was decided that the cannula was occluding the vena cava and aorta by crossing these two major vessels and thus reduced blood flow to the posterior limbs. A second problem which continually existed was to maintain functional cannulas. Since the rats were young, longer cannulas had to be used to allow for growth during the experimental period. Usually within three days after surgery, the rat would either chew the end off the cannula or pull on the cannula enough to pull the PE 10 tubing out of the aorta, both resulted in a non-functional cannula.

The most important observation involving the chronically implanted aortic cannulas occurred when 18 of the 28 cannulated rats died over a seven day period. All of the dead rats were autopsied and cultures were examined by the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University. All the cultures examined were negative, but gross observation revealed that every rat had either a severe subcapsular hematoma around the left kidney, or a hematoma around the intestines, and small white dispersed nodules on the kidney not encapsuled by blood. In some rats the right kidney also had a subcapsular hematoma (see Figures 7 and 8). The only explanation for the high mortality, by either gross or microscopic evaluation, was the possibility that emboli entered the renal or mesenteric arteries causing infarction and possible rupture of the small vessels. This could have occurred because the end of the PE 10 tubing, which was anterior to the renal artery, was flushed (every two days) to remove any small clots that were lodged in the tip of the PE 10 tubing. Only those rats which no longer had the cannula in the aorta

Figure 7. Hematoma around lower part of left kidney with small nodules on the area not involved

Figure 8. Severe hematoma around intestine





survived. This information helped establish the importance for a successful surgical technique and to further emphasize the difficulties involved with the direct blood pressure method.

Thus, the rats which were referred to in Materials and Methods as Group II were ordered to replace the rats which initially died, and the experiment was redesigned using only the indirect blood pressure method. Hence, the selection of other rats (Group IV) was made for the direct and indirect blood pressure evaluation.

Effects of Choline Chloride on Indirect Blood Pressure

An analyses of variance and t-test were conducted (at the five percent level) to evaluate the effects of the experimental diet on indirect blood pressure. The effect of the diet was not statistically significant at the five percent level, although the ten percent level of significance was approached during several weeks of the experiment. It appeared that the experimental diet caused a slight but non-significant blood pressure rise during six of the nine weeks of the experiment and a slight but non-significant decrease the other three weeks. The inability of the experimental diet to significantly affect blood pressure is in agreement with Hunt (1915) and Simonart (1932) who both reported that injectable choline chloride probably has no physiological importance, but is converted to derivatives which are more active.

The influence of sex on blood pressure was demonstrated to be very significant (one percent level), with the males having higher values than females. This was in disagreement with Medoff and Bongiovanni (1945) who

found that sex caused no variation in blood pressures. A table summarizing these data are shown below.

	Blood pressure (mm Hg)	
	Control diet	Experimental diet
Males	133.8	133.4
Females	120.4	121.7

An aspect which should be considered in greater detail in further experimentation would be to block choline oxidase with drugs and supplement the experimental diet with chlortetracycline. This would increase choline absorption. Luecke and Pearson (1945), Bligh (1952), and Appleton and Levy (1951) reported that blood choline levels remain fairly constant regardless of the amount ingested or infused. de la Huerga and Popper (1952) found that chlortetracycline increased choline absorption in the intestinal tract. Bianchi and Azzone (1964) found that rotenone inhibited the oxidation of choline by choline oxidase to betaine aldehyde and betaine. Conducting an experiment with these approaches, the physiological relationship of choline and blood pressure could possibly be examined.

Effects of Choline Chloride on Heart Rate

An analyses of variance and t-test (five percent level) were computed and it was found that the experimental diet, sex, and sex-diet interaction did not have any significant effect on heart rate. A summary table is shown below.

	Heart rate (beats/minute)	
	Control diet	Experimental diet
Males	417.5	402.2
Females	434.5	423.1

Effects of Choline Chloride on Blood Constituents

Hemoglobin

An analyses of variance and t-test (one percent level) indicated that sex, diet, and sex-diet interaction all significantly influenced the hemoglobin level.

It was found that the males had significantly higher hemoglobin values than the females. McKibbin et al. (1944), Reid (1955), and Engel (1948) demonstrated that a choline deficiency decreased the hemoglobin concentration while Steigman (1952), in a short term experiment, found that injected levels of choline did not affect hemoglobin values. The results observed in the study described in this thesis found that the supplemental dietary choline chloride significantly increased hemoglobin values. A significant sex-diet interaction was observed in hemoglobin values from males on experimental diet when compared with the control while the values of females on control diet were significantly less. A summary table is shown below.

	Hemoglobin (gms percent)	
	Control diet	Experimental diet
Males	13.6	13.7
Females	11.5	13.0

Packed cell volume

The statistical analyses of the packed cell volume showed results similar to that obtained from the hemoglobin data. The males had a significantly (one percent level) higher packed cell volume than the females. The experimental diet also significantly (five percent level) increased the packed cell volume, but no sex-diet interaction was found. These values were again opposite to those reported on the choline deficient diets by McKibbin et al. (1944) and Reid (1955), and in disagreement with Steigman (1952). No physiological explanation can be given at this time, but the answer to the packed cell volume increase would undoubtedly elucidate the hemoglobin increase. A summary table is shown below.

	Packed cell volume (volume percent)	
	Control diet	Experimental diet
Males	43	44
Females	37	40

Total white blood cells

The total white blood cell count was not significantly affected by sex, diet, or sex-diet interaction. These results agree with Steigman (1952). The summary table is shown below.

	Total white blood count (mm^3)	
	Control diet	Experimental diet
Males	12,400	10,980
Females	8,575	8,309

Prothrombin time

The statistical analyses revealed that the experimental diet significantly (five percent level) decreased prothrombin time. This finding was opposite to the results reported in choline deficient rats and puppies by Honorato and Ivanovic (1944) and McKibbin et al. (1944), respectively. Since choline is highly active in other biochemical mechanisms in the liver, it is possible that choline could also influence the blood coagulation mechanism. A summary table is shown below.

	Prothrombin time (seconds)	
	Control diet	Experimental diet
Males	16.5	15.5
Females	16.4	15.3

Total cholesterol

The experimental diet significantly (five percent) increased the total plasma cholesterol levels. These results disagree with those reported by Firstbrook (1950), who found that choline chloride lowered the plasma cholesterol in rabbits. No sex or sex-diet interaction effects were found to be significant. The elevation in plasma cholesterol probably resulted from an increase in the mobilization of cholesterol esters from the liver. A summary table is presented below.

	Plasma cholesterol (mg percent)	
	Control diet	Experimental diet
Males	73.0	89.0
Females	79.8	86.0

The Effects of Choline Chloride on Body Weight

Although the statistical analyses (five percent level) did not show a significant body weight response to the experimental diet, a significant (ten percent level) decrease in body weight was found from the third week of the experiment to the conclusion. The reduction in body weight did not appear as dramatic as the weight reductions of the rats fed choline chloride as reported by Hodge (1945). As expected, a very significant (one percent level) sex difference was found with the males steadily outgrowing the females. A summary table is shown below.

	Body weight (gm)	
	Control diet	Experimental diet
Males	458	430
Females	306	270

Mortality

Only those rats which died during the experiment evaluating the effects of the experimental diet on blood pressure, hematology, and body weight were recorded. A table listing the deaths is presented below.

Rat no.	Sex	Date of death	Comments
10	Female	June 13, 1967	Unknown cause - probably circulatory failure occurring during measurement of blood pressure
118	Female	June 4, 1967	
123	Male	May 19, 1967	

Rat no.	Sex	Date of death	Comments
13	Male	May 3, 1967	Cardiac puncture during blood sampling
46	Female	May 3, 1967	
119	Male	June 5, 1967	Observed to be abnormal from beginning of experiment

SUMMARY AND CONCLUSIONS

The data obtained from the pneumatic pulse pickup for determining the indirect blood pressure of rats correlated with simultaneous blood pressures recorded from chronic aortic cannulas. Vasopressin was used to elevate the blood pressure for another correlation study of the two blood pressure techniques. It was found that the normal indirect systolic blood pressure significantly correlated with the direct mean and systolic blood pressures. Results of the vasopressin injection showed that a significant correlation existed between the indirect systolic blood pressure and the direct mean blood pressure, but not between the indirect systolic blood pressure and the direct systolic blood pressure. There was a possibility that the vasopressin may have interfered with the correlation at the elevated blood pressure level by restricting blood flow in the tail and inhibiting an accurate indirect pulse pickup. Linear equations were computed which could be used to determine the direct blood pressure from the indirect blood pressure. The conversion equations only apply to the small sample of rats used in this experiment, and it should be emphasized that further work with a larger number of rats should be done to compute more valid equations that could be used in other blood pressure experiments with rats.

Analysis of variance and t-test at the five and one percent levels were performed on all variables with the computer. The indirect blood pressure method was used to examine the effects of an orally fed three percent choline chloride diet on the blood pressure and heart rates of rats. No significant blood pressure or heart rate responses were caused

by the experimental diet, sex, or sex-diet interaction.

Blood samples were taken from the rats after completion of the indirect blood pressure experiment. The hemoglobin and packed cell volume values were both significantly increased by the experimental diet. It was also found that the male rats had a significantly higher hemoglobin value and packed cell volume than the females. No significant changes were observed in the total white blood cell count by the experimental diet, sex, or sex-diet interaction. The prothrombin time was significantly decreased by the experimental diet, but no other significant changes from sex or sex-diet interaction were noticed. The total plasma cholesterol was significantly increased by the experimental diet, but was also not affected by sex or sex-diet interaction.

The three percent choline chloride diet did not significantly (five percent level) influence the body weight, but the diet did cause a decrease in body weight at the ten percent level of significance. There was a significant increase in the final body weight of males over females.

It is apparent that the experimental diet had little effect on most of the physiological parameters examined in this study. Those variables which were influenced by choline cannot be explained, but should indicate that further work is necessary. Even though blood pressure was not significantly altered, the blood pressure changes could have been examined more thoroughly if a choline oxidase inhibitor and chlortetracycline would have been used. This would have involved increasing the intestinal absorption and decreasing systemic catabolism of choline. Further study in this area could elucidate properties of choline chloride which could be used in controlling pathologic and adverse changes in blood pressure.

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APPENDIX

Table 1. Indirect and direct blood pressure comparisons (mm Hg)

Rat no.	Normal			Vasopressin		
	Indirect systolic	Direct systolic	Direct mean	Indirect systolic	Direct systolic	Direct mean
46	104.3	147.2	130.6	116.0	--- [a b]	138.0
200	110.9	--- [b]	128.2	133.7	--- [b]	136.3
202	107.8	--- [b]	133.4	--- [c]	--- [c]	--- [b]
203	106.7	--- [b]	123.7	138.0	--- [b]	160.0
204	92.0	--- [b]	107.6	118.7	--- [b]	123.7
206	85.7	--- [b]	128.7	161.1	188.8	167.7
207	107.8	--- [b]	124.3	151.8	--- [b]	173.5
208	129.7	--- [b]	134.9	165.0	--- [b]	171.5
209	112.2	--- [b]	126.7	139.2	--- [b]	157.3
210	123.0	--- [b]	134.0	157.5	--- [b]	157.9
212	92.0	--- [b]	125.0	155.2	--- [b]	172.1
213	128.7	154.3	141.3	149.5 [c]	176.8 [c]	161.8 [bc]
214	115.4	131.4 [b]	115.6	---	--- [b]	---
215	121.5	---	121.7	171.50	---	154.8
216	92.6	130.0	119.4	140.3	182.8	163.7
217	112.1	147.7	129.1	150.3	206.7	180.7
218	81.4	124.6	103.2	114.6	169.8	143.2
219	96.4	105.6	88.4	174.9	186.3	156.4
220	126.8	154.0	134.2	152.5	185.5	160.3
Average	107.7	136.9	123.7	145.9	184.2	157.6

[a] Pressure recorded on a different day then the normal.

[b] Dampening of pulse wave prevented accurate recording of the systolic blood pressure.

[c] No values could be obtained because of movement artifact or vessel constriction.

Table 2. Indirect blood pressure of male rats on control diet (mm Hg)

Rat no.	Week of experiment 1	2	3	4	5	6	7	8	9	Average
13[a]	110.5	Died								
27	126.0	132.0	142.0	125.1	157.0	166.0	135.0	128.7	145.3	139.7
55	137.8	139.5	125.0	130.0	138.4	141.5	137.8	131.6	143.7	136.0
101	117.8	127.0	123.4	148.8	124.0	134.8	148.3	137.4	125.3	131.9
105	139.3	125.0	126.0	120.3	134.0	149.6	166.3	143.1	118.0	135.8
109	137.0	137.0	136.2	136.0	136.9	140.5	136.8	139.8	143.4	138.2
111	138.6	123.6	132.4	124.0	166.8	128.0	156.9	127.2	126.0	135.9
113	124.0	126.8	135.5	131.5	135.2	132.0	130.0	155.8	130.0	133.1
115	130.7	125.2	126.8	135.8	139.7	132.7	129.0	141.8	124.0	131.7
119[a]	131.2	132.8	133.9	95.0	Died					
123[a]	118.5	118.0	Died							
126	124.8	132.0	116.8	118.5	140.8	129.7	137.5	133.7	123.0	128.5
128	112.3	114.5	128.0	136.0	145.2	135.3	143.4	131.0	133.0	130.9
130	118.3	123.0	126.0	120.0	139.7	132.3	128.5	146.1	141.0	130.6
Average	127.9	127.8	128.9	129.6	141.6	138.4	140.9	137.8	132.1	133.8

[a]Not counted in average.

Table 3. Indirect blood pressure of female rats on control diet (mm Hg)

Rat no.	Week of experiment 1	2	3	4	5	6	7	8	9	Average
10[a]	135.8	103.0	115.3	112.4	108.2	Died				
39	133.3	105.3	123.9	125.3	106.0	120.0	122.4	120.4	119.8	119.6
41	95.0	113.5	112.4	140.3	122.1	108.3	128.3	114.0	120.2	117.1
43	123.1	119.0	116.4	108.0	131.0	138.5	122.8	140.6	106.3	122.9
51	107.3	98.7	104.8	113.0	99.6	106.0	119.2	103.8	107.3	106.6
52	114.3	121.3	126.0	130.3	109.0	123.3	81.0	109.7	113.0	114.2
100	112.3	144.8	111.0	102.8	110.5	124.5	117.3	116.2	118.2	117.5
104	124.8	119.0	129.4	114.3	126.2	114.3	127.5	125.0	124.0	122.7
108	137.3	157.1	124.6	138.8	167.2	130.3	121.6	129.2	122.8	136.6
114	126.8	125.5	137.1	103.7	130.7	124.3	125.6	127.3	124.0	125.0
118[a]	111.5	135.0	106.0	116.7	144.9	Died				
122	126.14	136.0	104.8	109.8	124.0	128.0	132.0	126.0	118.3	121.9
Average	119.6	124.1	119.0	118.6	122.6	121.8	119.8	121.2	117.4	120.4

[a]Not counted in average.

Table 4. Indirect blood pressure of male rats on experimental diet (mm Hg)

	Week of experiment									
Rat no.	1	2	3	4	5	6	7	8	9	Average
8	156.0	131.5	160.3	161.0	156.7	150.7	142.0	143.0	127.2	147.6
45	140.0	109.8	120.0	142.5	112.0	123.2	115.9	128.3	124.1	124.0
53	138.0	120.5	154.8	175.6	136.0	139.3	152.3	143.3	156.8	146.3
56	144.4	129.0	145.0	147.7	148.3	134.7	150.0	127.8	133.4	140.0
57	144.4	138.8	136.3	182.5	130.2	139.1	140.0	152.8	137.3	144.6
103	130.4	125.8	125.3	113.0	126.7	128.7	138.4	129.8	118.0	126.2
107	135.6	121.7	141.9	131.6	132.0	133.1	134.0	132.5	126.5	132.1
110	121.6	123.3	125.0	115.3	136.7	136.0	130.0	128.0	133.9	127.8
112	135.3	138.1	122.9	121.9	133.4	125.5	149.7	129.4	127.7	131.5
117	137.0	135.4	142.2	128.0	151.3	128.7	149.3	137.0	142.0	139.0
121	132.7	123.3	132.7	127.5	130.7	138.8	116.7	139.8	124.0	129.6
125	110.7	116.0	127.7	122.6	132.7	117.8	136.3	126.0	125.2	123.9
127	134.2	131.2	133.0	127.3	132.0	133.0	141.0	153.2	150.5	137.3
129	130.0	115.2	119.3	118.0	128.0	127.0	143.0	125.6	125.0	125.7
131	120.5	126.5	125.8	124.4	120.5	126.0	138.3	126.0	122.0	125.6
Average	134.1	125.7	134.1	135.9	133.8	132.1	138.5	134.8	131.1	133.4

Table 5. Indirect blood pressure of female rats on experimental diet (mm Hg)

Rat no.	Week of experiment									
	1	2	3	4	5	6	7	8	9	Average
9	123.0	112.0	126.8	129.4	129.7	126.0	128.7	132.0	158.1	129.5
40	86.4	114.2	123.3	122.0	110.3	123.3	127.5	141.3	126.4	119.4
42	122.0	131.2	123.3	127.2	123.6	123.2	125.7	120.3	112.1	123.2
46[a]	96.2	Died								
48	119.5	115.7	123.9	117.2	111.0	128.3	115.0	136.0	125.1	121.3
54	99.6	80.0	125.7	122.0	99.2	111.8	100.0	118.0	126.0	109.1
58	128.3	114.3	129.0	128.0	147.0	127.0	126.5	146.3	130.7	130.8
59	140.3	115.9	118.3	134.2	123.0	131.0	129.0	128.0	120.7	126.7
60	97.0	114.0	108.8	115.6	110.3	113.0	130.8	123.5	114.3	114.2
102	121.3	132.0	106.0	122.5	112.0	118.7	122.3	124.0	132.5	121.3
106	129.0	125.6	124.6	135.5	133.5	139.2	137.0	137.5	122.8	131.6
116	117.6	117.5	115.7	117.0	119.4	110.0	120.0	124.0	128.0	118.8
120	130.3	135.0	113.6	107.7	138.3	126.0	136.8	126.5	130.7	127.2
124	117.7	110.4	103.8	93.3	120.0	125.5	100.0	106.5	106.0	109.2
Average	117.1	116.8	118.7	120.9	121.3	123.3	123.0	128.0	125.6	121.7

[a]Not counted in average.

Table 6. Effect of control diet on heart rate of male rats (beats/min)

	Week of experiment									
Rat no.	1	2	3	4	5	6	7	8	9	Average
27	420	420	420	360	420	480	420	480	420	426.7
55	420	480	480	420	420	420	480	480	420	446.7
101	480	420	360	420	420	480	480	480	420	440.0
105	480	420	420	480	480	480	480	480	420	460.0
109	420	480	420	420	480	480	540	480	420	460.0
111	360	360	360	360	540	420	420	420	360	400.0
113	360	360	360	360	480	480	480	360	420	406.7
115	420	420	360	420	420	420	420	420	420	413.3
126	360	480	360	360	420	420	420	420	420	406.7
128	360	300	360	360	420	480	420	420	360	386.7
130	300	300	360	360	360	360	360	360	360	346.7
Average	398.2	403.6	387.3	392.7	441.8	447.3	447.3	436.3	403.6	417.5

Table 7. Effect of control diet on heart rate of female rats (beat/min)

	Week of experiment									
Rat no.	1	2	3	4	5	6	7	8	9	Average
39	360	360	360	300	360	360	420	420	360	366.7
41	480	420	420	480	480	540	540	540	540	493.3
43	360	420	360	420	420	420	420	540	360	413.3
51	480	480	480	420	420	420	480	420	420	446.7
52	420	420	420	420	420	480	540	420	420	440.0
100	480	420	420	360	420	420	480	420	480	444.4
104	420	420	480	360	480	480	480	480	480	453.3
108	420	480	420	420	480	480	420	420	420	440.0
114	420	360	360	360	480	480	480	540	420	433.3
122	420	360	420	360	420	420	480	420	420	413.3
Average	426.0	414.0	414.0	390.0	438.0	450.0	474.0	462.0	432.0	434.5

Table 8. Effect of experimental diet on heart rate of male rats (beat/min)

Rat no.	Week of experiment									Average
	1	2	3	4	5	6	7	8	9	
8	360	360	360	360	360	420	420	360	360	373.3
45	420	420	420	420	420	360	420	420	420	413.3
53	480	420	420	360	420	360	360	480	360	406.7
56	420	360	420	420	480	420	420	420	420	420.0
57	360	360	300	480	360	360	420	300	300	360.0
103	480	360	360	360	480	420	420	420	480	420.0
107	540	360	480	360	480	420	540	480	480	460.0
110	480	360	360	420	540	420	480	420	420	433.3
112	480	420	480	480	480	480	420	420	360	446.7
117	480	360	360	360	420	420	420	360	480	406.7
121	480	300	360	420	420	420	420	420	360	400.0
125	360	300	300	360	420	360	360	360	420	360.0
127	420	360	360	360	420	360	360	360	360	373.3
129	300	360	360	300	360	420	420	420	420	373.3
131	420	360	420	300	360	420	420	420	360	386.7
Average	432.0	364.0	384.0	384.0	428.0	404.0	420.0	404.0	400.0	402.2

Table 9. Effects of experimental diet on heart rate of female rats (beat/min)

Rat no.	Week of experiment									Average
	1	2	3	4	5	6	7	8	9	
9	420	420	420	420	420	420	420	420	420	420.0
40	420	420	480	420	420	420	480	480	360	433.3
42	420	420	360	420	420	420	420	420	420	413.3
48	480	420	360	420	420	420	420	480	420	426.7
54	360	360	360	360	480	420	360	360	420	386.7
58	420	420	480	480	480	540	420	480	480	466.7
59	540	480	420	420	420	360	360	420	420	426.7
60	420	420	480	360	420	420	420	480	360	420.0
102	360	480	420	420	540	540	480	480	480	466.7
106	420	360	420	480	540	480	420	480	480	453.3
116	420	360	360	360	420	420	420	420	360	393.3
120	420	360	420	420	420	420	420	420	420	413.3
124	360	300	360	360	480	420	360	360	420	380.0
Average	420.0	401.5	410.8	410.8	452.3	438.5	415.4	438.5	420.0	423.1

Table 10. Hematology of male rats on control diet

Rat no.	Packed cell volume %	Hemoglobin gm %	White blood cell count mm^3	Prothrombin time sec.	Cholesterol mg %
27	42	13.5	16,600	15.9	60
55	37	13.1	12,950	16.6	66
101	43	13.2	10,425	16.6	80
105	42	12.5	15,425	18.2	78
109	46	14.6	10,575	16.6	67
111	42	13.0	10,800	15.2	80
113	44	13.2	13,825	14.0	64
115	36	13.1	6,225	13.7	92
126	50	14.8	13,575	13.7	72
128	46	14.5	11,475	20.6	68
130	43	13.6	14,525	20.7	85
Average	43	13.6	12,400	16.5	73

Table 11. Hematology of female rats on control diet

Rat no.	Packed cell volume %	Hemoglobin gm %	White blood cell count mm^3	Prothrombin time sec.	Cholesterol mg %
39	38	13.5	9,400	17.7	63
41	29	9.9	6,800	14.2	76
43	41	13.0	8,375	14.4	73
51	34	11.5	16,850	20.7	88
52	36	11.0	9,075	19.4	70
100	40	11.5	5,375	15.7	--[a]
104	40	11.3	6,700	15.7	100
108	40	11.7	5,275	15.2	--[a]
114	35	10.2	9,325	14.7	108
122	--[b]	--[b]	--[b]	--[b]	60
Average	37	11.5	8,575	16.4	79.8

[a]Lack of sample for AutoAnalyzer.

[b]Values were not obtained because of blood sample coagulation.

Table 12. Hematology of male rats on experimental diet

Rat no.	Packed cell volume %	Hemoglobin gm %	White blood cell count mm^3	Prothrombin time sec.	Cholesterol mg %
8	42[a]	13.0[a]	13,525[a]	17.75[a]	80
45	--[a]	--[a]	--[a]	--[a]	85
53	42	11.5	11,150	17.3	92
56	42	13.9	8,525	15.7	95
57	43	13.9	9,125	15.0	92
103	42	13.5	10,900	13.0	80
107	47	14.6	12,875	13.5	98
110	46	14.8	18,575	13.8	84
112	45	14.3	10,075	16.2	106
117	42	11.9	5,425	16.3	80
121	45	13.2	15,975	16.1	100
125	48	15.3	10,675	14.9	82
127	44	13.8	10,050	15.2	98
129	44	13.8	9,200	17.0	98
131	46	14.5	7,650	14.8	70
Average	44	13.7	10,980	15.5	89

[a]Values were not obtained because of blood sample coagulation.

Table 13. Hematology of female rats on experimental diet

Rat no.	Packed cell volume %	Hemoglobin gm %	White blood cell count mm^3	Prothrombin time sec.	Cholesterol mg %
9	38	13.5	9,400	17.7	63
40	39	12.2	7,225	16.3	106
42	41	13.0	8,375	14.4	83
48	35	12.2	9,275	13.0	84
54	41	12.2	10,875	15.5	94
58	39	13.1	6,100	14.8	74
59	43	13.7	6,725	21.5	126
60	38	12.4	4,650	15.7	--[a]
102	40	12.7	13,025	12.1	76
106	43	13.2	8,175	13.2	84
116	44	14.3	10,075	16.2	76
120	42	13.1	6,525	14.5	80
124	39	13.2	7,600	14.5	90
Average	40	13.0	8,309	15.3	86

[a]Lack of sample for AutoAnalyzer.

Table 14. Body weight of male rats on control diet (gms)

Rat no.	Week of experiment 1	2	3	4	5	6	7	8	9
27	450	461	474	508	538	525	551	545	557
55	534	531	556	581	600	600	620	628	632
101	240	292	334	358	368	375	394	388	381
105	265	311	349	365	374	388	412	423	430
109	268	294	332	356	372	387	417	418	410
111	275	295	365	395	418	458	455	458	475
113	267	313	354	378	398	420	440	438	448
115	263	307	350	365	395	415	438	444	450
126	258	310	344	358	371	388	400	408	400
128	264	314	356	378	387	408	420	420	405
130	264	307	348	372	378	418	421	430	452
Average	304.4	339.5	378.4	401.3	418.1	434.7	451.6	454.5	458.0

Table 15. Body weight of female rats on control diet (gms)

	Week of experiment								
Rat no.	1	2	3	4	5	6	7	8	9
39	297	300	302	312	312	320	324	325	330
41	278	297	316	325	338	335	333	320	335
43	310	324	346	365	368	374	388	397	400
51	272	272	272	274	275	285	287	287	287
52	362	354	374	364	383	392	400	412	412
100	170	198	220	232	245	251	255	265	268
104	184	205	225	228	243	247	250	257	265
108	198	225	254	260	280	294	294	299	285
114	161	177	192	201	214	224	225	235	235
122	168	194	208	220	227	225	232	240	245
Average	240.0	254.6	270.9	277.9	288.5	294.7	298.8	303.7	306.0

Table 16. Body weight of male rats on experimental diet (gms)

	Week of experiment								
Rat no.	1	2	3	4	5	6	7	8	9
8	435	425	424	450	450	467	477	470	473
45	472	474	485	512	495	524	546	526	530
53	474	460	450	458	448	460	470	471	484
56	480	455	448	465	453	480	510	510	509
57	435	444	442	448	445	459	465	468	453
103	232	240	272	294	328	351	345	366	394
107	237	242	266	270	322	344	346	365	387
110	235	237	270	275	308	328	330	345	362
112	251	259	291	302	345	361	350	367	379
117	246	260	304	315	345	371	380	400	410
121	248	252	303	302	346	340	344	372	406
125	258	268	303	327	375	399	410	423	450
127	248	255	291	296	337	350	355	365	395
129	241	245	285	291	330	338	331	364	400
131	247	247	296	315	338	375	380	393	425
Average	315.9	317.5	342.0	354.7	377.7	396.5	402.6	413.7	430.0

Table 17. Body weights of female rats on experimental diet (gms)

	Week of experiment								
Rat no.	1	2	3	4	5	6	7	8	9
9	293	276	275	278	274	285	285	283	283
40	289	282	280	290	285	285	287	285	290
42	300	286	271	278	269	302	292	305	304
48	300	296	303	318	315	332	335	325	330
54	278	270	263	268	258	277	267	270	267
58	328	316	255	270	260	265	271	265	280
59	268	262	307	305	302	318	320	315	325
60	263	257	254	274	265	277	280	265	270
102	174	185	205	210	217	232	230	225	233
106	135	140	165	170	192	204	207	210	225
116	154	162	194	198	216	222	224	230	225
120	120	174	202	202	222	225	232	240	245
124	157	159	180	187	215	218	219	225	235
Average	235.3	235.8	242.6	249.8	253.1	264.8	265.3	264.8	270.0